A STUDY OF SOME MECHANISMS OF EXPERIMENTAL SALT-INDUCED HYPERTENSION IN RATS

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Certification

This is to certify that the dissertation titled : *A study of some mechanisms of experimental salt-induced hypertension in rats* submitted to the School of Postgraduate Studies of the University of Lagos for the award of the degree of Ph.D. in Physiology, is a record of original research carried out by P.C.M. Obiefuna in the Department of Physiology, College of Medicine of the University of Lagos, and has not been previously submitted elsewhere for a similar purpose.

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INTERNAL EXAMINER

EXTERNAL EXAMINER

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Dedication

This dissertation is dedicated to the affection of:

my father CHIEF M.A.N. OBIEFUNA, the biggest and the best;

PROFESSOR E.N. UGOCHUKWU, whose idea it first was;

and the love of my life,

MRS IDONGESIT P. OBIEFUNA

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Abstract

A relationship between high blood pressure and dietary salt intake has been postulated for over a century. Dietary salt ingestion is now known to positively correlate with arterial blood pressure in man and experimental animals. The underlying mechanisms are, however, not clear. This study examined vascular smooth muscle activity in a Sprague-Dawley rat model of salt- induced hypertension.

Hypertension was induced in Sprague-Dawley rats of either sex made to eat a diet containing 8% NaCl for 6 weeks, during which control rats ingested normal rat chow (0.3% NaCl). Feed and tap water were presented to the animals *ad libitum*. Body weight and food intake were measured periodically and direct arterial pressure (BP) and heart rate (HR) were estimated terminally under anaesthesia in a random sample of each group. Serum sodium and potassium concentrations were also estimated terminally.

Vascular smooth muscle mechanisms were studied *in vitro* on ring preparations of the thoracic aorta from control and salt- loaded rats. Time-course of contraction of the rings to noradrenaline (NA) and to high-K^+ were determined with 10^{-5}M NA and 100mM KCl. Time-course of relaxation of NA- and K^+ - precontractions were determined by Ca^{2+} - withdrawal with Ca-free EGTA solutions. Dose-response tests to NA

(10^{-10}M to 10^{-5}M), 5- hydroxytryptamine (5-HT, 10^{-9}M to 10^{-5}M) and high-K^+ (4.7mM to 100mM) solutions were determined on freshly equilibrated tissues.

The differential roles of the Ca-entry channels - the receptor- operated (ROC) and the potential sensitive (PSC) channels were studied with specific physiological agents: The ROC with 10^{-5}M NA and the PSC with 100mM K+. Intracellular Ca^{2+} dynamics was studied, by monitoring phasic response, in freshly equilibrated rings to 10^{-5}M NA in a Ca^{2+}o-free medium. Rate of tone loss following phasic contraction was also studied.

The modulatory role of extracellular Mg^{2+} was studied by Mg^{2+}o-induced (range 0mM to 9mM) relaxation of established 10^{-7}M NA- and 40mM K^+-contractions. Ca^{2+}-Mg^{2+} interactions were studied as the effect of calcium agonists, Bay K8644 and CGP 28392, on Mg^{2+}o- induced relaxation of 40mM K^+-contractions.

The comparative effects of the potassium channel opener BRL 34915, the specific calcium channel blockers diltiazem and nifedipine and also the non-specific blocker hydralazine were tested on aortic rings from both rat groups, precontracted with either 10^{-7}M NA or 60mM KCl.

Calcium-induced relaxation (3mM to 25mM) was estimated as an index of the membrane stabilising property of high extracellular calcium ion concentration.

Endothelial function was estimated by recording relaxation responses to acetylcholine (ACh) and histamine in both intact and endothelium-denuded rings from both rat groups.

The results at the end of the feeding experiments showed that food consumption per unit body weight was similar in both rat groups but there was a significant weight loss in rats fed a high salt diet (body weight: control 154±6g, salt- loaded 125±5g; $P<0.05$). Salt loaded rats developed mild hypertension (Mean BP: control 92.0±2.6mmHg, salt-loaded 159±4.3mmHg; $P<0.05$), hypokalaemia (Serum K: control 5.7±0.2mM, salt-loaded 4.0±0.1mM; $p<0.05$). There were, however, no significant changes in HR and serum Na concentration due to salt loading.

Aortic rings from salt-loaded rats appeared to contract more slowly than normotensive aortic rings to NA ($P<0.05$). Moreover, maximum responses induced by NA in salt-loaded rat rings (1191±92mg) was lower ($P<0.05$) than that in control rings (1710±129mg). Similar observations were made for KCl (salt-loaded 896±84mg, control 1325±147; $P<0.05$). Maximal responses to 5-HT did not differ between the two rat groups. There were no significant differences, between the two rat groups, in the rates of relaxation of the aortic rings to Ca^{2+} withdrawal following established NA or KCl contractions.

Salt loading appeared to significantly ($P<0.05$) shift the dose- response curve to NA to the left {EC_{50}(M): control $4.4(\pm1.0)\times10^{-8}$, salt-loaded $3.7(\pm0.8)\times10^{-9}$}. Similar observations were made for KCl (EC_{50}(M): control 21.36±1.68, salt-loaded 13.27±0.98). The results imply possible salt-induced increase in vascular sensitivity to NA and KCl. Dose response test to 5-HT showed no difference between the control and salt-loaded rats. There appeared to be no significant effect of salt-loading on maximal phasic tone but rings from salt-loaded rats appeared to lose tone less readily than control rings. Calcium chloride concentration response curve was shifted to the left

by salt loading if Ca^{2+} entry was restricted to ROCs but during PSC-mediated Ca^{2+} entry, no difference was observed between the two rat groups.

Serum from salt-loaded rats appeared to contain more vasoactive substances than that from control rats. Moreover, salt-loading appeared to inhibit ouabain-sensitive vascular Na,K-ATPase pump activity.

$Mg^{2+}{}_o$-induced relaxation of KCl contractions and 10^{-7}M NA contractions were both significantly attenuated by salt loading. It, however, appears that increased calcium influx in both control rings and rings from salt-loaded rats affected $Mg^{2+}{}_o$-induced relaxation in a similar way.

During K^+-stimulation, BRL 34915 induced a significantly biphasic response in the control group and a contractile response in rings from salt-loaded rats. The difference between the two rat groups was statistically significant ($P < 0.05$). During NA stimulation, BRL 34915 produced dose-dependent relaxations in both rat groups. Maximum responses were reached at BRL 34915 concentrations of 10^{-5}M, at which 88.37±13.37% relaxation was produced in rings from salt-loaded rats and 105.5±3.6% observed for the control group ($P < 0.05$).

Diltiazem, hydralazine and nifedipine all caused relaxation of contractions to NA and high-K^+ in both rat groups with salt- loading showing attenuated relaxation responses. Differences in relaxation to diltiazem of both NA- and high-K^+ contractions were significant between the two rat groups ($P < 0.05$). Attenuated relaxation responses to hydralazine in the salt-group were significant during NA stimulation but not during KCl stimulation. The differences in relaxation to nifedipine on the other hand were not

significant under the various experimental conditions. There was no noticeable effect of salt loading on membrane stabilisation to Ca^{2+}_{o}.

Aortic rings from salt-loaded rats showed attenuation of endothelium-dependent relaxation responses to histamine {IC_{30}(M): control $4.1 \pm 1.3 \times 10^{-4}$, salt-loaded $1.5 \pm 0.2 \times 10^{-3}$; $P < 0.05$}. Endothelium-dependent relaxation to acetylcholine did not appear to be affected by salt loading.

In summary, contractile processes – NA-induced contraction, depolarization contraction, serum contraction and ROC activation – were enhanced while relaxant processes – endothelium-dependent histamine-induced relaxation, membrane stabilization, Mg^{2+}-relaxation, Na-K-ATPase pump activity and Ca^{2+} sequestration – were attenuated by salt loading. Some processes, on the other hand, were not affected by salt loading. These were acetylcholine-relaxation, 5-HT-contraction and Ca^{2+}-entry via PSC.

The results generally suggest that salt-loading may cause blood pressure elevation by altering vascular smooth muscle modulatory processes such that some vasorelaxant components are attenuated and some vasoconstrictor mechanisms enhanced.

CHAPTER 1
INTRODUCTION

1.1. DIETARY SALT AND HYPERTENSION: BROAD OVERVIEW

A relationship between high blood pressure and dietary salt intake has been postulated since antiquity (Ruskin, 1856). Experimental evidence for this was first put forward in the early part of this century by Ambard & Beaujard (1904) who found that the blood pressure of patients with renal failure could be influenced by the amount of salt they were given to eat. They, however, thought chloride was responsible for the effect they observed on blood pressure; they probably did so because they could assay chloride but not sodium. After World War II, Kempner (1948) published his observations that patients with malignant hypertension responded with a decrease in blood pressure and improvement in end-organ damage if they were given a diet consisting almost solely of boiled rice and fruit. This diet offered about 10mmol/day of sodium and chloride and was also extremely low in protein and high in potassium.

Dahl (1961) gave great impetus to the salt hypothesis of hypertension in several ways. He developed two strains of rats from Sprague-Dawley (SD) stock by feeding SD rats an 8% salt diet. He mated rats that developed high blood pressures with each other and rats with low blood pressures with each other. Within three generations, he was successful in demonstrating not only the salt sensitivity and salt resistance of hypertension in rats, but also was able to show the influence of genetic variance on the salt sensitivity of blood pressure. By showing that animals were heterogeneous in their blood pressure responses to a high-salt diet, and that these responses were heritable, Dahl made a particularly unique contribution (Dahl, 1972, 1977).

Later, others would show that humans are also heterogeneous with respect to the blood pressure- increasing effects of salt (MacGregor, Best, Cam, Markandu, Elder, Sagnella & Squires, 1982; Berglund, 1983; Miller, Daugherty, Weinberger, Grim, Christian & Lang, 1983; Watt, Tudor, Hart & Foy, 1983b; DeWardener, 1991). This heterogeneity in the response of blood pressure of humans to salt intake explains, in part, the difficulty in demonstrating a straightforward relationship between salt intake and blood pressure within populations (Miall, 1959; Oldham, Pickering & Roberts *et al.*, 1960; Simpson, Waal-Manning, Bolli, Phelan & Spears, 1978; Bing, Thurson

& Swales, 1979; Elliot & Stamler, 1988; DeWardener, 1990b; DeWardener, 1991).

In further experiments, including cross transplantation studies, Dahl demonstrated the role of the kidney and the importance of circulating humoral factors in the development of salt-sensitive hypertension (Dahl, Heine & Thompson, 1974; Dahl & Heine, 1975). On the basis of these findings and a body of research generated by numerous other investigators, notable among which was that of Gleibermann (1973), experts in the field suggested that if the population ingested a diet containing less than 60mmol/day of sodium, high blood pressure would cease to be a public health problem (Freis, 1976).

The earlier epidemiological data, their pitfalls and shortcomings, have been reviewed and criticised (Prineas & Blackburn, 1985). In the last decade a newer body of important data has been published that cast light on the issue of dietary salt level and hypertension. A new, comprehensive, worldwide epidemiological study has been performed and published that supplanted the earlier and partly erroneous survey relied on by Dahl (1960) and Gleibermann (1973). Experimental animal and cellular investigations have been performed which shed new light on various possible mechanisms by which salt intake and sodium may influence blood pressure (Gavras, 1986; Miyajima & Bunag, 1987; Maxwell & Waks, 1987; Luft, 1989; Morgan, DiBona &

Mark, 1990; Widimsky, Kuchel, Debinski & Thibault, 1990). These mechanisms are reviewed in section 1.3 (page 12).

Salt sensitivity and salt resistance have been characterised in normal humans and in those with hypertension. Prospective dietary intervention trials have been conducted in patients with essential hypertension; these trials have delineated the therapeutic role of dietary salt restriction in the treatment of hypertension (Beard, Cooke, Gray & Barge, 1982; MacGregor *et al.*, 1982; Silman, Locke, Mitchell & Humpherson, 1983; Watt, Edwards, Hart, Hart, Walton & Foy, 1983a; Richards, Nicholls, Espiner, Ikram, Maslowski, Hamilton & Wells, 1984).

Although considerable work remains to be done, substantial progress has been made that provides a more realistic perspective of the sodium-hypertension issue.

1.2. ROLE OF SODIUM IN BLOOD PRESSURE REGULATION

1.2.1. EPIDEMIOLOGICAL STUDIES

Since the publications of Dahl (1960) and Gliebermann (1973), numerous epidemiological studies have sought a relationship between dietary salt intake and blood pressure (Prineas & Blackburn, 1985). Most of these investigations were mostly within populations, and most were not

able to identify a relationship between salt intake and blood pressure. A more recent analysis of data from the National Health and Nutrition Examination Survey I (USA) suggested an inverse relationship between salt intake and blood pressure (McCarron, Morris, Henry & Stanton, 1984). To overcome serious methodological problems from previous surveys and to provide information from across- population and within-population analyses, a large investigative team, termed Intersalt, headed by Rose and Stamler (Intersalt cooperative research group, 1988) set about a more carefully controlled study. Their aim was to address most of the epidemiological hypotheses of salt-hypertension.

Fifty-two centres in 32 countries throughout the world participated in the Intersalt study which involved 10,079 men and women (Stamler, Rose, Elliot, Dyer, Marmot, Kesteloot & Stamler, 1991). Two hundred subjects ranging in age from 20 to 59 years were recruited at each centre. Twenty-four-hour urine samples were carefully collected. They found that in four unacculturated centres, sodium ingestion (as reflected by urinary sodium excretion) was found to be very low (less than 50mmol/day), blood pressure tended not to increase with age, and very little hypertension was identified in those populations. In the remaining 48 centres, sodium excretion ranges from 100mmol to 246 mmol/day. When data from all 52 centres were included in the

analysis, there was a significant, direct correlation between sodium excretion and median blood pressure levels; however, this relationship was lost if the four unacculturated centres were deleted from the analysis. The same was true for the relationship between the prevalence of hypertension and median sodium excretion of the centres. Potassium excretion, on the other hand, was negatively correlated with blood pressure in individual subjects after adjustment for confounding factors (Elliot & Stamler, 1988).

One potential limitation of Intersalt may be related to the relatively narrow age range of the subjects in the study. The span of 20-59 years ignores the segment of the population considered to be 'elderly'. It is primarily this population that is at the greater risk for the sequels of hypertension and is also the population that is most likely to exhibit changes in blood pressure with alterations in dietary salt intake (Swales, 1988).

Aside from these limitations, the Intersalt experiments still hold great significance in relation to similar studies. Maddocks (1967) compared different populations in New Guinea and found that, while in the highland no hypertension was found and blood pressure did not increase with age, in coastal communities in which people consumed salted canned foods, blood pressure increased with age and hypertension was present. Similar studies in other primitive societies con-

firmed a relationship between blood pressure and sodium intake (Lowenstein, 1961; Prior, Grimley, Harvey, Davidson & Lindsey, 1968; Shaper, 1972; Sinnett & Whyte, 1973; Page, Danion & Mollering, 1974; Oliver, Cohen & Neal, 1975; Chuwa, 1987).

A recent study of the Yi people in Japan (mean sodium excretion ranged between 74-190mmol/24hr) is consistent with the view that a diet low in sodium and high in potassium, calcium and magnesium may prevent the development of hypertension (He, Tell, Tang, Mo & He, 1991).

In contrast to cross-cultural studies, studies of the correlation between habitual sodium intake and blood pressure within industrialised populations have been almost uniformly negative (Dahl & Love, 1954; Oldham et al., 1960; Dawber, Kanel & Kagan, 1967).

The reason why no relationship was found between sodium intake and hypertension in industrialised societies may be because of the relatively high range of habitual sodium intake in these populations: about 100 - 200 mEq per day, in contrast to unacculturated societies with sodium intake of less than 50mEq per day. In the Akita Prefecture of northern Japan where sodium intake is extremely high, with a range of 500 - 600 mEq per day, the prevalence of hypertension is 48 per cent,

and stroke is the most prominent cause of death (Fukuda, 1954; Takahashi, Saaski & Takeda, 1957).

It is possible that only a susceptible minority within a population will experience an increased blood pressure when exposed to a high sodium intake. Thus, if the range of sodium intake within that population is relatively small, even though the sodium intake itself is large, no correlation may be found between arterial pressure and sodium intake. The body of evidence that supports this opinion was recently reviewed by Adigun and Akinyanjuola (1989).

1.2.2. SODIUM LOADING AND RESTRICTION IN MAN

1.2.2a. SODIUM LOADING

Kirkendall and his colleagues (Kirkendall, Conner & Abboud, 1976) found no rise in mean blood pressure in normotensive volunteers after increasing their daily intake of sodium from 10 to 410 mEq per day for 4 weeks. Luft and Coworkers (Luft, Rankin, Bloch, Weyman, Willis, Murray, Grim & Weinberger, 1979) varied sodium intake from 10 to 1500 mEq per day in normotensive volunteers and found that blood pressure was not affected at sodium intakes of less than 800 mEq per day, and even at that level of consumption not all subjects had elevated blood pressures. The blood pressure rise was either avoided or attenuated when potassium balance was maintained. These results

are in accordance with population studies described above, and suggest that the effect of dietary sodium on blood pressure level can be demonstrated only at extremes of intake.

Hypertensive subjects demonstrate variable response to sodium loading. There was a modest rise in blood pressure in subjects with borderline hypertension after increasing sodium intake from 10 to 410 mEq per day (Mark, Lawton, Abboud, Fitz, Conner & Heistad, 1975). In a study by Kawasaki and his colleagues (Kawasaki, Delea, Barther & Smith, 1978), out of a group of 19 hypertensive subjects 9 subjects showed significantly increased blood pressure (salt- sensitive), while the other 10 subjects showed negligible changes in pressure (salt-resistant). Similar observations were made by Fujita, Henry, Barther, Lake & Delea (1980) who showed that 9 of their 18 hypertensive patients were salt-sensitive.

Most pertinent in the relationship of sodium to hypertension may be the demonstration that the blood pressure of normotensive teenagers with one hypertensive parent rose in response to increased salt intake, whereas teenagers with normotensive parents did not have a rise in their blood pressure after increasing their sodium intake (Falkner, Onesti & Hayes, 1982).

1.2.2b. SODIUM RESTRICTION

Studies on the effect of moderate sodium restriction in subjects with mild hypertension which were reported in the 1970s claimed that it produced as great a drop in blood pressure as that achieved by the use of diuretics (Gleibermann, 1973). More recent, carefully controlled, double-blind crossover studies comparing low and high sodium intake (MacGregor *et al.*, 1982; Watt *et al.*, 1983a) showed a heterogeneity of subject responses, so that blood pressure might increase, decrease, or stay the same. Richard's group (1984) and Longworth's (Longworth, Drayer & Weber, 1979) found that reduction of sodium intake by about 50 per cent resulted in no change in mean blood pressure. Parfrey, Markandu, Roulston, Jones, Jones & MacGregor (1981) found a small but significant reduction of mean arterial pressure on a very low sodium intake. Recently, Klemm, Gordon, Tunny & Finn (1990) reported that severe dietary sodium restriction corrected hypertension and hyperkalaemia in two patients with Gordon's Syndrome.

The heterogeneity in response to salt restriction may be mirrored by the blood pressure response to salt loading, in which some subjects are salt- sensitive and some are salt-resistant. It has been proposed that salt-sensitive hypertensive subjects represent a subset of subjects with essential hypertension (Kawasaki *et al.*, 1978).

1.2.3. EXPERIMENTAL HYPERTENSION

Certain forms of experimental hypertension may be induced in animals by increasing salt intake (Lenel, Katz & Rodbard, 1948; Koletsky, 1959; Meneely & Dahl, 1961; Dahl, Leitt & Heine, 1972), with or without the administration of mineralocorticoids or reduction of renal mass (Saperstein, Brandt & Drury, 1950; Koletsky & Goodsitt, 1960; Carretero & Romero, 1977; Dina, Sofola, Egbe & Owolabi, 1986; Obiefuna, Sofola & Ebeigbe, 1991). It is easier to produce salt-induced hypertension in young rats than in adult rats (Maxwell & Waks, 1987; Luft, 1989). Dahl (1972, 1977) demonstrated variability in the response to salt loading in normal rats and was able to distinguish an apparent genetic basis for sodium-dependent hypertension in this species. Through selective breeding he developed two strains, one salt-sensitive, the other salt-resistant. Resistant strains were also resistant to other forms of experimental hypertension except for experimental renovascular hypertension (Dahl, 1977).

A major shortfall of animal studies is that the relative amount of salt required to induce hypertension in animals is far in excess of the usual salt content of human diets. For example, the 8% NaCl fed to rats to induce hypertension is equivalent to human consumption of 40g of sodium daily (Maxwell & Waks, 1987). Even very high salt intakes

do not consistently result in hypertension in all or even in most animals, implying heterogeneity of response. In the Kyoto spontaneously hypertensive rat, which is considered to be the closest counterpart of human hypertension, similar clear-cut salt sensitivity has not been demonstrated (Luft, 1989).

Although experimental salt-induced hypertension does not necessarily support a primary role for sodium in the pathogenesis of human essential hypertension, the model may be applicable for a subset of genetically susceptible individuals.

1.3. MECHANISM OF SALT-INDUCED HYPERTENSION

In seeking to explain how dietary salt induces hypertension in humans and experimental animals, researchers have proposed quite a number of mechanisms. While some of these proposed mechanisms are based on empirical evidence, a number of them are largely speculative. Presented below are bodies of evidence which support the various mechanisms: (a) neurogenic, (b) baroreflex, (c) renal, with emphasis on the atrial natriuretic factor (ANF), and (d) vascular.

1.3.1. NEUROGENIC MECHANISMS

1.3.1a. PERIPHERAL MECHANISM

The use of new, sensitive, and specific radioenzymatic assays for the measurement of circulating catecholamines as well as the existence of a significant correlation between the blood pressure and circulating noradrenaline (NA) levels in DOCA-salt rats and in sodium-loaded human subjects (Nicholls, Kiowski, Zweifler, Julius, Schork & Greenhouse, 1980; Bouvier & DeChamplain, 1986) point towards a possible involvement of neurogenic mechanisms in the genesis of salt-hypertension. Similar studies have further shown that the elevated circulating NA concentration observed in salt-hypertension was mainly due to an increase in NA spillover rate and could not be accounted for by a reduction in the NA clearance rate (Bouvier & De Champlain, 1985). These observations as well as the findings of increased sympathetic nerve electrical activity (Triuchijima, Mizogami & Sokabe, 1975; Katholi, Naftilan & Oparil, 1980) strongly support the hypothesis that the development of hypertension is closely associated with the activation of the sympathetic system (DeChamplain, Eid, Drolet, Bouvier & Foucart, 1989).The presynaptic modulation of neurotransmitter release from sympathetic fibres by alpha and beta-adrenergic autoreceptors (Yamaguchi, DeChamplain & Nadeau, 1977; Langer, 1981; Majewski & Rand, 1986) as well as the local modulation of

catecholamine release from chromaffin cells by alpha-2 adrenoceptors (Bouvier & DeChamplain, 1983; Foucart, Nadeau & DeChamplain, 1987) and by beta-2 adrenoceptors (Foucart, Nadeau & DeChamplain, 1988) constitute putative additional sites where dysfunction could lead to the development of an increased sympathoadrenal tone and reactivity. Since it has been demonstrated that those local adrenergic modulatory mechanisms are highly dependent on the frequency of stimulation as well as on the local concentrations of catecholamines (Foucart *et al.*, 1987, 1988), it may be expected that a chronic increase in catecholamine release from sympathetic fibres and adrenal medulla might alter the sensitivity and the reciprocal interactions of those modulatory mechanisms. Under physiological conditions, presynaptic beta-2 receptors facilitate catecholamine release at low frequency of stimulation, whereas postsynaptic alpha-2 receptors inhibit the release at higher frequencies of stimulation (Borkowski, 1988). The development of an imbalance between the sensitivity of those two systems could, therefore, result in marked alterations in the process of catecholamine liberation.

The possibility of an attenuation of the postsynaptic beta-adrenoceptor function has been suggested by several studies on experimental and human hypertension (Borkowski, 1988).

Specific radioligand studies have consistently demonstrated a reduction of β-adrenergic sites in the heart and vessels of hypertensive animals (Limas & Limas, 1978, 1979; Woodcock, Olsson & Johnston, 1980; Fujimoto, Dohi, Aoki & Matsuda, 1988).

These studies support the hypothesis of a dominance of alpha-1 adrenergic receptor functions in hypertension. This condition combined with a resetting of baroreceptor sensitivity, as is reviewed later, could account to a good extent, for the elevated blood pressure and for the abnormal haemodynamic responses observed in experimental and human salt-sensitive hypertension.

1.3.1b. CENTRAL MECHANISM

The hypothesis of an involvement of brainstem alpha-2 adrenoceptors in salt-hypertension was proposed by Gavras (1986). The theory depends largely on the knowledge that: (i) sodium excess causes sympathetic overactivity (Triuchijima *et al.*, 1975; Nicholls *et al.*, 1980), (ii) the normal function of central catecholaminergic neurons located in the brainstem is to exert a constant tonic inhibition of sympathetic vasoconstrictor tone (Chalmers, 1975; Nathan & Reis, 1977; Carey, Dacey, Jane, Win, Ayers & Tyson, 1979; Reis, 1981), and (iii) the alpha-2 adrenergic receptor function of those catecholaminergic neurons can be attenuated by the sodium ion *in vitro*, which

decreases the receptor sensitivity to locally occurring agonist neurotransmitters (Greenberg, U'Prichard, Sheehan & Snyder, 1978; Tsai & Lefkowitz, 1978; Rouot, U'Prichard & Styder, 1980; Glossman, Lubbecke, Belleman, Sattler & Doell, 1982; Bylund & U'Prichard, 1983).

Gavras (1986) proposed that it is possible for sodium ions to act in a similar manner *in vivo* and thus, loading with sodium chloride may decrease the affinity of alpha-2 adrenergic receptors located in the central nervous system for naturally occurring local agonists. This would lead to diminished tonic inhibition of central sympathetic outflow to the periphery.

In fact, Gavras and his colleagues have recorded lasting pressor responses in rats following microinjection of hypertonic sodium chloride into the medulla of these animals (Gavras, Bain, Bland &Gavras, 1983; Gavras, Bain, Bland, Vlahakos & Gavras, 1985).

One salient requirement for this hypothesis, however, is for sodium ion level to increase in brain tissues following salt loading. Miyajima & Bunag (1984), in an intracerebroventricular (ICV) infusion experiment similar to those of Gavras *et al.*(1983, 1985), observed similar pressor responses but found no discernible alteration in either cerebrospinal fluid sodium concentration or responsiveness to

anterior hypothalamic stimulation in rats fed for five weeks on a high-salt diet.

It would, therefore, appear that central mechanisms such as those activated by intracerebroventricular infusion of salt solutions are not involved in baroreceptor inhibition produced by dietary salt loading.

1.3.2. THE BAROREFLEXES

Excessive salt intake and abnormal baroreflex regulation have been commonly implicated in causing hypertension, but exactly how they interact to elevate blood pressure is still unknown.

Rocchini and his colleagues in 1977, first observed that pressor responses to carotid occlusion were inhibited in conscious dogs by low-sodium diets (Rocchini, Cant & Barger, 1977). Ferrario, Brosnihan, Takishita, Szilagyi & Smeby (1982) subsequently confirmed blunting of baroreflex pressor responses by salt depletion and further showed that this was due to sympathetic inhibition resulting from increased vagal afferent activity from cardiopulmonary receptors. Another early attempt to correlate dietary salt intake with baroreceptor function was made by Gordon, Matsuguchi & Mark (1981), who found that although Dahl salt-sensitive rats became hypertensive only when fed high-salt diets, reflex bradycardia in response to injected phenylephrine was consistently reduced, thereby suggesting that

baroreflex sensitivity was impaired, whether the salt-sensitive rats were hypertensive or not.

Miyajima & Bunag (1985) fed high-salt and low- salt diets to different groups of normotensive Sprague-Dawley rats. They found that the high salt intake caused the development of mild hypertension with diminished baroreflex sensitivity in the animals. Baroreceptor resetting as suggested by DeChamplain *et al.*, (1989) cannot fully account for the diminished baroreflex sensitivity following salt loading: Ferrari & Mark (1987) were able to produce salt-induced baroreflex damping in Dahl salt-sensitive rats when blood pressure rise was prevented by chemical sympathectomy with 6-hydroxy dopamine. More compelling evidence for an action of salt on aortic baroreceptors is the finding by Miyajima & Bunag (1985; 1987) that responses to stimulation of the central cut end of the aortic nerve were unaffected by salt loading. This implies that parts of the reflex arc that are promixal to the cut end are not involved in the salt-induced aberrations of the baroreflex activity.

Calhoun, Wyss & Oparil (1991) have, however, relently shown enhanced arterial baroreceptor reflex in NaCl-sensitive spontaneously hypertensive rats. Calhoun *et al* (1991) tested arterial baroreceptor reflex-mediated changes in lumbar nerve activity and heart rate in conscious, unrestrained rats during phenylephrine-induced and

nitroprusside-induced changes in mean arterial pressure.They opined that the NaCl-induced augmentation of arterial baroreceptor reflex control of lumbar sympathetic nerve activity in salt-sensitive spontaneously hypertensive rats would serve to buffer the NaCl-induced increase in mean arterial pressure. Calhoun *et al* (1991) did not, however, attempt to localise the effect they observed to either the peripheral or central components of the central arterial baroreceptor reflex arc.

Baroreflex dysfunction could result from conditions that induce reduced vessel wall distensibility (Angell-James, 1973, 1974), baroreceptor abnormalities (Sapru & Krieger, 1979), local ionic changes (Haddy, Pamnani & Clough, 1978), or constriction by humoral agents such as angiotensin (Pettinger, Marchell & Augusto, 1971) or vasopressin (Cowley, Merril, Osborn & Barber, 1984). In fact, the discharge properties of baroreceptors of an *in vitro* perfused aortic arch preparation and the reflex effects originating from the vascularly isolated carotid sinus have been shown to be directly related to the concentration of sodium in the perfusion fluid (Kunze, Saum & Brown, 1977; Andressen, Kuraoka & Brown, 1979). However, although recent observations of Ferrari & Mark (1987) may corroborate these findings, they in addition measured sodium and potassium ion concentration in serum of their experimental animals and found no diet-related differ-

ences. Moreover, intracellular Na^+ and K^+ in arterial smooth muscle cells have also been found to be unaffected by Na^+ intake in Dahl rats (Abel, Trepani, Matsuki, Ingram, Ingram & Hermsmeger, 1981).

1.3.3. RENAL HANDLING OF SODIUM

Transplant experiments have shown the importance of the kidney in the salt model of experimental hypertension (Dahl *et al*, 1974; Bianchi, Baer, Fox, Duzzi, Pagetti & Griovanetti, 1975). Tobian and others (Tobian, Iwai, Hiller, Johnson & Goossens, 1979) showed that kidneys of young still normotensive, Dahl salt-sensitive rats excreted only half as much sodium as kidneys from resistant rats at equal levels of inflow pressure. Sensitive Dahl rats also failed to develop hypertension while receiving an 8% salt diet if sodium retention was prevented by the administration of a thiazide diuretic (Tobian *et al*, 1979).

An important finding was recently reported by Morgan, DiBona & Mark (1990). They transplanted the kidneys of Dahl salt-sensitive (DS) and salt-resistant (DR) strains of rat among one another. After two weeks of 8% salt loading, both Dahl salt-sensitive rats with DR kidneys and salt-resistant rats with DS kidneys developed hypertension. Although this observation supports the long-known renal mechanism, development of hypertension in the DS strains with DR kidney transplant is revolutionary. It is a renal evidence for the invol-

vement of extrarenal factors in the development of salt-hypertension.

The renal mechanism as postulated by DeWardener & MacGregor (1980) proposes that salt sensitivity is due to an inherited defect in the ability of the kidney to eliminate sodium. Further empirical evidence in favour of this hypothesis has been reported (Scrabal, Herholz, Neumayr, Hamberger, Ledochowski, Sporer, Hortnagl, Schwarz & Schonitzer, 1984; DeWardener, 1990a, 1991). The defect becomes more obvious with increased sodium intake. The difficulty in eliminating sodium, induces an increase in the concentration of atrial natriuretic factor (ANF), a sodium-transport inhibitor (Poston, 1984; Haddy, 1990). The ANF affects sodium transport across cell membranes. In the kidneys, it adjusts urinary sodium excretion so that sodium balance is near that of normal subjects on the same intake of sodium (DeWardener & MacGregor, 1981). It is speculated that the effects of ANF on vascular cells would be to inhibit Na, K-ATPase activity and hence increase vascular reactivity and blood pressure. This process has been shown to occur in essential hypertension (Tobian & Binion, 1952; Overbeek, Derifield, Pamnani & Sozen, 1974).

ANF (or more currently, atrial natriuretic peptide; ANP), peptide with several biological actions like natriuresis, diure vasorelaxation, hypotension, and involvement in the regulation sympathetic outflow (DeBold, Borenstein, Veress & Sonnenberg,

1981; Garcia, Thibault, Cantin & Genest, 1984; Kuchel, Debinski, Racz, Buu, Garcia, Cusson, Larochelle, Cantin & Genest, 1987), is released from the mammalian heart (Lang, Tholken, Ganten, Luft, Ruskoaho & Unger, 1985; DeBold, DeBold & Sarda, 1986; Ruskoaho, Tholken & Lang, 1986). Its active carboxylic terminal form, consisting of 28 amino acids circulates in the blood, from where it is rapidly eliminated (Murthy, Thibault, Schiffrin, Garcia, Chartier, Gutkowska, Genest & Cantin, 1986).

Recent reports have shown that prolonged high salt intake, for 5 weeks but not for 3 weeks, significantly decreases plasma ANF concentration as well as dissociates the correlation between right atrial pressure and plasma ANF in Sprague-Dawley rats (Debinski, Kuchel, Buu, Thibault, Tremblay & Hamet, 1988; Widimsky, Kuchel, Debinski & Thibault, 1990). These findings may either imply that the direct effect of ANP in the first three weeks are hardly reversible, or that other factors are responsible for keeping blood pressure above normal during the later stages of salt loading.

Acute infusion of ANF has been shown to produce an enhanced depressor response in salt-sensitive spontaneously hypertensive (SHR-S) rats fed a high NaCl diet compared with control SHR-S fed a normal NaCl diet (Jin, Yang, Chen & Oparil, 1988) and that dietary NaCl loading is associated with significantly increased circulating ANF

levels in Wistar-Kyoto rats but not in SHR-S (Jin, Chen, Yang, Meng & Oparil, 1988). Also, further studies by Jin, Chen & Oparil (1991) have shown that chronic infusion of ANF prevents NaCl-sensitive hypertension in spontaneously hypertensive rats.

It would be interesting to study vascular Na,K- ATPase activity following salt loading. Na^+ induced ATPase pump inhibition has been reported in erythrocytes of normotensive men (Quintanilla, Weffer, Koh, Rahman, Molteni & DelGreco, 1988).

1.3.4. VASCULAR RESPONSES

The changes that occur in the vascular smooth muscle cell in various forms of hypertension are fairly well understood and were reviewed by Triggle & Laher (1985). These changes generally centre around receptor malfunction and abnormalities in calcium ion handling both intracellularly and extracellularly (Holloway & Bohr, 1973; Aoki, Kondo, Mochizuki, Yoshida, Kato, Kato & Takikawa, 1978; Kwan & Daniel, 1981).

On the other hand, only a few studies have examined vascular changes in salt-hypertension. Limas, Westrum, Iwai & Limas (1982) have shown certain morphological changes in the aorta of salt- dependent genetic hypertension. Winquist, Bunting, Baskin & Wallace (1984) reported attenuated endothelium-dependent relaxation in New Zealand genetically hypertensive rats. In hypertensive Dahl rats, en-

dothelium-dependent relaxations in response to acetylcholine, adenosine 5'-phosphate, and thrombin are reduced (Luscher, Raij & Vanhoutte, 1987). Recently, Fukuda and his colleagues measured adenylate cyclase activity in the vascular smooth muscle in early and established DOCA-salt hypertension (Fukuda, Honda, Minato, Soma, Izumi & Hatano, 1990). They found that the activity of this "second messenger" was significantly higher in salt-loaded rats than in the controls.

The electrogenic Na,K-ATPase pump activity have recently been reported to rise in DOCA-salt hypertension (Soltis & Field, 1986; Friedman, McIndoe & Tanaka, 1988). It is, however, not known if 8% salt loading to SD or Dahl rats would produce similar effects.

Generally, apart from a few isolated reports in the literature, the role of the vascular smooth muscle in salt-hypertension is mostly unknown.

1.4. VASCULAR SMOOTH MUSCLE PROCESSES

1.4.1. ADRENERGIC AND SEROTONERGIC MECHANISMS

1.4.1a. ADRENERGIC MECHANISMS

The pressor effects of adrenaline were first demonstrated in 1906 by Dale and by Elliot & Durham. Subsequent attempts to understand

the mechanism of action of adrenaline gave rise to the concept of adrenoceptors. Adrenoceptors were classified into alpha and beta, by virtue of their responses to selective agonists and antagonists (Ahlquist, 1948; Lands, Arnold, McAuliff, Luduena & Brown, 1967).

The alpha-1 and alpha-2 adrenoceptors, first subdivided by Starke & Langer (1979) on the basis of their selectivities to agonists and antagonists are known to mediate contractile responses in vascular smooth muscles (Docherty, McDonald & McGrath, 1979; Drew & Whiting, 1979; Docherty & McGrath, 1980; DeMey & Vanhoutte, 1981b; McGrath, 1982). Circulating catecholamines constrict vascular resistance vessels by activating postjunctional alpha-1 and alpha-2 adrenoceptors (Flavahan & McGrath, 1980; McGrath, Flavahan & McKean, 1982; Wilffert, Timmermans & Van Zwieten, 1982).

This pharmacological classification of alpha adrenoceptors appears to be the most valid approach as long as the molecular structure of the receptors remains unknown. Drugs frequently chosen for their high degree of selectivity include the alpha-2 agonists clonidine and guanabenz, the alpha-2 antagonists yohimbine and rauwolscine, the alpha-1 agonist phenylephrine, and the alpha-1 antagonist prazosin (Bylund & U'Prichard, 1983). On the basis of this approach, the vascular smooth muscle has been shown to contain the classical postsynap-

tic alpha-1 as well as postsynaptic alpha-2 adrenoceptors (Drew, 1980; Langer, 1981).

Since postsynaptic ß-adrenoceptors predominantly mediate smooth muscle relaxation, the net vascular effect of either the exogenously added adrenaline or that produced by adrenal gland stimulation appears to be a balance between alpha-adrenoceptor pressor and beta-adrenoceptor-mediated depressor effects (Flavahan, Grant, Greig & McGrath, 1985).

Evidence for postjunctional beta-receptor activity following direct sympathetic stimulation is not available and this could be as a result of lack of beta-adrenoceptors at the junctional areas (Osswald & Guimaraes, 1983) or due to the inability of noradrenaline (NA) to activate beta-2 adrenoceptors (Lands *et al.*, 1967). Intravenous NA is unaffected by propranolol, a $ß_2$-adrenoceptor antagonist (Barnett, Docherty, Flavahan, Hart & McGrath, 1980).

The phasic and tonic components of vascular smooth muscle contractile responses to NA have been explained to reflect the release of intracellular calcium and the utilisation of extracellular calcium respectively (Golenhofen, 1976; Hester & Carrier, 1977). These tonic and phasic components have been shown to represent responses to stimulation of alpha-2 and alpha-1 adrenoceptors respectively (Van-Meel, DeJong, Lalkman, Wilffert, Timmermans & Van Zwieten,

1981). This is consistent with the observation that the response to stimulation of postjunctional alpha-2 adrenoceptors was susceptible to calcium-entry channel blockade, whereas that of alpha-1 adrecoceptors was relatively resistant (Van Meel *et al.* 1981; Cavero, Shepperson, LeFevre-Borg & Langer, 1983; Curro & Greenberg, 1983; Van Zwieten, Van Meel & Timmermans, 1983). These observations are, however, inconsistent with existing clinical evidence that calcium antagonists are efficacious in lowering arterial pressure in prazosin-insensitive hypertensive patients (Colucci, 1983). Similarly, further *in vivo* and *in vitro* studies have shown similarities in the mechanisms by which the alpha- adrenoceptor subclasses stimulate calcium ion influx (Cooke, Rimele, Flavahan & Vanhoutte, 1985; Morita, Maniwa, Satoh & Taira, 1985): alpha-adrenoceptors open a population of calcium channels referred to as receptor-operated channels.

1.4.1b. SEROTONERGIC MECHANISMS

The pressor response to 5-hydroxytryptamine (5-HT), which is due mainly to direct 5-HT_2 receptor- mediated action on vascular smooth muscle, and probably also by potentiation of the effects of other vasoconstrictors (Van Nueten, Janssen, Van Beek, Xhonneux, Verbeuren & Vanhoutte, 1981), could be demonstrated *in vivo* after ganglionic blockade (Paintal, 1973). The blockade prevents the fall in

heart rate and blood pressure that results from changes in autonomic nerve activity (Paintal, 1973).

5-HT produces net vasodilatation *in vivo* (Feniuk, Humphrey & Trevethick, 1981a; Feniuk, Hymphrey, & Watts, 1981b) by inhibition of neurogenic tone (Page & McCubbin, 1953; Feniuk & Humphrey, 1980; Feniuk, Humphrey & Watts, 1981a). It could also inhibit contractile responses to certain agents in isolated mesenteric smooth muscles (Moreland, VanBreemen & Bohr, 1985), or cause direct relaxation itself in certain other vessels like the coronary vessels and porcine vena cana (Eyre, 1975; Feniuk *et al.*, 1981a,b). The receptor involved in 5-HT relaxatory response (5-HT_1) are different from the 5-HT_2 subclass that mediates contraction (Feniuk *et al.*, 1981a).

Both the relaxatory and the contractile responses to 5-HT are blocked by methysergide (Coerletti & Doepfner, 1958; Feniuk *et al.*, 1981c) while ketanserine is specific for inhibiting contractile responses via 5-HT_2 receptors (DeCree, Leempoels, DeCock, Gouken & Verheagen, 1981; Leysen, Awouters, Kennis, Laduron, Vandenberk & Janssen, 1981; Van Nneten *et al.*, 1981; Fozart, 1982; Kalman, Timmermans & Van Zwieten, 1982; Kalkman, Harms, Van Gelderen, Batink, Timmermans & Van Zwieten 1983; Wenting, Woittiez, Man in't Veld & Schalenkamp, 1984).

1.4.2. CALCIUM

Current concepts of the role of calcium in contractile regulation in smooth muscle have progressed considerably in two decades. The demonstration that an increase in Ca^{2+} can trigger contraction in glycerinated (permeabilized) smooth muscle (Filo, Bohr & Ruegg, 1965) dispelled earlier notions that contraction was directly regulated by monovalent ions like Na^{+} and K^{+} (Somlyo & Somlyo, 1968). It is now generally believed that the major mechanism of regulation is through binding of Ca^{2+} to calmodulin (Cal) and of the $Ca^{2+}{}_4$-Cal complex to the catalytic subunit of the myosin light chain kinase, followed by myosin light chain phosphorylation that permits the activation of myosin ATPase by actin (Kamm & Stull, 1985; Hartshone, 1987).

The first step in the excitation-contraction process of vascular smooth muscles is an increase in the level of free intracellular (activator) calcium ions - $[Ca^{2+}]_i$ (Somlyo & Himpens, 1989). The source of $[Ca^{2+}]_i$ is dual: influx from the extracellular compartments or release from intracellular stores (Bohr, 1973; Steinsland, Furchgott & Kirpekar 1973; Ebeigbe, 1982a & b). The nucleus and nuclear membrane, endoplasmic reticulum, mitochondria, and cell membranes could all bind calcium to varying degrees (Somlyo & Somlyo, 1971; Popescu, Diculescu, Zelck & Ionescu, 1974; Somlyo, Somlyo, Devine,

Popescu, Diculescu, Zelck & Ionescu, 1974; Somlyo, Somlyo, Devine, Peters & Hall, 1974; Heumann, 1976; Somlyo & Somlyo 1976). The microsomes have a higher affinity but lower capacity for calcium than the mitochondria (Batra & Daniel, 1971; Fitzpatrick, London, Debbis & Hurwitz, 1972; Baron & Kreye, 1973; Aoki, Yamashita & Hotta, 1976; Allen, 1977; Daniel, Kwan, Matlib, Crankshaw & Kidwai, 1977). Stimulant drugs like NA have been shown to decrease microsomal calcium sequestration (Baudouin-Legros & Meyer, 1973), while relaxant drugs like papaverine increase it (Anderson, 1972).

The sarcoplasmic reticulum (SR) is the major intracellular source of $[Ca^{2+}]_i$; mitochondria do not play a significant role in the physiological regulation of $[Ca^{2+}]_i$. Under pathological conditions, however, the mitochondria can reversibly accumulate large amounts of calcium (Somlyo & Himpens, 1989). Large deposits of calcium occur in perinuclear regions, consistent with the location of the Golgi apparatus in permeabilized, pulmonary artery smooth muscle loaded with Ca^{2+} (Kowarski, Shuman, Somlyo & Somlyo, 1985)

Resting $[Ca^{2+}]_i$ usually ranges from 80 to 200 nM whereas the extracellular concentration is about 10^{-3}M; the difference across the cell membrane is more than 10,000-fold (Bohr, 1973). Total cytoplasmic calcium increases by approximately 2×10^{-1}M during a maximal sustained K^+-contraction in the presence of extracellular Ca^{2+}

(Bond, Shuman, Somlyo & Somlyo, 1984), indicating a considerable Ca^{2+} buffering capacity.

A rise in intracellular free calcium concentration is generally known to mediate contractile responses of all muscle types (Bohr, 1963; Somlyo & Somlyo, 1968; Bohr, 1973; Endo, Kitazawa, Yagi, Iino & Kakuta, 1977). In the vascular smooth muscle, the calcium concentration threshold for contraction is 10^{-7}M and maximal contraction occurs at 10^{-5}M; relaxation occurs whenever calcium concentration decreases over the range of 10^{-5}M to 10^{-7}M (Bohr, 1973).

Calcium sequestration within the smooth muscle is important not only for terminating contraction, but also because such bound calcium could be available for subsequent contraction after release.

Exposure of a vascular smooth muscle preparation to a calcium-free solution reduces the tonic but not the phasic phase of NA-induced contractions (Bohr, 1963; Godfraind & Kaba, 1972; Bevan, Gartka, Su & Su, 1973; Freeman & Daniel, 1973). This implies that the initial phasic response to NA is induced by the release of calcium from intracellular sites in these vascular tissues.

Contractions induced by high-K^+ solutions are largely due to increased influx of extracellular calcium ions (Hinke, Wilson & Burnham, 1964; Hiraoka, Yamagishi & Sano, 1968; Van Breemen, Farinas, Gerba & McNaughton, 1972).

Contractions of smooth muscle can be initiated by changes in membrane permeability after either the binding of agonist to their receptors or the exposure of tissues to high-K^+ solutions (Bolton, 1979). Calcium influx for these contractions could occur either through voltage-sensitive or receptor-linked calcium ion channels (Bolton, 1979). Voltage- sensitive channels open in response to cell membrane depolarisation while the receptor-linked channels open upon receptor activation by receptor agonists (Bolton, 1979). The increase in permeability to calcium ions produced by different stimulants is unequal. Several different agents may have their respective populations of receptor-operated calcium ion-entry channels (ROCs; Somlyo & Somlyo, 1968).

Drugs classified as Ca^{2+}-entry blockers belong to a class of compounds that act predominantly on the cardiovascular system where they reduce contractile force of the heart, relax vascular smooth muscle and damp the calcium-dependent activity of cardiac pacemakers (Fleckenstein, 1977; Zsoter, 1980) by inhibiting the entry of Ca^{2+} into the cell.

A wide variety of Ca^{2+}-entry blockers are now known. Spedding (1984) proposed three major classes of the drugs: (i) the dihydropyridines like nifedipine which bind to sites on the channel, (ii) drugs which regulate the dihydropyridine site by allosteric modulation,

examples are verapamil and diltiazem, and (iii) the diphenylalkylamines. Although Ca^{2+}-entry blockers may have effects on the various sources and stores of activator Ca^{2+}, their predominant and characteristic actions, however, are mediated via selective inhibition of Ca^{2+} entry through voltage-dependent channels (Fleckenstein, 1977; Godfraind, 1983; Godfraind, Miller & Wibo, 1986). At therapeutic concentrations, they have no effect on intracellular mobilisation of Ca^{2+} (Nayler & Poole- Wilson, 1981). At higher concentrations, diltiazem and nisoldipine have been shown to cause inhibition of Ca^{2+} release from an intracellular store in the rabbit mesenteric artery (Saida & Van Breemen, 1983).

Generally, Ca^{2+}-entry blockers are more potent on K^{+}-depolarised than on NA-contracted smooth muscles (Kadza, Knorr & Toward, 1983).

More recent evidence have further shown that changes associated with chronic treatment with calcium channel ligands are not limited to those changes on PSCs but may overlap with other receptor systems. For instance, chronic treatment with calcium antagonists can alter ß-adrenoceptors (Hedberg, Kempf, Josephson, Molinoff, 1985; Gengo, Skattebol, Moran, Gallant, Hawthorn & Triggle, 1988; Ferrante & Triggle, 1990). Chronic administration of nifedipine, with or without ß-receptor antagonists, to patients with heart disease prior to

cardiac bypass surgery, resulted in increased ß-adrenergic receptor number in arterial tissue (Herdberg *et al.*,1985). Conversely, in rats treated chronically with nifedipine, decreased ß-adrenergic receptor numbers were observed in the heart and brain (Gengo *et al.*, 1988). There was no change in ß-adrenoceptor density in rats given verapamil for 6 weeks (Nayler, Dillon, Sturrock & Buckley, 1988). However, there was a reduction in cardiac noradrenaline levels. The differences between these results may represent the differences between human and animal models. It is obvious, however, that more studies are needed to examine the relationship between long-term calcium antagonist treatment and ß-adrenoceptor expression.

1.4.3. IONIC INTERACTIONS

1.4.3a. SODIUM, POTASSIUM-ADENOSINE TRIPHOSPHATASE

The cell membrane of the vascular smooth muscle is characterised by: (i) a low permeability to Na^+, (ii) a high permeability to K^+, and (iii) a transmembrane potential with the inside of the cell being negative relative to the outside. The Na^+ pump plays an important role in the maintenance of these membrane cation and electrical potential gradients. The net effect of the pump is to extrude Na^+ against an electrochemical gradient but down an electrical gradient (Webb, Lockette, Vanhoutte & Bohr, 1981).

The stoichiometry of the reaction is three Na^+ moved out of the cell in exchange for two K^+ pumped in per molecule of ATP hydrolysed (Schwartz, Lindemayer & Allen, 1975). As a consequence, the intravascular environment of the vascular smooth muscle cell contains low concentrations of Na^+ and high concentrations of K^+, even though these cells are bathed in a medium containing high Na^+ and low K^+ (Jones, 1980).

Under steady state conditions, the contribution of the electrogenic sodium pump to the resting membrane potential is small. The electrogenic action of the pump is proportional to the concentration of intracellular Na^+ (Schwartz *et al.*, 1975; Jones, 1980; Webb *et al.*, 1981). Loading the cell with Na^+, thus, results in overactivity and membrane hyperpolarisation (Bonaccorsi, Hermsmeyer, Aprigliano, Smith & Bohr, 1977). The simplest method by which to produce this loading is to decrease Na^+ extrusion by removal of external K^+ which inhibits Na, K-ATPase (Webb & Bohr, 1979; Ebeigbe, Ezimokhai & Aloamaka, 1985).

Once the vascular smooth muscle cells are loaded with Na^+, the pump activity could be studied by: (a) K^+-induced relaxation of an NA-precontraction (Webb & Bohr, 1978a,b,1979), (b) transmembrane electrical potential (Anderson, 1976; Johansson & Somlyo, 1980;

Jones, 1980) and (c) ion flux measurements (Heidlage & Jones, 1978).

K^+-induced relaxation is the simplest and most commonly used of the three techniques. The magnitude of relaxation is known to be a functional measure of the electrogenic pumping of Na^+ and K^+ (Webb & Bohr, 1978b). Treatment of vascular smooth muscle with ouabain, an inhibitor of Na,K-ATPase (Schwartz *et al.*, 1975), decreases the amplitude of K^+-induced relaxation (Bonaccorsi *et al.*, 1977; Reiner, 1978; Webb & Bohr, 1978b, 1979, 1980; DeMey & Vanhoutte, 1979).

It has been possible using this method, to determine that the Na^+, K^+-ATPase plays an important role in controlling the activity of the vascular smooth muscle in health (Webb *et al.*, 1981) and disease (Webb & Bohr, 1979; Ebeigbe *et al.*, 1985).

1.4.3b. SODIUM-CALCIUM INTERACTIONS

If a vascular smooth muscle preparation is placed in a Na-free solution, tension develops (Bohr, Seidel & Sobieski, 1969; Reuter, Blaustein & Haeusler, 1973). Tension development under this condition is due to intracellular accumulation of calcium ions (Sitrin & Bohr, 1971; Blaustein, 1977b). Reuter *et al.* (1973) and Blaustein (1977a) showed that $^{45}Ca^{2+}$ efflux and relaxation of precontractions were

slowed when external Na^+ was removed, even in the absence of external Ca^{2+}.

Two possible mechanisms have been adduced for this interaction: (a) that under normal conditions, sodium ions compete with calcium ions for Ca^{2+} binding sites and thus prevent calcium loading (Luttgau & Niedergerke, 1958) and (b) that normal levels of Na^+ in the extracellular fluid facilitate Ca^{2+} efflux largely enough that the Ca^{2+} released from the stores by Ca-free solutions is immediately extruded from the cell (Reuter & Seitz, 1968).

Reduced extracellular fluid Na^+ is known to facilitate Ca^{2+} release from intracellular stores as well as inhibit Ca^{2+} outward movement (Bohr *et al.*, 1969; Sitrin & Bohr, 1971).

Similarly, it is believed that a Na^+-Ca^{2+} antiport mechanism exists on the vascular smooth muscle. The role of this pump in the maintenance of vascular tone has not, however, been evaluated.

1.4.3c. CALCIUM-MAGNESIUM INTERACTIONS

Withdrawal of extracellular $[Mg^{2+}]_o$ potentiates contractile responses in the rat and rabbit arterial and arteriolar smooth muscle preparations (Altura, 1971, 1974, 1976a,b, 1977, 1978a,b; Goldstein & Zsoter, 1978; Krishnamurty & Mukherjee, 1981; Ebeigbe &

Aloamaka, 1986). The reverse, attenuation of contractile response, is observed when $[Mg^{2+}]_o$ is increased (Altura & Altura, 1980).

Acute hypomagnesemia in animals and man is often associated with increased peripheral vascular resistance and blood pressure (Seelig, 1978; Marier, Neri & Anderson, 1979). A number of disease states like diabetes mellitus, renal hypertension and eclampsia that are often associated with elevated blood pressure also often feature decreased plasma levels of magnesium (Seelig & Heggtveit, 1974; McNair, Christiansen, Madsbad, Lauritzen, Faber, Binder & Transbil, 1978; Seelig, 1978; Wacker, 1980).

Increased $[Mg^{2+}]_o$ is believed to depress vascular contractility by inhibiting calcium influx as well as decreasing total exchangeable and membrane- bound calcium (Turlapaty & Altura, 1978; Turlapaty, Weiner & Altura, 1981). Available data suggest that the surface membrane of arterial smooth muscles contain functional Mg^{2+}-Ca^{2+} exchange sites (Altura, 1978). Mg^{2+} displaces a functional pool of calcium on the muscle cell membrane. This calcium pool may be verapamil resistant ((Turlapaty *et al.*, 1981).

Arteries are known to have a larger density of these Mg^{2+}-Ca^{2} exchange sites than veins (Altura & Altura, 1971, 1974, 1976; Turlapa., & Altura, 1978; Turlapaty *et al.*, 1981).

High $[Mg^{2+}]_o$ paradoxically increases the magnitude of NA-induced contractions in Ca-free environments (Ebeigbe & Aloamaka, 1986). This may be due to increased calcium antagonism at intracellular and membrane sequestration sites, thereby making more calcium available to NA for contractile response.

Krishnamurty & Mukherjee (1981), working on rat aorta, reported that high $[Mg^{2+}]_o$ had no effect on the maximal response to NA, whereas, Mg^{2+}-free exposure depressed it.

These effects of Mg^{2+} were attributed to increased or decreased Mg^{2+}-antagonism which resulted in decreased or increased uptake of cytosolic Ca^{2+} in high $[Mg^{2+}]_o$ or Mg^{2+}-free medium respectively.

1.4.4. ENDOTHELIUM-MEDIATED VASCULAR RESPONSES

Every blood vessel has a continuous, flattened monolayer of intimal endothelial cells. Aside from its vegetative function of regulating the growth of the underlying medial smooth muscle cells (Fisherman, Ryan, Karnovsky, 1975; Gajdusek, DiCorleto, Ross & Schwartz, 1980; Castellot, Addonizid, Rosenberg & Karnovsky, 1981), the vascular endothelium also mediates and modulates responses of many vasoactive agents (DeMey & Vanhoutte, 1982; Carrier & White, 1984).

An intact vascular endothelial cell layer is required for the vasodilatory effect of several pharmacological agents like acetylcholine (Furchgott & Zawadzki, 1980; Furchgott & Jothianandan, 1983), histamine (Van de vorde & Leusen, 1983; Carrier, White & Kirby, 1984; Obiefuna *et al.*, 1991), hydralazine (Spokas, Folco, Wuilley, Chander & McGiff, 1983), and adenosine nucleotides (DeMey & Vanhoutte, 1981a). The relaxatory responses induced by these agents are greatly reduced or completely abolished by endothelial removal.

Endothelial removal is also known to potentiate vascular smooth muscle contractile responses. It did not significantly alter the maximum responses to NA but maximal responses to phenylephrine and methoxamine (selective alpha-1 agonists) as well as clonidine and B-HT 920 (selective alpha-2 agonists) were significantly enhanced (Carrier & White, 1984). Endothelium-dependent relaxation responses to acetylcholine, adenosine triphosphate, bovine thrombin and arachidonic acid were prominent in arteries but only transient in the veins. In the veins, however, arachidonic acid or thrombin caused endothelium- dependent contraction in NA-precontracted vessels (Ingelman-Wojensky, Silver & Smith, 1981).

The vasodilatation induced by acetylcholine and other agents is mediated indirectly by the release of an endothelium-derived relax. factor (EDRF; Furchgott & Zawadzki, 1980). Some of the substan...

which have been proposed to represent EDRF include lipoxygenase pathway intermediates (Furchgott, 1981), an oxygen-derived free radical (Gryglewski, Palmer & Moncada, 1986) and nitric oxide (NO; Furchgott, 1988). The possible existence of more than one EDRF has also been postulated (Vanhoutte, 1987).

EDRF action is calcium-dependent (Long & Stone, 1985; Tayo & Bevan, 1987) and is associated with a rise in the cellular level of cyclic GMP (Holzmann, 1982; Furchgott & Jothianandan, 1983; Furchgott, Cherry, Zawadzki & Jothianandan, 1984).

1.4.5. BLOOD-BORNE VASOCONSTRICTOR AGENTS

Various components of blood have been postulated to have vasoconstrictor effects (Moussatche, 1954; Zucker & Borreli, 1955a,b; Raynor, McMurtry & Pool, 1961; Yamamoto, Feindel, Wolf, Katoh & Hodge, 1872; Osaka, 1977; Osaki & Mullan, 1979; Tanishima, 1980). Of these components, serotonin and haemoglobin are the most potent (Reid & Bick, 1942; Tanishima, 1980).

Tanishima (1980) demonstrated pronounced constructive effect of haemoglobin on cerebral, femoral and coronary arteries of the dog. Ebeigbe & Aloamaka have consistently observed similar effects on the rat tail artery (Aloamaka, 1987).

Serotonin was thought to be the only vasoconstrictor agent in blood plasma or serum samples (Rapport, Green & Page, 1948; Zucker & Borreli, 1955a,b; Allen, Henderson, Chouz French, 1974). Plasma or serum serotonin is released from platelets (Reid & Bick, 1942; Zucker & Borreli, 1955a,b); careful removal of platelets from serum or plasma samples immediately after collection abolishes the vasoconstrictor effect of serum or plasma (Reid & Bick, 1942).

1.5. AIM OF PRESENT STUDY

Dietary salt loading of Sprague-Dawley (SD) rats causes elevation of blood pressure. Many mechanisms have been adduced for this. Although most hypertensive conditions are known to be associated with increased peripheral resistance, evidence is lacking on the precise role of vascular mechanisms in this model of hypertension.

The aim of this study is to, first, reproduce hypertension in SD rats by dietary salt-loading and then test the functional levels of various vascular processes in the hypertensive model in comparison with controls. These mechanisms include: receptor- drug interactions, via noradrenaline and 5-HT- sensitive receptors; membrane depolarisation by high KCl solutions; ion pumps and ionic interactions through the Na-K adenosine triphosphatase enzyme activity and Ca^{2+}-Mg^{2+}

interactions; potassium channel status using the potassium channel agonist; the calcium status and calcium entry regulation, by the use of calcium channel blockers and calcium antagonists, calcium channel agonists and selective voltage-sensitive and receptor-regulated calcium channel manipulations; vascular effects of serum, and the role of the endothelium monitored as relaxatory responses to known endothelium-dependent vasorelaxants.

It is hoped that some mechanisms would ultimately evolve, which involve possible vascular smooth muscle dysfunction in the elevation of blood pressure which follows excessive salt intake in the rat.

CHAPTER 2
MATERIALS AND METHODS

2.1. ANIMALS

Inbred, 6-week-old Sprague-Dawley (SD) rats weighing between 70g and 100g and of either sex were obtained from the laboratory animal centre of the College of Medicine of the University of Lagos.

2.2. SALT LOADING

Salt-hypertension was induced in the SD rats by a previously described method (Miyajima & Bunag, 1985; Nwaigwe & Sofola, 1989; Obiefuna *et al.*, 1991). Rats were randomised into two groups. The control group was fed on normal rat chow (NaCl content of 0.3g/100g chow; Pfizer, Nigeria). The salt group was fed on rat chow with NaCl content of 8g/100g chow. The 8% NaCl diet was prepared by adding 7.7g NaCl (May & Baker) to 100g Pfizer cubes which already contained 0.3g NaCl per 100g of chow. The mixture was mashed in enough water to make for adequate binding and was pressed into lumps. The lumps were then oven-dried at 80°C.

Feed and tap water were presented to both groups of rat for about 5-6 weeks *ad libitum*. Body weight was measured weekly and food intake every three weeks.

2.3. POLYGRAPH BALANCING AND THE CALIBRATION OF PRESSURE AND ISOMETRIC TRANSDUCERS

The driver amplifier (Model 7DAG) and preamplifier (Model 7P1F) of the Grass polygraph (Model 7D; Grass Instruments, Quincy, Mass, U.S.A.), the blood pressure transducer (Statham Gould, Model P23ID, Hato Rey, Puerto Rico) and isometric force transducer (Model FT.03C; Grass Instruments) were calibrated at the beginning of each day's experiments as described below.

2.3.1. CALIBRATION OF THE DRIVER AMPLIFIER

The driver amplifier magnifies signals from the preamplifier of the polygraph sufficiently enough to drive the writing pen.

All controls were first turned to the zero position and the polygraph switched to "STANDBY" and left for, at least, 15min. The control switch was then turned to the "ON" position and the "POLARITY" switch selected as desired: the "-UP" position was used for blood pressure recording and the "+UP" position for isometric

force recording. The half- amplitude frequency was selected with the "½ AMP- FREQ" switch. This position could be changed during recording to control 'noise'. A convenient baseline was chosen using the "BASELINE POSITION CONTROL". This sets the pen to a position corresponding to zero input from the preamplifier. The "POLARITY" switch was then placed at "CAL" position. The "—100mV" knob was depressed and with the aid of the sensitivity dial, the pen deflection was adjusted to 2cm; this step was by-passed when calibrating for isometric measurements since sensitivity was best chosen during transducer calibration (section 2.3.4, page 47). The sensitivity dial, in any case, was locked to prevent accidental changes in sensitivity. The "POLARITY" switch was then turned to "USE". In this position, the driver amplifier would be under the direct control of the preamplifier and the pen position usually shifted from baseline. The preamplifier could then be calibrated.

2.3.2. CALIBRATION OF THE PREAMPLIFIER

Either of the blood pressure or the isometric force transducers was coupled to the preamplifier through the "IN" jack on the preamplifier console. The "INPUT" selector was then switched to "BRIDGE 2K" position. The "BRIDGE 2K" position is used for low resistance strain gauge bridges such as the transducers used in the

present experiments. To represent 100mV with a 2cm pen deflection, the "CALL -2mV" knob was depressed and fine adjustments were made with the "SENSITIVITY" switch. Maximal sensitivity was then obtained by turning the "SENSITIVITY" switch to 0.01mV/cm. This gave maximum pen deflection and the "BALANCE VOLTAGE" knob was adjusted until the pen returned to the baseline. A convenient sensitivity was then chosen, usually between 0.5mV/cm to 2.0mV/cm.

2.3.3. CALIBRATION OF THE PRESSURE TRANSDUCER

The blood pressure transducer was calibrated after the calibration of the driver amplifier and preamplifier. The transducer was filled with heparinised saline, using a hypodermic syringe connected to the side port of the dome. Care was taken to expel all air bubbles from the fluid chamber after which it was locked with the aid of stopcocks. A mercury manometer was connected to the central port of the pressure dome and a step function calibration, in 20mmHg steps, of the transducer carried out with the central port stopcock open to the manometer-transducer path.

2.3.4. CALIBRATION OF THE ISOMETRIC TRANSDUCER

The isometric transducer, now connected to a balanced preamplifier was calibrated as follows. A 1g weight (Grass Instru-

ments) was suspended by a hook on the transducer. The hook used must be on the transducer during balancing of the preamplifier. The pen sensitivity was then adjusted to a convenient position. Usually, a 20-25mm deflection per gram was adequate for most measurements. The 1g weight was then replaced with the isolated tissue. Later on, the balance voltage knob was used to return the pen to baseline following tissue stretch (see section 2.7, page 58).

2.4. STANDARDISATION OF THE FLAME PHOTOMETER

The Corning digital flame photometer (Corning Model 410C) was standardised before each measurement, using a standard Na/K solution. The solution was prepared from Corning Na and Corning K standards and contained 160mM Na^+ and 8mM K^+ both as chloride salts. On each day of the experiments, 200μl of this stock was diluted 200 times, to get a working standard, using a micropipette and an automatic fixed-volume dispenser. The diluent was distilled, double-deionised (DDD) water. The Na or K filters were selected in turn. With the filter set to Na, the working standard was used to calibrate the photometer, against a DDD water blank, to display "16.0". This was done with the "COARSE" and "FINE" adjustment knobs on the console of the photometer. This process was repeated until the

photometer consistently displayed "0.0" for DDD water and "16.0" for working standard. The photometer was then ready for Na assay.

Similarly, with the K filter selected, the working standard was used against DDD water to calibrate the photometer to consistently display "0.0" for DDD water and "8.0" for working standard. The photometer was then ready to assay for K^+ in serum samples.

2.5. DRUGS AND SOLUTIONS

2.5.1. DRUGS

Drugs used were: acetylcholine hydrochloride (Sigma Chemical Co.), Bay K8644 (Bayer AG), BRL 34915 (Sandoz, Basel), CGP 28392 (Ciba-Geigy, Basel), α-chloralose (BDH), diltiazem hydrochloride (Marion), ethyleneglycol o-o'-bis-(ß-amino-ethyl) N,N,N',N'-tetracetic acid (EGTA; Fluka AG), heparin sodium (Upjohn, U.S.A.), histamine hydrochloride (Sigma), hydralazine (Apresoline; Ciba-Geigy, Basel), 5-hydroxytryptamine creatine sulphate (5-HT; serotonin; Sigma), nifedipine (Bayer AG), (-)-noradrenaline bitartrate (Hoechst), ouabain (Sigma), phentolamine mesylate (Rogitine; Ciba) and urethane (BDH).

2.5.2. SOLUTIONS

2.5.2a. DRUG SOLUTIONS

Solutions of the various drugs used in isolated tissue experiments were freshly prepared in saline, except Bay K8644, BRL 34915, CGP 28392 and nifedipine. Stock solutions of 10^{-2}M were normally prepared, except for histamine, and desired dilutions made from this. A stock solution of 10^{-1}M histamine was prepared, from which further dilutions were made. NA solutions contained 0.1% ascorbic acid as antioxidant.

Stock solutions of Bay K8644 and nifedipine were made in 70% ethanol. A further dilution to 10^{-3}M was made in 70% ethanol but further dilutions were made in saline. All operations using Bay K8644 and nifedipine were carried out in semi-darkness.

A stock solution of CGP 28392 was mixed with 0.1ml of dimethylsulphoxide (DMSO) and 1ml of 50% ethanol. The resulting 10^{-2}M stock solution was further diluted to a 10^{-3}M solution with 50% ethanol. Further dilutions from the 10^{-3}M solution were made with saline. The stock solutions of Bay K8644, CGP 28392 and nifedipine were sometimes stored at -20°C for up to 48hours.

Stock solutions (10^{-2}M) of BRL 34915 were made in 50% ethanol. Further dilutions were made in saline.

2.5.2b. HEPARINISED SALINE

Two strengths of heparinised saline (HS) were used. The general purpose HS, used for filling transducers and during surgery was made by mixing 50 Units of heparin with 1ml of saline (153.85mM NaCl). The HS which was usually infused into the cannulated artery (see 2.6.3, page 56) was made twice as concentrated as the general purpose HS, since very small volumes of the former were usually used.

2.5.2c. NORMAL PHYSIOLOGICAL SALT SOLUTION (PSS)

Normal PSS was prepared by mixing volumes of 1M stock solutions of the various salts as shown in Table 1 (page 54). Calcium chloride and glucose were, however, added as pre-weighed salts on the day the PSS would be used, calcium chloride being added to dilute solutions of the rest of the salts. This was to avoid the precipitation of calcium carbonate ($CaCO_3$) which may occur if calcium chloride ($CaCl_2$) was allowed to mix with a strong solution of sodium bicarbonate ($NaHCO_3$). Varieties of PSS were prepared as described below and shown in Table 1 (page 54).

2.5.2d. HIGH-K^+ PSS

Different high-K^+ solutions containing gradations of K^+ were prepared by equimolar substitution of NaCl with KCl; the extent of

substitution depended on the desired concentration of K^+. It was provided, however, that the sum of [Na^+] and [K^+] was constant (Casteels & Kuriyama, 1966) so as to maintain normal osmolarity. High-K^+ solutions contained $3X10^{-7}$M phentolamine to exclude the influence of NA, released from adrenergic nerve endings in the tissue wall, on high-K^+-induced contractions (Vanhoutte, Verbeuren & Webb, 1981).

K^+-free PSS was PSS that contained no added potassium.

2.5.2e. CALCIUM-FREE PSS

This had the same composition as normal PSS except that it contained no added $CaCl_2$. Ca^{2+}-free PSS sometimes contained 1mM EGTA.

2.5.2f. MAGNESIUM-FREE AND HIGH-Mg^{2+} PSS

These solutions had similar compositions with normal PSS except for variations in the concentration of Mg^{2+}. Mg^{2+}-free PSS contained no added Mg^{2+} whereas high-Mg^{2+} PSS contained 4.8mM Mg^{2+}.

2.5.2g. LOW - BICARBONATE PSS

This solution contained 6mM $NaHCO_3$ instead of 14.9 mM $NaHCO_3$ contained in normal PSS. The low-HCO_3 PSS had a pH of 7.3 and was used in experiments designed to study the membrane stabilising effect of calcium. The low-bicarbonate environment prevented precipitation of $CaCO_3$ when high concentrations of calcium chloride were added to the bath (Webb & Bohr, 1978a; Ebeigbe & Aloamaka, 1986).

Table 1. Composition of Physiological Salt Solution (PSS)

TYPES OF PSS	CONCENTRATION (mM)								
	NaCl	KCl	KH_2PO_4	NaH_2PO_4	$MgSO_4$.$7H_2O$	$NaHCO_3$	$CaCl_2$.$2H_2O$	Glucose	EGTA
Normal PSS	119	4.7	1.2	—	1.2	14.9	1.6	11.5	—
Ca^{2+}-free PSS	119	4.7	1.2	—	1.2	14.9	—	11.5	—
Ca^{2+}-free EGTA	119	4.7	1.2	—	1.2	14.9	—	11.5	1.0
K^+-free PSS	123.7	—	—	1.2	1.2	14.9	1.6	11.5	—
High-K^+—PSS*	x	y	—	1.2	1.2	14.9	1.6	11.5	—
Low-HCO_3 PSS	128	4.7	1.2	—	1.2	6.0	1.6	11.5	—
Mg^{2+}-free PSS	119	4.7	1.2	—	1.2	14.9	1.6	11.5	—
High-Mg^{2+} PSS	119	4.7	1.2	—	4.8	14.9	1.6	11.5	—

*High-K+ PSS contained $3x10^{-7}$M phentolamine; y = 10,15,20,30,40,60,80,100 mM KCl; x = (123.7—y) mM NaCl

2.6. BLOOD PRESSURE AND HEART RATE MEASUREMENTS

At the end of the period of salt loading, 5 rats from each group were anaesthetised with 25% (w/v) urethane: 1% (w/v) alpha-chloralose mixture (section 2.6.1). The blood pressure (BP) and heart rate (HR) were determined invasively by cannulating the left common carotid artery. Mean arterial pressure (MAP) was determined either as diastolic pressure plus one-third pulse pressure (mmHg), or by electronically damping the pressure pulse with the "½-AMP. FREQ" control of the driver amplifier. Heart rate (beats/min) was determined from the number of arterial pulses in 15 seconds. Details of the methods are given below.

2.6.1. INDUCTION OF ANAESTHESIA

The rats were anaesthetised with a mixture of 25% (w/v) urethane and 1% (w/v) alpha-chloralose dissolved in distilled water and injected intraperitoneally at a dose of 5ml/kg body weight of rat. The anaesthetic mixture, which was always stored at 4°C was first warmed up to 25°C, in a water bath, before administration. Anaesthesia was determined by observing either the absence of corneal reflex or lack of

response to painful stimuli. Dissection usually followed immediately after the induction of anaesthesia.

2.6.2 DISSECTION AND CANNULATION OF THE TRACHEA

The anaesthetised rat was laid supine on a dissecting table and the limbs were fastened to the table via cotton loops connected to dissecting pins which were fixed on the board. A midline incision was made in the neck, with a pair of scissors, from the chin to the upper part of the sternum; the underlying fascia, connective tissue and muscle were thus exposed. The fascia and connective tissue were neatly teased out by blunt dissection and the trachea was located. The trachea was separated from the surrounding tissue with a blunt pair of curved forceps. Two loops of ligature were placed around the trachea. An incision was then made between the loops on the trachea and a plastic tracheal cannula, about the size of the trachea was inserted and firmly tied in place with the ligatures. This manoeuvre allowed uninterrupted air flow into and out of the lungs throughout the experiment.

2.6.3. DISSECTION AND CANNULATION OF THE LEFT COMMON CAROTID ARTERY

The left common carotid artery lies deep alongside the trachea. In order to locate the artery, the sternocleidomastoid muscle and left

omohyoid muscles lateral to the trachea were separated by blunt dissection. The artery was identified as it pulsated in its sheath. The sheath was carefully teased open and the vagus nerve was separated from the common carotid artery. A proximal loop was tied firmly on the artery while a bulldog clip was clamped as distally as possible on the same artery. A loose loop was then placed near, but proximal to, the bulldog clip. A cut was made on the artery, with a pair of fine scissors, close to the proximal end of the exposed artery.

A polythene cannula (Luer 3FG; OD 0.7mm; Portex Ltd, Kent), filled with heparinised saline and stoppered with a three-way stopcock, was inserted into the cardiac end of the artery and pushed gently until it was close to the bulldog clip. The loose ligature was then loosely tied onto the cannula and the bulldog clip removed. The cannula was pushed further forwards until the tip rested beyond the point on the artery where the bulldog clip was clamped. The ligature was finally tightly tied in place and the cannula flushed with about 0.2ml of heparinised saline.

The stopcocks on the cannula and transducer were tightly linked to each other. With the taps closed against both artery and transducer, air bubbles trapped at the point of connection of the stopcocks were flushed out with heparinised saline. This enabled a continuous liquid

column between the blood vessel and the transducer membrane when the stopcocks were open to each other.

The exposed tissue on the rat was constantly moistened with cotton wool soaked in heparinised saline.

2.7. AORTIC RING ISOLATION, PREPARATION AND MOUNTING

At the end of the 5 or 6 weeks of salt loading, the rats which were now weighing about 200-300g were killed by stunning. An abdominal incision was made on the left side of the animal, through which the thoracic cavity was exposed. The thoracic aorta - from the aortic bifurcation to the diaphragm - was carefully freed of adhering connective tissue with the aid of microdissecting forceps and scissors. The aorta was then removed and quickly placed in a trough of PSS. The lumen was gently flushed free of blood and the vessel transferred to a dissecting microscope, still fully bathed in PSS. Under the calibrated field of the microscope, any remaining connective tissue was removed and the artery was then cut into 2mm ring segments. The rings were made from the aortic end of the vessel. On the other hand, physical contact with the tissue was restricted to the diaphragm end. Care was, however, taken not to handle the tissue unduly throughout.

In some of the experiments, the endothelium of the ring was damaged by gently rubbing the intima of the ring with a fine wire. Otherwise, special care was taken to avoid contact with the intimal surface. The ring was then mounted between two fine L-shaped stainless steel wires.

This was then transferred onto the transducer and suspended to dip vertically into a 20ml organ bath containing PSS at 37°C which was bubbled with 95%O_2: 5%CO_2 gas mixture. The PSS pH under these conditions was 7.35-7.40. The bubbling also constantly stirred the bath solution. The bath temperature was maintained by a water jacket connected to a thermostatic circulator. Fresh PSS was pre-warmed to 37°C before adding to the bath. An array of, at least, four organ baths was used and so, pairs of arterial rings from both control and salt-loaded rats were studied simultaneously. All baths had a parallel connection to the source of PSS. (see Figure 1a, page 100).

The free end of the ring preparation was connected to a solid support at the base of the organ bath. With the aid of micrometer screws, a passive tone of 2g was applied to the ring by stretching. The "BALANCE VOLTAGE" fine adjustment knob of the preamplifier was used to return the transducer input to zero. The ring was then ready for further experiments.

2.8. BLOOD COLLECTION AND SERUM EXTRACTION

To collect blood for serum electrolyte assay or isolated vessel experiments, rats which would later on be used for isolated tissue experiments were anaesthetised with ether by inhalation. The thoracic cavity was quickly opened up and blood collected by cardiac puncture using a 19G hypodermic needle. The blood was transferred to test tubes and allowed to clot at 4°C. After about 1 hour, the serum was taken out with a Pasteur pipette. It was used immediately if needed for isolated vessel experiments, or was stored for up to one week at -20°C if needed for flame photometry.

2.9. SERUM ELECTROLYTE ASSAY

The serum was usually taken from the freezer where it was stored, and was left to thaw at room temperature. It was then diluted 200 times with DDD water. The flame photometer was first calibrated and standardised for Na (section 2.4, page 48). The dilute serum was read against a "16.0" digital readout of 160mM Na^+ standard. The readout value for serum was then multiplied by 10 and expressed as mM Na^+. Similarly, with the photometer standardised for K^+, the dilute serum was read against an "8.0" digital readout of 8mM K^+ standard. The readout value was expressed directly as mM K^+.

2.10. TISSUE BATH EXPERIMENTS

The following treatments always preceded all tissue bath experiments. After the tissue had been given passive tone of 2g as described earlier, it was then allowed to equilibrate in the PSS for, at least, 90 min. During this period, it was rinsed every 15min with fresh PSS. The tissue was also given about three stabiliser-doses of either 10^{-7}M NA or 40mM K^+ PSS to obtain a reproducible stimulant-response value. Each stabilising stimulation lasted about 3min after which it was washed off. Sometimes, but quite rarely, more than three stabiliser doses were needed. This occurred when using 12-hour stored tissues in which three stabiliser doses of contractile agent may not be enough to produce uniform consecutive contractions; uniformity of consecutive contractions were used as an index of adequate stabilisation of the tissue in PSS.

At the end of the 90-min period, the various *in vitro* protocols described below were then carried out.

2.10.1. TIME-COURSE OF NA AND HIGH-K^+ CONTRACTIONS

Time-course of NA contractions was determined by exposing the tissue to 10^{-5}M NA while the polygraph chart speed was set at 50mm/min. Tension development was estimated every 12 seconds for 1 min. and then at 2min, 5min and 10min durations.

Time-course of high-K^+ contractions was determined by exposing the stabilised tissue to 100mM K^+ PSS. Tone was similarly determined as for NA but a 15th-minute determination was taken in addition, about which time the K^+-contractions usually peaked.

2.10.2. TIME COURSE OF RELAXATION OF NA AND HIGH-K^+ CONTRACTIONS DUE TO Ca^{2+} WITHDRAWAL

Relaxation rates were determined as follows: At the peak of maximal contractile responses to 10^{-5}M NA or 100mM K^+ PSS, the bath solution was replaced with Ca^{2+}-free EGTA solution. Relaxation rate was determined and expressed as percentage of maximal contraction. Relaxation to NA was determined every minute for 12min while relaxation to high K^+-PSS, which was much faster, was determined every half-a- minute for 5 min.

2.10.3. CONCENTRATION RESPONSE TESTS TO NA, 5-HT AND HIGH-K^+ PSS

For NA and 5-HT, the agents were added to the bath cumulatively; a higher concentration being added when the effect of the previous concentration had been fully established. A higher concentration was achieved by adding its difference from a previous concentration. Concentration response test to NA was done in NA concentration

range of 10^{-10}M - 10^{-5}M and for 5-HT, in the 5-HT range of 4×10^{-8}M to 4×10^{-5}M.

Concentration response test to high-K^+ PSS was done non-cumulatively to avoid osmolarity increases that would otherwise occur if cumulative values of 4.7 - 100mM K^+ were added to the organ bath PSS. Seven different high-K^+ PSS solutions were therefore prepared. They contained 10mM, 20mM, 30mM, 40mM, 60mM, 80mM and 100mM KCl and were made iso-osmotic by equimolar replacement of sodium (section 2.5.2d, page 51). Concentration response tests were carried out by double washout of a previous high-K^+ PSS with the next stronger solution, starting from normal PSS (K^+ = 4.7mM).

2.10.4. CONCENTRATION RESPONSE TESTS TO CALCIUM CHLORIDE DURING NA AND HIGH-K^+ PSS STIMULATION

The protocol for these experiments is illustrated in Figure 1b (page 102). Rings were exposed to 10^{-5}M NA. At the peak of contraction, the bathing medium was washed with Ca^{2+}-free EGTA PSS for 30min. Fifteen minutes into this period, the rings were exposed to another dose of 10^{-5}M NA to further deplete the cell of calcium stores; a second application of NA was almost ineffective. At the end of the 30min, the rings were exposed to either Ca^{2+}-free PSS (without EGTA) containing 10^{-5}M NA or Ca^{2+}-free high-K^+ PSS containing

100mM KCl but containing no EGTA. Tone hardly developed due to lack of Ca^{2+} in or outside the cell. Five minutes later, calcium chloride was added to the bath cumulatively in the concentration range of 2.5×10^{-5}M - 2.5×10^{-2}M. The resulting, stepwise increases in tension were then recorded.

2.10.5. PHASIC CONTRACTION TO NA IN Ca^{2+}-FREE PSS

Freshly stabilised rings were exposed to Ca^{2+}- free EGTA PSS for 15min. They were then exposed to 10^{-5}M NA. The phasic contractile response so recorded is attributable to the intracellularly mobilised Ca^{2+} since the contraction occured in a Ca^{2+}- free extracellular environment. The phasic response was expressed as percent of maximum response to NA in normal PSS as well as in mg of observed tone.

2.10.6. CONTRACTILE RESPONSE TO SERUM

To test the presence of vasoactive agents in the sera of the two rat groups, normotensive aortic rings removed from control rats were used. Stabilised rings were exposed to 10^{-2} units final bath concentration of serum; undiluted serum was taken arbitrarily as 1 unit. Peak responses were expressed both as mg tone and as percent of maximum

response to NA. All rings used in this experiment had comparable initial responses to 10^{-7}M NA ($P<0.05$).

2.10.7. K^+-INDUCED RELAXATION

If a contractile response to NA is produced in a potassium-free PSS, relaxation achieved by adding K^+ to the medium is a functional indicator of Na,K-ATPase pump activity (section 1.4.3a, page 34). The protocol used is illustrated in Figure 20 (page 140). Rings were exposed to K^+-free PSS for 15min. They were then made to contract to 10^{-7}M NA. At the peak of contraction, cumulative concentrations of KCl were added, and relaxation responses recorded.

In another set of experiments, the cumulative addition of KCl was preceded by addition of 10^{-5}M ouabain. Ouabain was added at the peak of NA contraction, 5min before the addition of KCl. Ouabain is a cardiac glycoside and an inhibitor of Na,K-ATPase.

2.10.8. $[Mg^{2+}]_o$-INDUCED RELAXATION: EFFECTS OF BAY K 8644 AND CGP 28392

All rings used for these procedures were first exposed to Mg^{2+}-free PSS for 30mins. The protocols for these experiments are illustrated in Figure 22 (page 144).

Magnesium-induced relaxation of NA or KCl contractions were tested by precontracting the rings with 10^{-7}M NA or 40mM K^+-PSS. At the peak of contraction, $MgCl_2$ was added to the bath cumulatively to achieve up to a 9mM bath concentration.

In other experiments, the precontraction was produced with a mixture of either 40mM K^+-PSS and 4×10^{-8}M Bay K 8644 or of 40mM K^+-PSS and 4×10^{-5}M CGP 28392. The doses of agonists used represent the EC_{30} estimates of the agonist concentration response tests. Either of the calcium channel agonist was added as soon as the tissue in each bath was superfused with 40mM K^+-PSS.

2.10.9. RELAXATION RESPONSE TESTS TO BRL 34915, DILTIAZEM, HYDRALAZINE AND NIFEDIPINE

Rings were first precontracted in 60mM K^+-PSS or 10^{-7}M NA and at the peak of each contraction, cumulative concentrations of BRL 34915, diltiazem, hydralazine or nifedipine were added. No two drugs were tested on the same arterial ring. When rings were used for the same drug, an equilibration period of 1 hour, with constant rising, was allowed between the two stimulations. During the equilibration period, 10^{-7}M NA was repeatedly used to assess tissue recovery. (see section 2.10, page 61).

2.10.10. RELAXATION RESPONSES TO CALCIUM CHLORIDE

Calcium-induced relaxation was estimated as a measure of the membrane stabilising property of high extracellular fluid calcium ions.

Rings were first exposed to low-HCO_3 PSS for 15min after which they were then exposed to either 10^{-7}M NA or 60mM K^+-PSS. At the peak of contraction, 3mM-25mM $CaCl_2$ were cumulatively added to the bath and the relaxation response recorded. A low-HCO_3 medium prevents $CaCO_3$ precipitation.

2.10.11. ENDOTHELIUM - DEPENDENT RELAXATION TO ACETYLCHOLINE AND HISTAMINE

Dose-dependent relaxation to acetylcholine (ACh) or histamine was tested in both denuded and intact rings. Denuded rings were prepared as previously described (section 2.7, page 58). Loss of endothelium was confirmed by the absence of relaxation of NA-induced precontractions to acetylcholine (Furchgott & Zawadzki, 1980).

Rings from the two rat groups, i.e. with and without endothelium, were stabilised in normal PSS. The rings were made to contract to 10^{-7}M NA. At the peak of contraction, 10^{-5}M ACh was applied. Rings that relaxed to ACh by up to 50% of NA contracture were marked "+E", those that did not relax at all or relaxed by less than 2% of NA-contracture were marked "-E", while those that were in-between

were not used. The tissues were than rinsed and allowed to stabilise. At the end of the stabilisation, they were precontracted with 10^{-7}M NA and at the peak of pre-contraction, cumulative doses of ACh (10^{-9}M - 10^{-5}M) or histamine (10^{-6}M - 10^{-3}M) were added. At maximal responses to histamine, 10^{-5}M ACh was added to effect further relaxation to confirm a still intact endothelium (Obiefuna *et al.*, 1991).

2.11. DATA ANALYSES

Values presented are means ± SEM, for each group of rats, usually six in number. In our relaxation experiments, to avoid the possibility that any difference in relaxation rate between two groups of experiments may be due to differences in tension of precontractions, the results were considered valid only when *t*-test of precontraction between the two groups was not significant (i.e $P > 0.05$). Statistical analyses were by Student's *t*-test and where three or more means were compared simultaneously, and this would be indicated, one-way analysis of variance (ANOVA) was used. If ANOVA showed significant effect of the univariate property, Fisher's least square difference (LSD) procedure was used for *post hoc* analysis of differences. A P value less than 0.05 was considered to be statistically significant. n represents number of animals studied. Duplicate estimations were usually made for each rat and internal averages determined.

CHAPTER 3
RESULTS

3.1. FOOD INTAKE AND BODY WEIGHT

Food intake was measured at the third and sixth weeks of salt loading. The results are shown on Table 2 (page 84). There was no significant difference in rate of food consumption between the control and salt groups at the 3rd week. At the 6th week, salt-loaded rats consumed less food per day, per rat($P < 0.05$). When the week-6 food intake was corrected for body weight, which differed significantly at this time, both rat groups consumed identical quantities of chow per unit body weight (Table 2).

Figure 3 (page 106) shows the mean changes in body weight of salt and control rats during salt loading. Weight gain was identical in both rat groups in the first week of feeding. Control rats, however, showed faster growth rate from the second week. The gap became wider with time and became statistically significant in the 5th week ($P < 0.05$). The salt group showed a significant weight loss in the 6th week (ANOVA; $P < 0.05$).

The weight loss in salt-loaded rats contributed to the large difference in terminal mean body weight between the two groups (control 154±6g, salt 125±5g; $P < 0.05$).

3.2. BLOOD PRESSURE(BP) AND HEART RATE(HR)

Terminal BP and HR of randomly picked rats from both groups are shown on Table 3. Figure 4 shows representative tracings for both groups. Salt loading caused significant elevation of BP in SD rats($P < 0.05$). The mean BP in control rats was 92.0±2.6 mmHg while that of salt-loaded rats was 159.5±4.3 mmHg. There was no significant effect of six-week salt loading on HR in these experiments. Control rats had HR of 362.0± 11.3 beats/min while the salt-loaded rats had HR of 350.71±3.76 beats /min.

3.3. SERUM SODIUM AND POTASSIUM ION CONCENTRATION

Table 4 also shows the effect of salt loading on serum levels of Na^+ and K^+. There was no significant difference in Na^+ concentration between the control and salt groups (control 136.8±2.0mM, test 131.3±4.0mM) Salt loaded rats showed mild, relative hypokalaemia.

The value in the control rats was 5.7±0.2mM and in the test group, 4.0±0.1mM. The difference was statistically significant($P<0.05$).

3.4. NA, 5-HT AND HIGH-K^+ CONTRACTIONS

3.4.1. TIME-COURSE OF CONTRACTILE RESPONSES

The rapidity of contractions of aortic rings from both rat groups to 10^{-5}M NA and 100mM K^+-PSS is illustrated in Figures 5 and 6 respectively. Rings of both groups reached maximum contractile responses to NA after 10min and to high-K^+ after 15min. Salt- loaded rings appeared to respond more slowly than controls to both NA and high-K^+ PSS, in the first 120s, within which about 70% of total contractile responses were achieved in the rings.

3.4.2. MAXIMUM CONTRACTILE RESPONSES

The maximum contraction induced by 10^{-5}M NA in rings from salt-loaded rats (1191±92 mg) was lower than that in control rats (1710±129mg). Salt loading thus significantly reduced vascular reactivity to NA in the rat aorta ($P<0.05$).

Vascular reactivity to 10^{-5}M 5-HT was qualitatively lower in rings from salt rats (1011±175mg) than in rings from control rats (1213±328mg). The difference was, however, not significant.

Vascular reactivity to 100mM-K^+ PSS depolarisation was also attenuated by salt loading. The salt group showed 896±84 mg maximal tone while the control group showed 1325±147mg ($P < 0.05$).

Maximal contractile responses to the various agents are summarised in Figure 7 and Table 5.

3.4.3. RELAXATION RATES FOLLOWING Ca^{2+} WITHDRAWAL

These experiments were designed to investigate the rate of tissue relaxation as an index of the rate of calcium ion elimination from the intracellular environment, following washout of aortic rings with Ca^{2+}-free PSS at the peak of 10^{-5}M NA or 100mM-K^+ stimulation.

High-K^+ stimulation was fully relaxed in both rat groups after 4-5 min of exposure to Ca^{2+}-free EGTA solutions. NA-contractions took a longer time to relax; about 10-12 min. There were no significant differences in relaxation rates between the control and salt-loaded rats, of NA or high K^+ contractions under these conditions.

Tissues used for this experiment were selected such that there was no significant difference in initial contraction to NA or to KCl (Figure 8) between the two rat groups. This ensured that there was no influence of amount of precontraction on relaxation rates.

3.4.4. CONCENTRATION RESPONSE TESTS

Concentration-dependent contractile responses to NA and 5-HT of aortic rings from salt and control rat groups are summarised in Figures 9 and 10. Table 6 shows the EC_{50} values for NA and 5-HT. Salt loading caused a left-ward shift in the concentration response curve for NA. EC_{50} (NA) value for the salt group [$3.7(\pm 0.8) \times 10^{-9}$M] was significantly lower ($P < 0.05$) than that of the controls [$4.4(\pm 1.0) \times 10^{-8}$M]. There were no significant differences in the 5-HT curves between the two rat groups. The results indicate that salt loading induces increased sensitivity of aortic smooth muscle to NA but not to 5-HT.

Concentration-dependent contractions to K^+- induced depolarisation is shown in Figures 11 and 12. EC_{50} values for K^+-depolarisation are shown in Table 7 for both rat groups.

There was a left-ward shift of the curve due to salt loading. Aortae from salt-loaded rats showed a lower mean EC_{50} for KCl (13.27 ± 0.98mM) than controls (21.30 ± 1.68mM). A significant difference in sensitivity to KCl was observed at KCl concentrations below 60mM. These results suggest salt-induced increase in smooth muscle sensitivity to high-K^+ depolarisation.

3.4.5. PHASIC CONTRACTION TO NA

This experiment was designed to investigate the size and dynamics of the intracellularly mobilisable calcium pools in the aorta

smooth muscle from control and salt-loaded rats. The results, as summarised in Figures 13, 14 and 15, show that there is no significant difference in maximum phasic response to noradrenaline between the aortic preparations from control and salt-loaded groups. However, the phasic tone in control rings faded faster than in the salt-loaded rat rings (Figures 13 and 14).

3.5. CONCENTRATION RESPONSE TO CALCIUM CHLORIDE

Calcium ion influx through potential-sensitive channels (during K^+ stimulation) or receptor- operated channels (during NA stimulation) were investigated in aortic rings from control and salt- loaded rats. The results are shown on Figures 16 and 17 and on Table 8. Rings from salt-loaded rats showed increased Ca^{2+}-induced contraction during NA stimulation but there was no significant effect of salt-loading on Ca^{2+}-induced contraction during high-K^+ stimulation. These observations are also reflected in the EC_{50} values (Table 8).

Maximum contraction recorded during K^+ stimulation was 1325±147mg for controls and only 610±109mg in salt-loaded rat rings ($P<0.05$). During NA stimulation, salt-loaded rings showed 1131±99mg while controls showed 1411±83mg tone ($P<0.05$). It,

therefore, seems that salt loading tends to increase muscle tone at lower Ca^{2+}_o concentration in these experiments, although maximum attainable tension appeared to be generally reduced in salt-loaded rat rings.

3.6. CONTRACTILE RESPONSE TO SERUM

Figures 18 and 19 show the results of contractile responses of normotensive rat aortic rings to freshly collected, 10^{-2} units of serum from salt- loaded and control rats; 1 unit of serum representing the undiluted state. The rings elicited contraction in response to serum from either rat group. Serum from salt-loaded rats, however, induced significantly higher maximum contractions than serum from control rats. All serum effects were reversible by washout, as determined from the comparison of magnitudes of contraction to 10^{-7}M NA before and after the application of serum.

3.7. POTASSIUM-INDUCED RELAXATION

Potassium-induced relaxation of NA-contractions in K^+_o-free medium was observed as an indicator of sodium, potassium-adenosine triphosphatase activity (see section 1.4.3a, page 34). Attenuated K^+_o-induced relaxation was observed in rings from salt-loaded rats, in comparison with controls. Salt loading, therefore, appears to induce

an inhibition of Na,K-ATPase activity in aortic smooth muscle cells (Figures 20 and 21, Table 9).

K^+_o-induced relaxation was significantly inhibited by 10^{-5}M ouabain (Figure 21). The effect of ouabain was significant in the control group but not significant in the salt-loaded group (ANOVA, Fisher's LSD test). Ouabain also appeared to abolish the difference in K^+-induced relaxation between the control and salt-loaded rat aortae (Figure 21, Table 9).

3.8. MAGNESIUM-INDUCED RELAXATION: EFFECT OF BAY K8644 AND CGP 28392

Figures 22-28 and Tables 10 and 11 summarise the results of Mg^{2+}_o-induced relaxation of KCl-induced and NA-induced contractions in Mg^{2+}_o-free media. Mg^{2+}_o-induced relaxation of 40mM KCl contractions or 10^{-7}M NA contractions were both significantly attenuated by salt loading. There were no differences in the magnitudes of either K^+-induced or NA-induced precontractions between the control and salt-loaded rat groups (Figures 22 - 24).

K^+-precontractions induced in the presence of calcium channel agonists served to investigate the role of increased calcium entry through potential-sensitive channels on Ca^{2+}-Mg^{2+} interaction. Mean precontractions produced in the presence of 4 X 10^{-8}M Bay

K8644 or 4 X 10^{-5}M CGP 28392 were generally higher than those produced in the presence of 40mM KCl alone. The differences were, however, small in magnitude and not statistically significant (Figures 24 - 28).

Either Bay K8644 or CGP 28392 significantly attenuated Mg^{2+}_o-induced relaxation in both control and salt-loaded rat groups (ANOVA). With either calcium-channel agonist, rings from salt-loaded rats relaxed less to Mg^{2+}_o than the control rats (Fisher's LSD test). The results are summarised in Figures 24 - 28 and in Table 11. The degree of impact of agonist on any group is estimated as a ratio. The B/A ratio measures the impact of Bay K8644 while the C/A ratio measure the impact of CGP 28392. B, C and A stand for Bay K8644, CGP 28392 and "untreated" respectively. The B/A or C/A ratios (Table 11) did not differ significantly between control rings and rings from salt-loaded rats. It thus appears that increased calcium influx in both control rings and rings from salt-loaded rats affected Mg^{2+}_o-induced relaxation in an identical manner.

3.9. RESPONSES TO POTASSIUM CHANNEL OPENING AND CALCIUM ANTAGONISM

Responses of rings from salt and control groups to various antihypertensive agents were recorded from precontractions to 60mM

K^+ PSS and to 10^{-7}M NA. The magnitudes of precontraction, induced by either K^+ or NA, in the two rat groups were not statistically significant (Figures 29 - 36).

Apart from responses to BRL 34915 (Figures 29 and 30), which were significantly biphasic (ANOVA), responses to diltiazem, hydralazine and nifedipine were dose-dependent relaxation responses. The results are presented below.

3.9.1. RESPONSES TO THE K^+ -CHANNEL OPENER, BRL 34915

Responses of K^+ - and NA-contracted rings from salt-loaded and control rats to the potassium channel opener, BRL 34915, are shown in Figures 29 and 30 and in Table 12. During K^+ -stimulation, BRL 34915 induced a biphasic response in the control group. The biphasic response was statistically significant (ANOVA). This comprised an initial dose-dependent relaxation component between 10^{-8}M and 6.4 X 10^{-7}M BRL 34915 (which reached maximal relaxation of 25±10%) and a later dose dependent contraction component which, between 6.4 X 10^{-7}M and 10^{-5}M BRL 34915, progressively reversed the initial relaxation and finally caused additional 2.6% contraction over the initial precontraction (Figure 30). In the salt- loaded rat group, BRL 34915 response was essentially a contraction, at which peak (10^{-5}M BRL 34915), 18.35±1.65% mean contraction was

produced over the precontraction. The difference in BRL 34915 responses in the salt and control rat groups during K^+-PSS stimulation was statistically significant ($P<0.05$) at every drug concentration.

During NA-stimulation, BRL 34915 produced dose- dependent relaxations in both rat groups. Maximum responses were reached at BRL 34915 concentration of 10^{-5}M, at which 88.37±13.37% relaxation was produced in rings from salt-loaded rats while control rings relaxed beyond baseline by about 5% (i.e 105.5±3.6%). This slight overshoot of baseline was reversible by washout; baseline was restored within 30min of washout using normal PSS. The differences between salt and control groups at maximal and other concentrations of BRL 34915 (Figure 30), as well as differences in IC_{50} (M) values (Table 12) were all statistically significant ($P<0.05$).

3.9.2. DILTIAZEM

Relaxation responses of NA-contracted rings of both rat groups to the Ca^{2+}-entry channel blocker, diltiazem, were less than those produced from K^+ - contracted rings (Figures 31 and 32 and Table 13). Salt-loaded rat aortic rings showed comparatively reduced relaxation to diltiazem under all conditions.

Differences in relaxation to diltiazem of NA- contractions of salt and control rat rings were significant between 2.5 X 10^{-9}M and 6.4 x

10^{-7}M diltiazem. Differences in relaxation to diltiazem during K^+-contraction as well as IC_{30} (M) values, on the other hand, were more prominent (Figures 31 and 32) than for NA-contractions and were significant, with salt-loaded rat rings showing comparatively attenuated relaxation responses to diltiazem.

3.9.3. HYDRALAZINE

Between bath concentrations of 2.5 x 10^{-9}M and 4 x 10^{-5}M, hydralazine produced dose-dependent relaxation to K^+- and NA-contractions in both salt and control rat groups (Figures 33 and 34, Table 14). Rings from salt-loaded rats showed comparatively reduced relaxation to hydralazine under all conditions. The differences, however, during K^+- stimulation were not significant at most concentrations. The IC_{30}(M) values, however, were significantly different. On the other hand, differences between the two rat groups during NA-stimulation at most concentrations of hydralazine and between the IC_{30}(M) values under this condition were significant (Figure 34, Table 14).

3.9.4. NIFEDIPINE

Nifedipine, a classical blocker of the potential-sensitive calcium channels, produced dose- dependent relaxation responses to K^+-and

NA-induced precontractions in rings from both control and salt-loaded rats (Figures 35 and 36, Table 15). Maximal relaxation to nifedipine was observed at about 4 X 10^{-8}M nifedipine during K^+ -stimulation and about 2.5 X 10^{-6}M nifedipine during NA-stimulation. Relaxation to nifedipine in the salt group was lower than in the control group, under similar conditions. The differences at most nifedipine concentrations and the differences in IC_{30}(M) values, however, were not statistically significant (Figures 36, Table 15).

3.10. RESPONSES TO HIGH Ca^{2+}_o

Precontractions produced with 60mM K^+ PSS or with 10^{-7}M NA in low-HCO_3 PSS did not differ significantly between the salt-loaded and control rats. Ca^{2+}-induced relaxation due to membrane stabilisation was achieved under these conditions by increasing Ca^{2+}_o. Membrane stabilising effect of Ca^{2+}_o was a Ca^{2+}_o-dependent relaxation of NA precontractions in low-HCO_3 PSS (Figures 37 and 38, Table 16). There was comparatively smaller relaxation of NA-precontractions to $Ca^{2+}o$ in the salt-loaded rat aortic rings. These differences were significant at high $Ca^{2+}o$ concentrations (15mM - 25mM).

During K^+ -precontractions, the effect of increasing Ca^{2+}_o was biphasic - an initial contraction, up to about 9% over the precontrac-

tions, between 3mM and 5mM $Ca^{2+}o$ and a dose-dependent relaxation between 5mM and 25mM $Ca^{2+}o$. The biphasic response in either the salt or the control group was significant (ANOVA). The difference in relaxation between the two rat groups was essentially not significant (Figure 38).

3.11. ENDOTHELIUM-DEPENDENT RELAXATION TO ACETYLCHOLINE AND HISTAMINE

Differences in precontractions of endothelium- intact (+E) rings to 10^{-7}M NA were not significant between the salt and control rat groups (Table 17). Acetylcholine, in the range of 10^{-9}M to 10^{-5}M produced dose-dependent relaxation of NA-precontraction in both rat groups. This response was endothelium dependent, since it was abolished by endothelium removal (Figure 39). The differences at various doses of ACh between salt and control rat groups were, however, not significant (Figure 41). Moreover, the IC_{30}(M) values for ACh did not differ significantly between the salt-loaded and control groups.

Similarly, histamine (10^{-6}M - 10^{-3}M) produced dose-dependent relaxation of NA-precontraction in rings of both groups with intact endothelium but not in deendothelialised rings (Figure 40). The histamine response was smaller in magnitude than the ACh response. At

the peak of histamine response, 10^{-5}M ACh produced further relaxation in rings with intact endothelium (Figure 40) which is evidence that weak 'relaxation responses recorded for histamine were not due to mechanical endothelial denudation. Salt-loaded SD rats showed comparatively attenuated endothelium-dependent relaxation, of NA-precontractions, to histamine (Figure 41). The IC_{30}(M) values for histamine were significantly different for salt-loaded and control rings. These differences in precontraction between the two groups were not statistically significant (Table 17).

Table 2. Food intake of control and salt-loaded rat groups during salt loading

	Wk 3 Intake		Wk 6 Intake	
	(g/d/rat)	(g/d/kg rat)	(g/d/rat	(g/d/kg rat)
Control	12.2±1.0	84.3±6.6	14.3±0.8	91.4±6.2
Salt-loaded	11.0±0.6	82.1±5.6	12.4±0.7*	97.2±4.5

Mean± SEM; n = 15 rats per group; *p<0.05 *cf* controls

Table 3. Blood Pressure (BP) and Heart rate (HR) of control and salt-loaded rats

	BP (mmHg)		HR (beats.min^{-1})	
	Control	Salt-loaded	Control	Salt-loaded
	90	145	372	372
	100	161	336	402
	87	164	396	312
	92	169	324	318
	84	148	384	348
	99	170	360	352
Mean	92.0	159.5	362.0	350.7
SEM	2.6	4.3	11.3	13.7
P	<0.05		N.S	

N.S. not significant

Table 4. Terminal levels of Na and K in the serum of control and salt-loaded rats

	Na (mM)		K (mM)	
	Control	Salt-loaded	Control	Salt-loaded
	134	137	5.7	3.6
	137	113	5.4	4.6
	144	132	5.1	4.0
	130	134	6.0	4.2
	140	130	5.8	4.0
	136	142	6.2	3.8
Mean	136.8	131.3	5.7	4.0
SEM	2.0	4.0	0.2	0.1
P	N.S		<0.05	

N.S. not significant

Table 5. Maximum responses of aortic rings from control and salt-loaded rats to 10^{-5}M NA, 10^{-5}M 5—HT and 100mM KCl

	NA	5—HT	KCl
		(Mg)	
Control	1710±129	1213±328	1325±147
Salt-loaded	1191±92	1011±175	896±84
P	<0.05	N.S.	<0.05

Mean SEM; n = 6 for each test; N.S. not significant

Table 6. EC_{50} (M) values for NA and 5—HT dose response tests in control and salt-loaded rats

	NA	5—HT
Control	$4.4(\pm 1.0)\times10^{-8}$	$2.9(\pm 0.7)\times10^{-6}$
n	9	5
Salt-loaded	$3.7(\pm 0.8)\times10^{-9}$	$1.9(\pm 0.3)\times10^{-6}$
n	11	7
P	<0.05	N.S.

Mean (± SEM)

Table 7. EC_{50} (mM) values for KCl concentration response tests in control and salt-loaded rats

	Control	Salt-loaded
EC_{50}	21.36±1.68	13.27±0.98
n	6	6
P	< 0.05	

Mean ± SEM

Table 8. $EC_{50}(M)$ values for $CaCl_2$ concentration response of control and salt-loaded rings following 10^{-5}M NA and 100mM KCl stimulation

	NA	KCl
Control	$6.2(\pm 0.4)\times10^{-5}$	$1.3(\pm 0.3)\times10^{-4}$
Salt-loaded	$2.4(\pm 0.1)\times10^{-5}$	$3.1(\pm 0.7)\times10^{-4}$
n	5	5
P	<0.05	N.S

Mean ± SEM; N.S. not significant

Table 9. IC_{50}(mM) values for K^+-induced relaxation, in the presence and absence of 10^{-5}M ouabain, of NA contraction in control and salt-loaded rat aortic rings

	Control	Salt-loaded	P
No ouabain	0.75±0.05	1.30±0.11	<0.05
With ouabain	3.42±0.28	2.87±0.39	N.S.
n	6	6	
P	<0.05	<0.05	

Mean ± SEM

Table 10. IC_{30}(mM) values for Mg^{2+}-induced relaxation of 10^{-7}M NA- or 40mM K^{+}-induced contractions

	Control	Salt-loaded	P
NA stimulation	0.08±0.01	0.73±0.25	<0.05
KCl stimulation	1.24±0.19	2.96±0.65	<0.05
n	6	6	

Mean ± SEM

Table 11. Effects of $4x10^{-8}$M Bay K8644 and $4x10^{-5}$M CGP 28392 on the IC_{30}(mM) values for Mg^{2+}-induced relaxation of 40mM K^{+}-induced contraction

		Control	Salt-loaded	P
(A)	KCl	1.24±0.19	2.96±0.65	<0.05
(B)	KCl + Bay K8644	1.90±0.03	5.52±0.39	<0.05
	Ratio B/A	1.53±0.01	1.86±0.05	N.S.
(C)	KCl + CGP 28392	2.76±0.29	6.52±0.52	<0.05
	Ratio C/A	2.23±0.13	2.20±0.14	N.S.

Mean ± SEM; n=6; N.S. not significant

Table 12. Responses to BRL 34915 following 10^{-7}M NA- and 60mM K^+-precontractions

	Control	Salt-loaded	P
NA(IC_{50},M)	$1.09(\pm0.01)\times10^{-7}$	$6.97(\pm5.51)\times10^{-7}$	<0.05
K^+(IC_{30},M)*	$4.72(\pm1.23)\times10^{-6}$	$-1.10(\pm0.30)\times10^{-5}$	<0.05
n	6	6	

Mean SEM; *Extrapolated; (—)indicates contraction

Table 13. Relaxation responses to diltiazem following 10^{-7}M NA and 60mM K^+ -induced contractions

	Control	Salt-loaded	P
NA (IC_{30},M)	$1.32(\pm0.18)\times10^{-6}$	$9.32(\pm2.68)\times10^{-6}$	< 0.05
K^+ (IC_{50},M)	$1.49(\pm0.01)\times10^{-7}$	$6.03(\pm0.07)\times10^{-7}$	< 0.05
n	6	6	

Mean ± SEM

Table 14. Relaxation responses to hydralazine following 10^{-7}M NA and 60mM K^+-induced contractions

	Control	Salt-loaded	P
NA (IC_{30},M)	$1.37(\pm0.92)\times10^{-6}$	$6.08(\pm2.43)\times10^{-6}$	<0.05
K^+ (IC_{50},M)	$1.36(\pm0.83)\times10^{-6}$	$5.24(\pm2.43)\times10^{-6}$	<0.05
n	6	6	

Mean ± SEM

Table 15. Relaxation responses to nifedipine following 10^{-7}M NA and 60mM K^+-induced contractions

	Control	Salt-loaded	P
NA (IC_{30},M)	$1.63(\pm0.45)\times10^{-8}$	$1.72(\pm0.50)\times10^{-8}$	N.S.
K^+ (IC_{50},M)	$5.15(\pm1.53)\times10^{-9}$	$7.42(\pm1.21)\times10^{-9}$	N.S.
n	6	6	

Mean ± SEM; N.S. not significant

Table 16. Relaxation responses to high extracellular calcium concentration of 10^{-7}M NA and 60 mM K^+ -induced contractions

	Control	Salt	P
NA (IC_{50},mM)	3.84±0.59	5.49±1.44	N.S.
K^+ (IC_{30},mM)*	31.47±0.22	25.64±1.11	N.S.
n	6	6	

Mean ± SEM; *Extrapolated; N.S. not significant

Table 17. Values for IC_{30}(M) and maximal relaxation (Max. relax, %) in response to histamine and acetylcholine. Tension was induced by 10^{-7}M noradrenaline

	Histamine		Acetylcholine	
	Control	Salt-loaded	Control	Salt-loaded
IC_{30}	$4.1(\pm 1.3)\times10^{-4}$	$1.5(\pm 0.2)\times10^{-3}$*†	$2.9(\pm 0.9)\times10^{-8}$	$3.7(\pm 0.8)\times10^{-8}$
Max. relax.	48.8±5.7	22.8±3.8†	62.8±6.0	67.9±3.6
Tension	1320±148	918±145	1120±126	1014±130
n	6	6	6	6

Mean ± SEM; *Extrapolated; †P<0.05 as compared to control

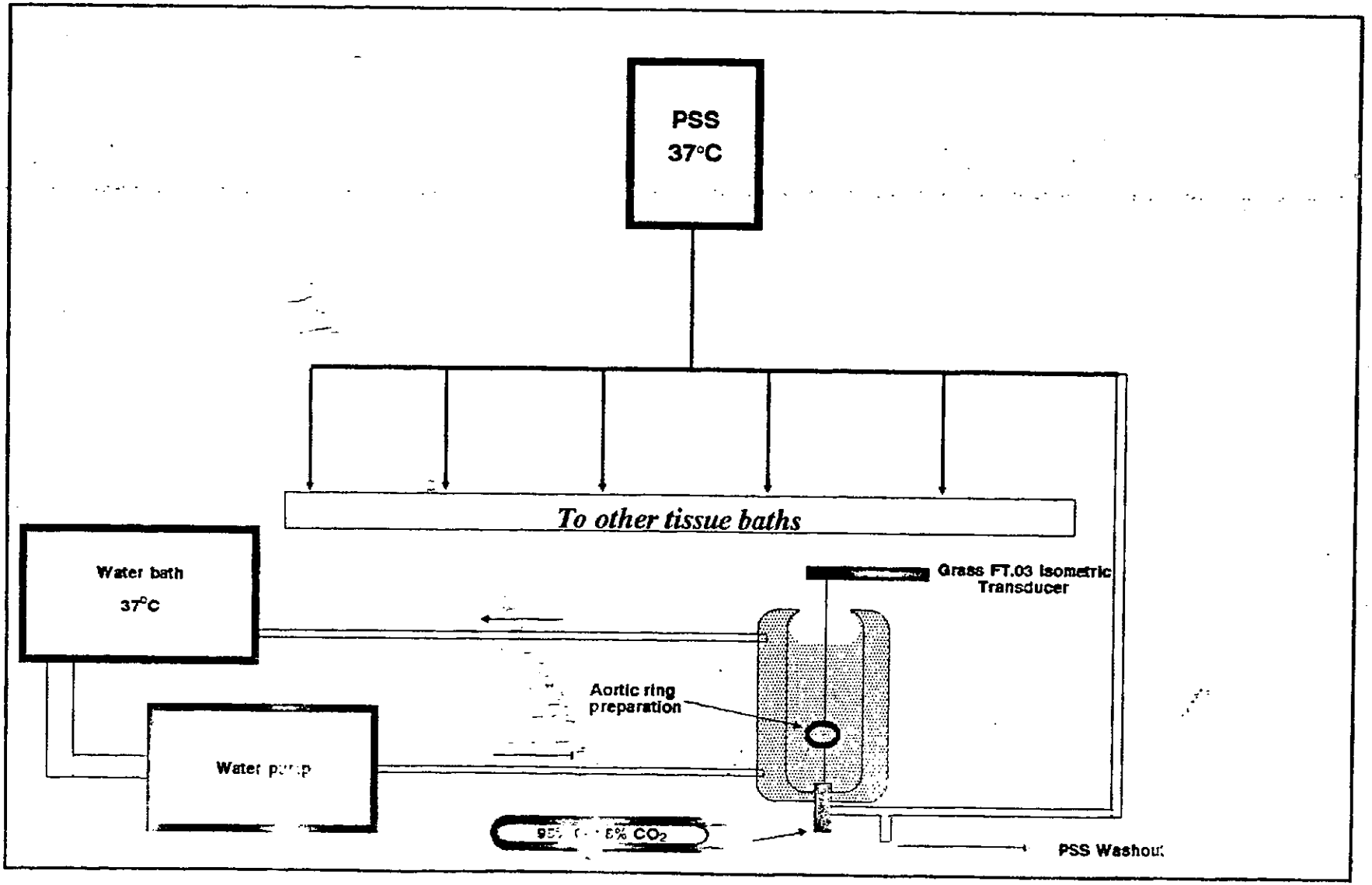

PSS
37°C
To other tissue baths
Water bath
37°C
Grass FT.03 Isometric
Transducer
Aortic ring
preparation
Water pump
PSS Washout

Figure 1a. Tissue bath assembly used for in vitro study of mechanical activity of the aortic rings (Not drawn to scale; PSS - Physiological salt solution).

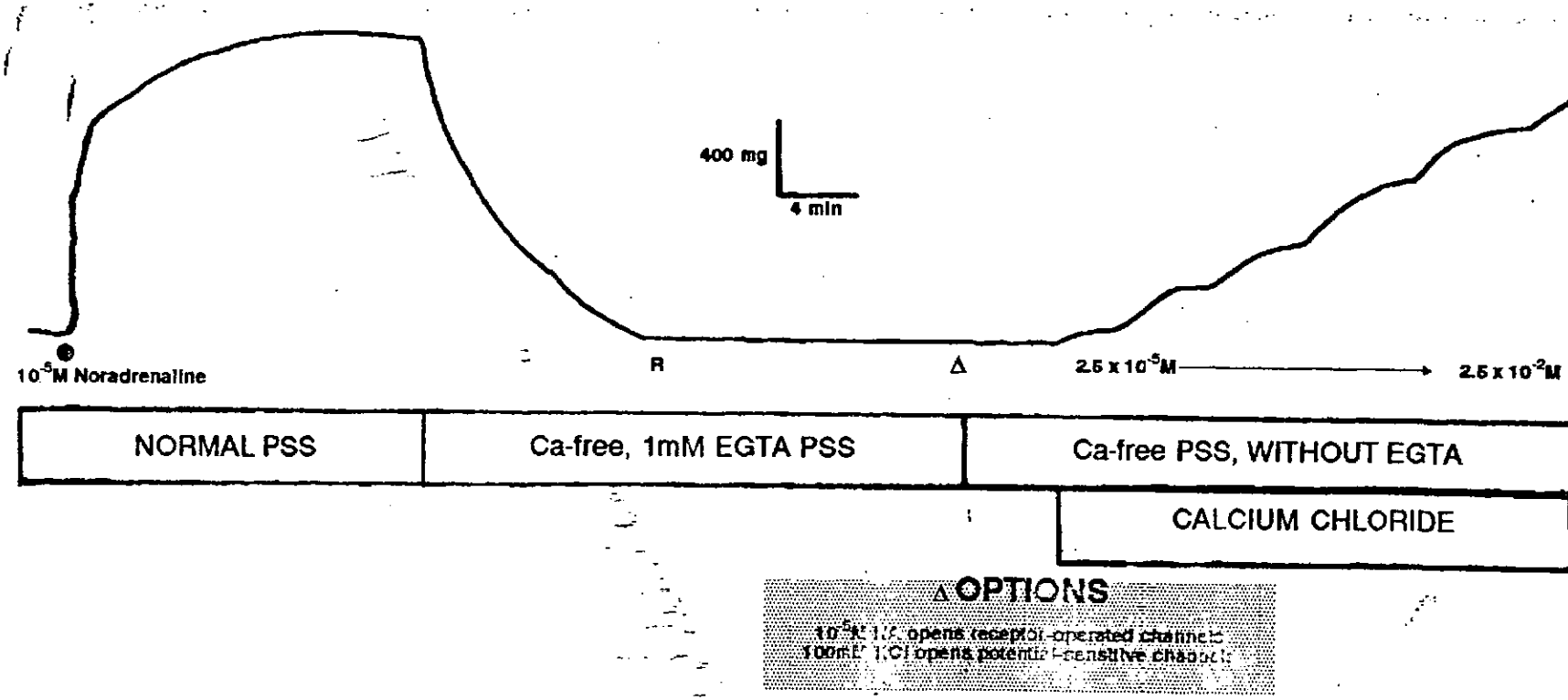

400 mg
4 min
10⁻⁵M Noradrenaline
R
Δ
2.5 x 10⁻⁵M
2.5 x 10⁻²M
NORMAL PSS
Ca-free, 1mM EGTA PSS
Ca-free PSS, WITHOUT EGTA
CALCIUM CHLORIDE
Δ OPTIONS
10⁻⁵M NE opens receptor-operated channels
100mM KCl opens potential-sensitive channels

Figure 1b. Schematic protocol for concentration response test of aortic rings to calcium chloride during stimulation of receptor- operated or potential-sensitive calcium-entry channels. R indicates washout

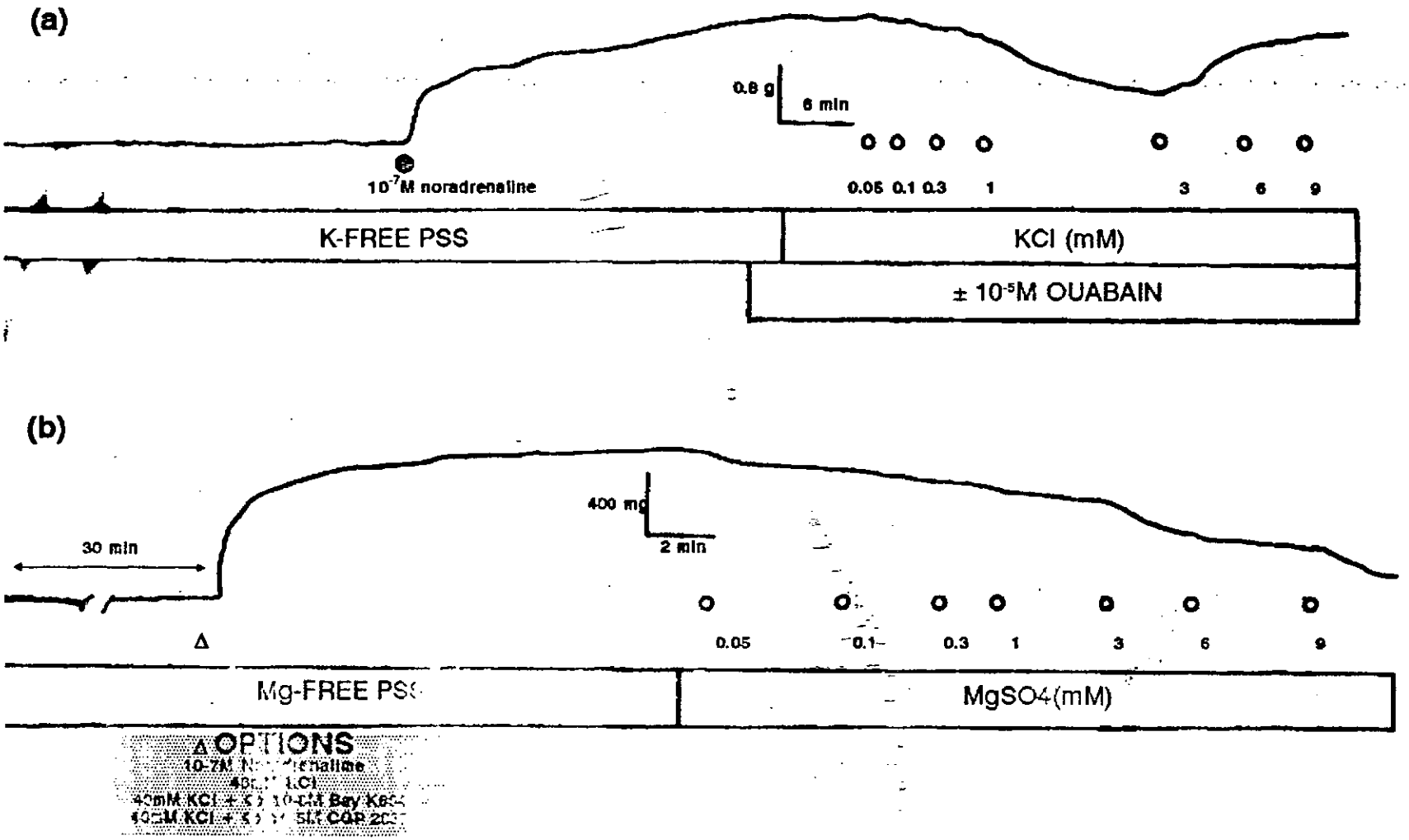

(a)
0.8 g
6 min
10⁻⁷M noradrenaline
0.05 0.1 0.3 1 3 6 9
K-FREE PSS
KCl (mM)
± 10⁻⁵M OUABAIN
(b)
400 mg
2 min
30 min
Δ
0.05 0.1 0.3 1 3 6 9
Mg-FREE PSS
MgSO4(mM)
Δ OPTIONS

Figure 2. Schematic protocols for $[K^+]_o$-induced (a) and $[Mg^{2+}]_o$-induced (b) relaxation responses

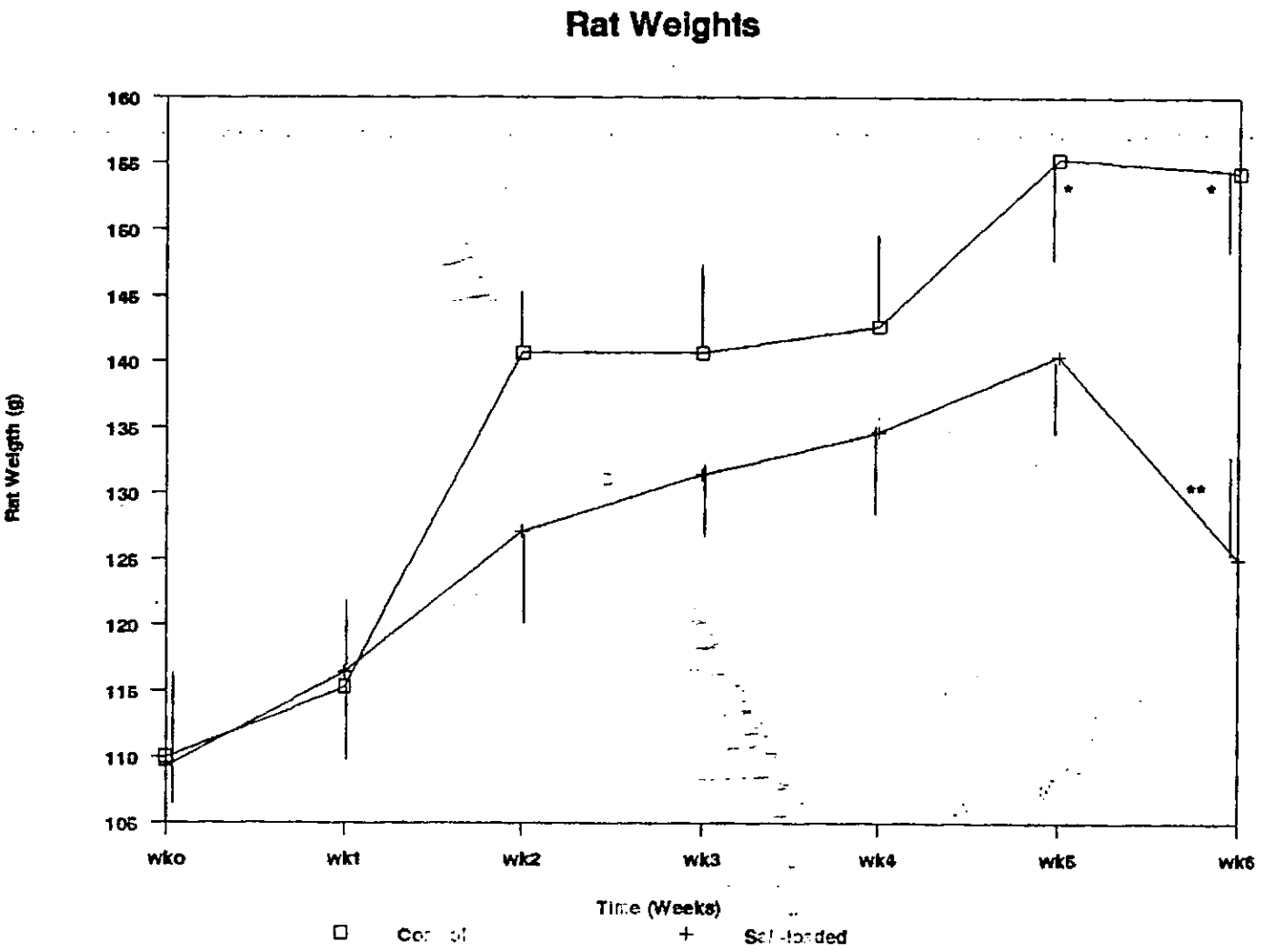

Rat Weights
160
155
150
145
140
135
130
125
120
115
110
105
Rat Weigth (g)
wko
wk1
wk2
wk3
wk4
wk5
wk6
Time (Weeks)
*
*
**
Control
Solitaire

*Figure 3. Weekly body weight profiles of control and salt-loaded rats during salt-loading. *Significant difference between groups. **Significant weight loss (ANOVA, $P<0.05$).*

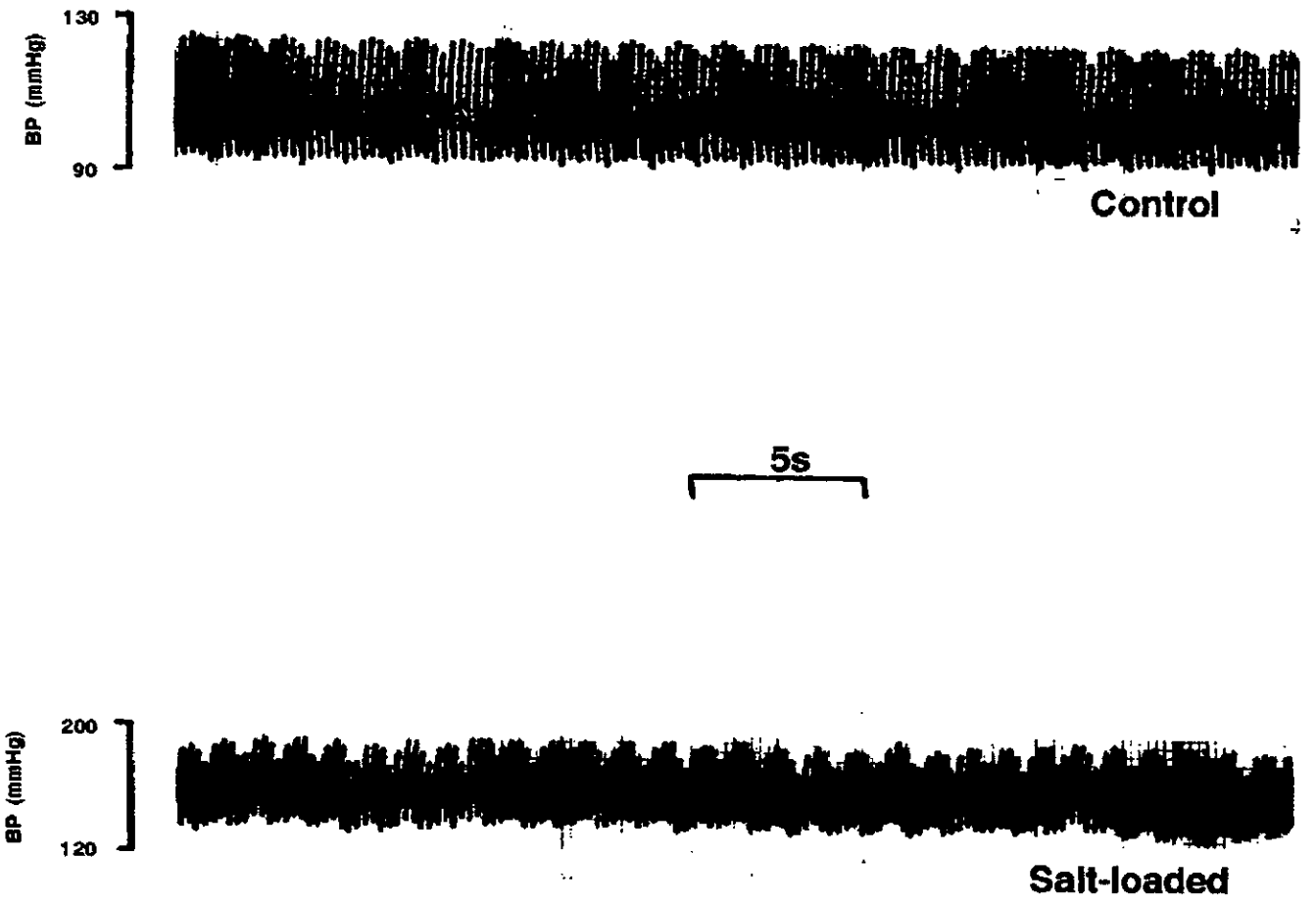

130
BP (mmHg)
90
Control
5s
200
BP (mmHg)
120
Salt-loaded

Figure 4. Terminal blood pressure tracings of control and salt-loaded rats.

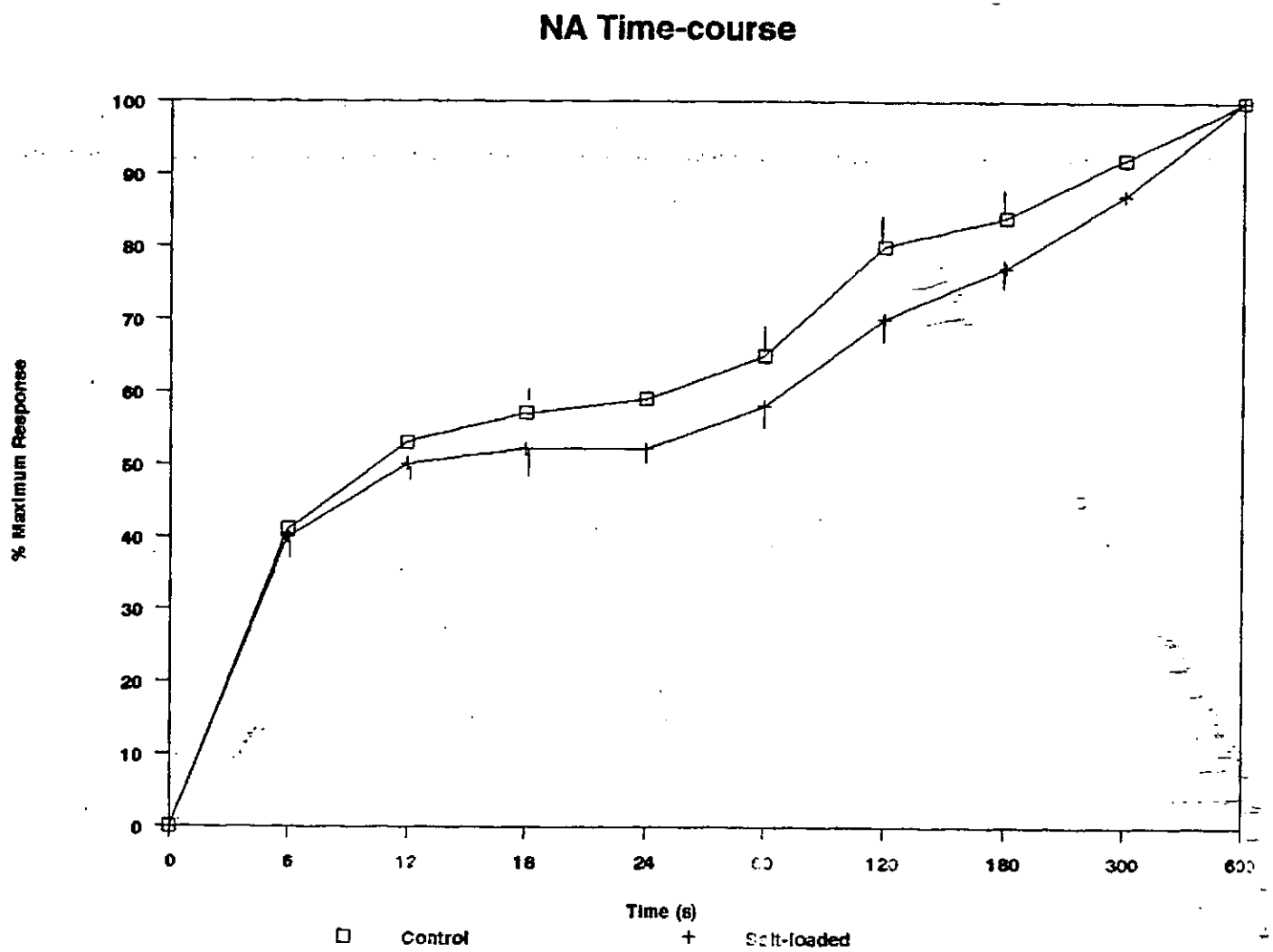

NA Time-course
% Maximum Response
100
90
80
70
60
50
40
30
20
10
0
0
6
12
18
24
60
120
180
300
600
Time (s)
Control
Salt-loaded

Figure 5. Time-course of contraction of aortic rings from control and salt-loaded rats to 10^{-5}M noradrenaline (Mean ±S.E.M.).

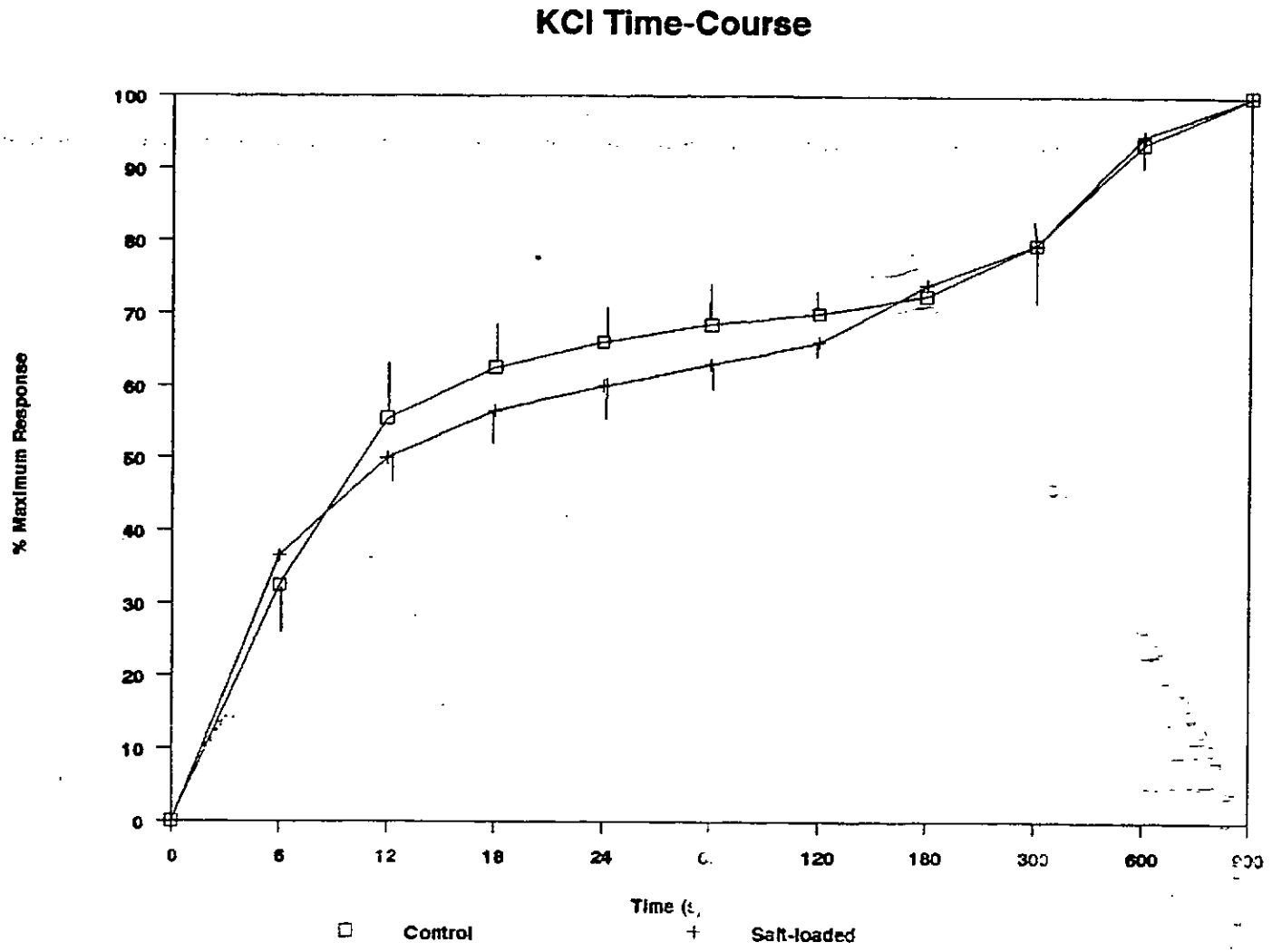

KCl Time-Course
% Maximum Response
100
90
80
70
60
50
40
30
20
10
0
0
6
12
18
24
120
180
300
600
Time (s,
Control
Salt-loaded

Figure 6. Time-course of contraction of aortic rings from control and salt-loaded rats to 100mM K^+-PSS (Mean ±S.E.M)

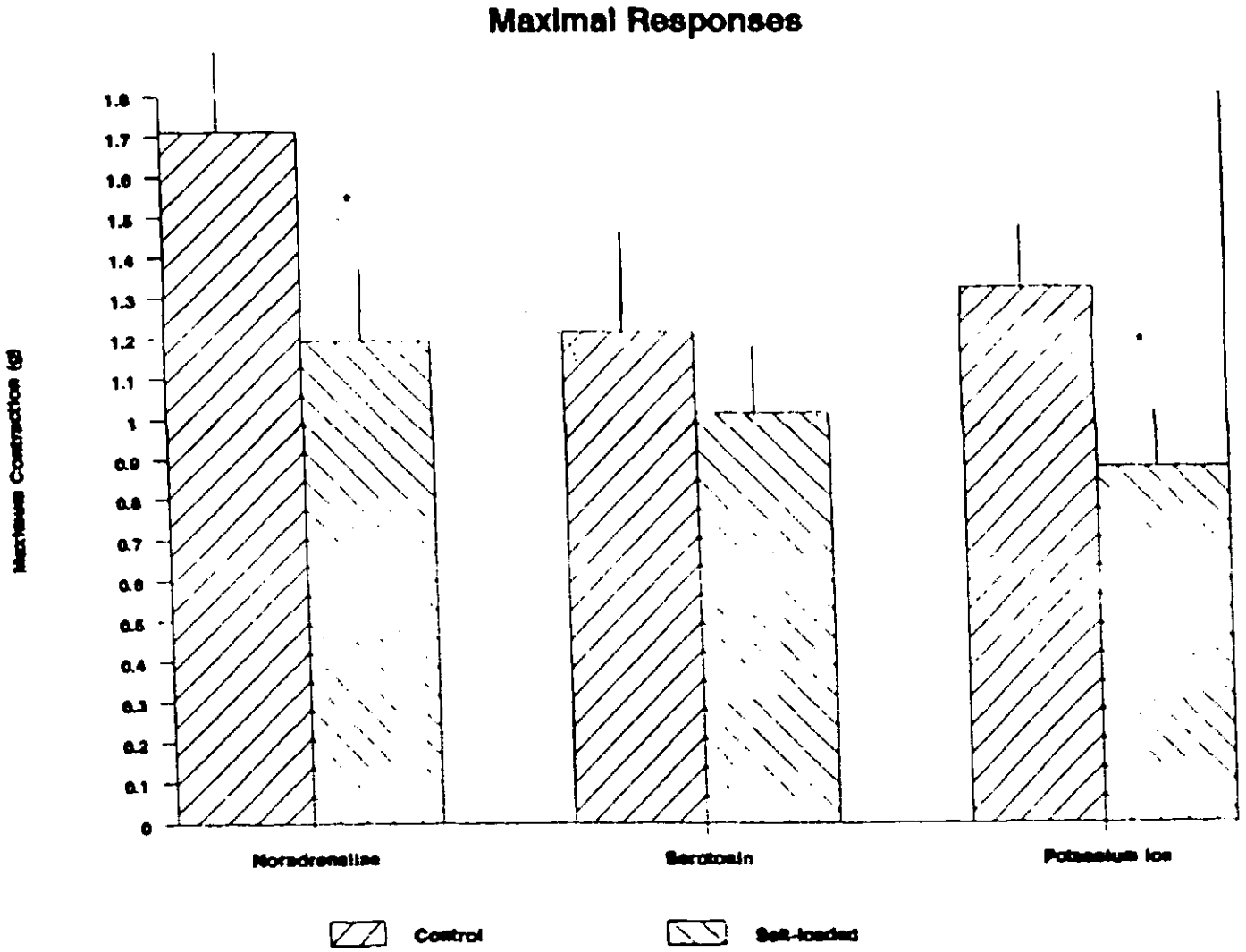
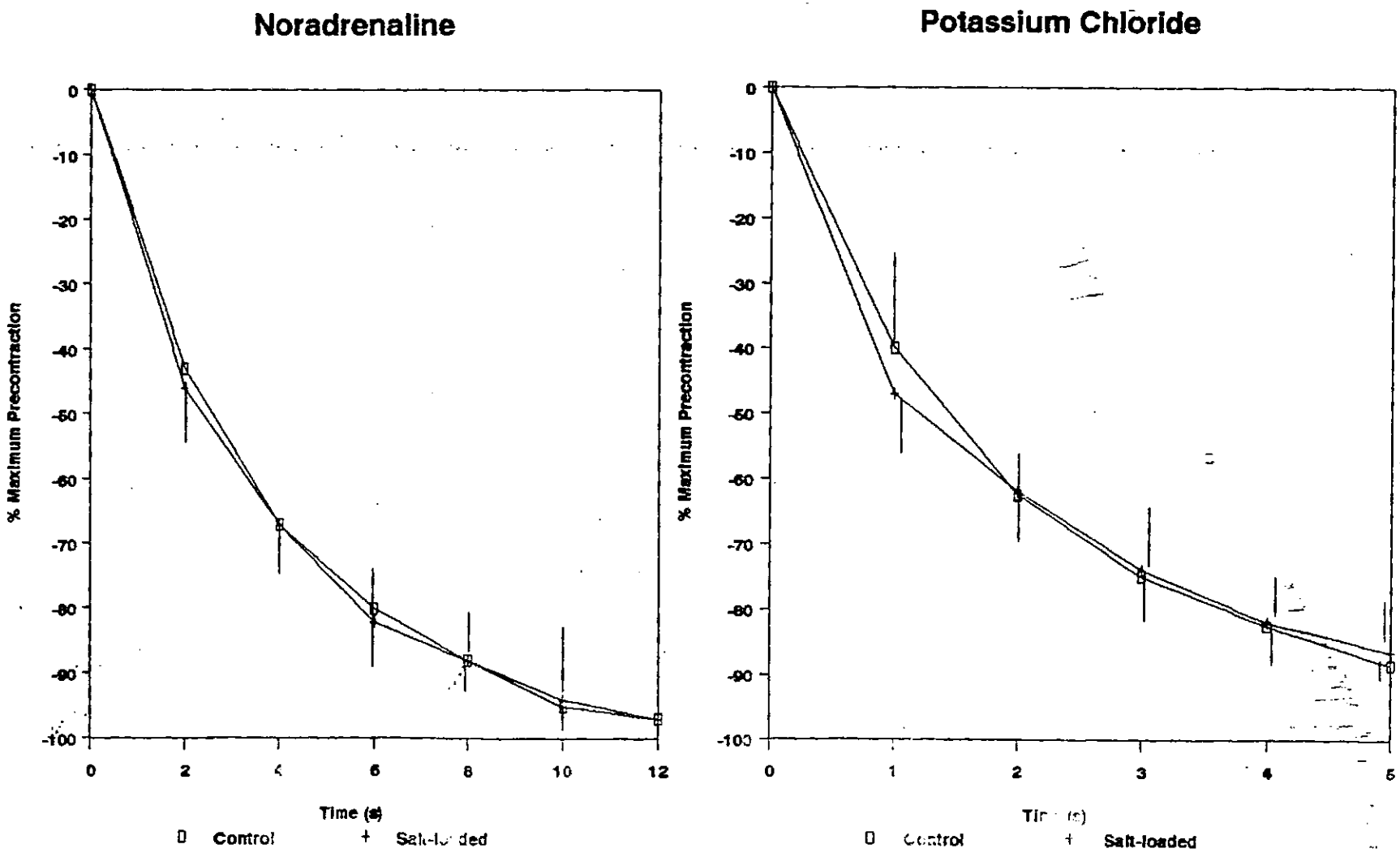

Maximal Responses
Maximum Contraction (g)
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Noradrenaline
Serotonin
Potassium ion
Control
Salt-loaded

*Figure 7. Maximal contractile responses of aortic rings from control and salt-loaded rats to noradrenaline, 5-hydroxytryptamine and KCl. *denotes significant difference (Mean ±S.E.M.).*


Noradrenaline
% Maximum Precontraction
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
0
2
4
6
8
10
12
Time (s)
Control
Salt-loaded
Potassium Chloride
% Maximum Precontraction
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
0
1
2
3
4
5
Control
Salt-loaded

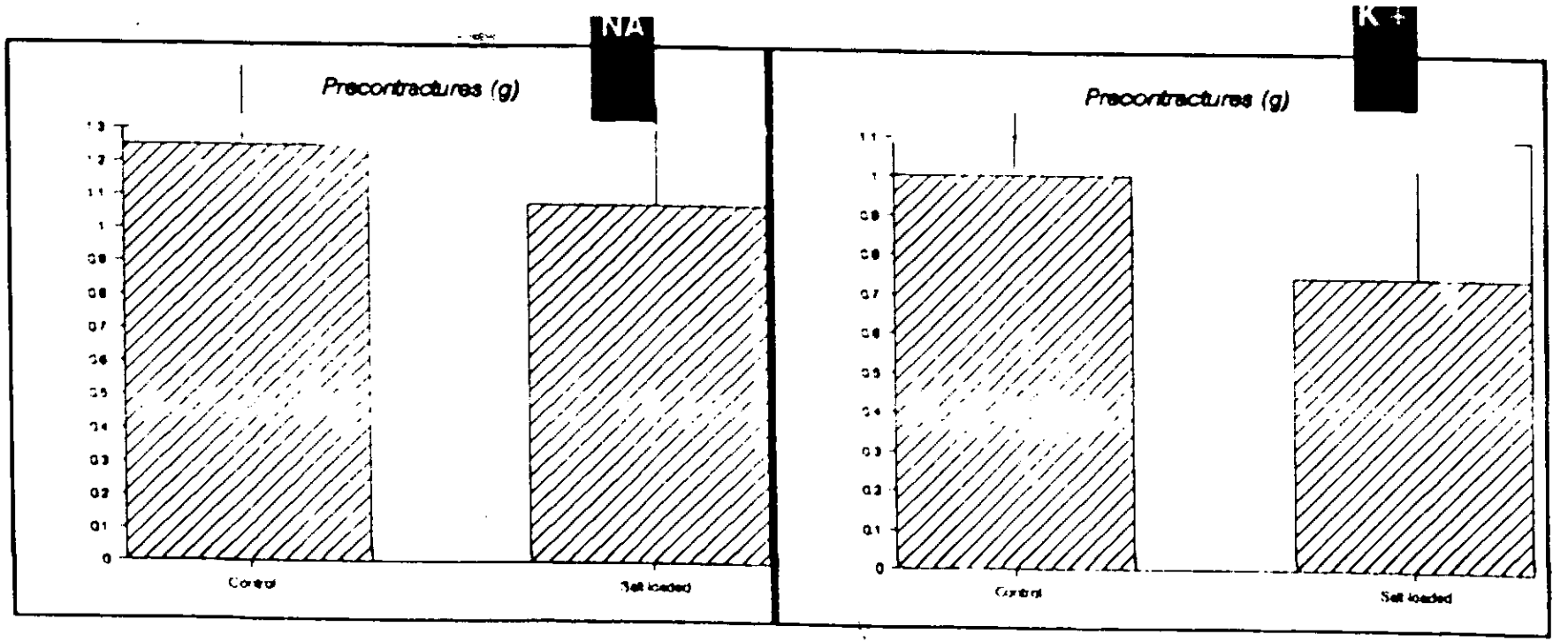


Figure 8. Time-course of relaxation of maximal contraction to NA and KCl due to $[Ca2+]_o$ withdrawal. Withdrawal procedures were performed in control and salt-loaded rat aortic rings that attained statistically similar maximal contraction (Mean ±S.E.M.)

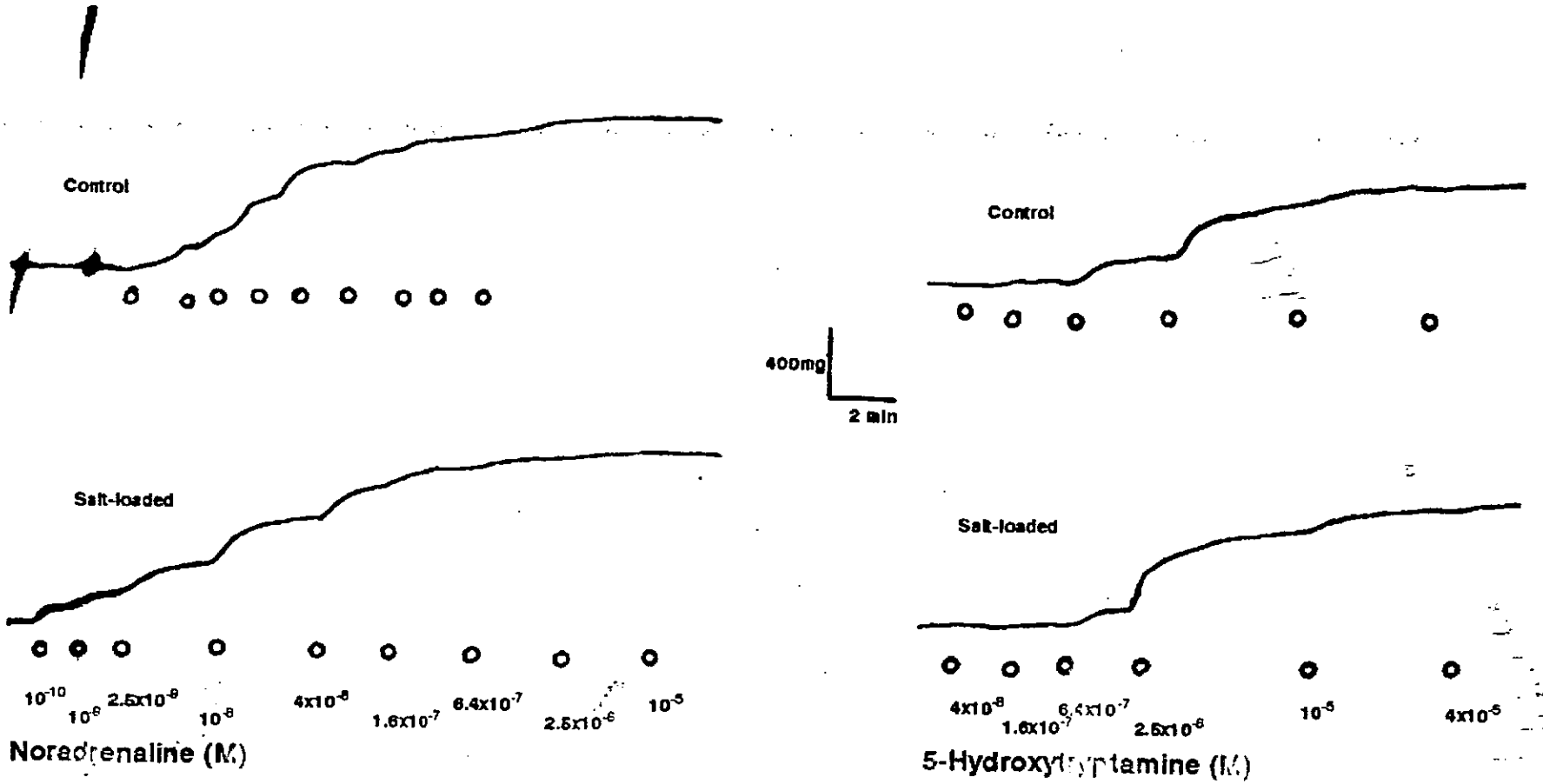

Control
Salt-loaded
10^{-10}
10^{-9}
$2.5x10^{-9}$
10^{-8}
$4x10^{-8}$
$1.6x10^{-7}$
$6.4x10^{-7}$
$2.5x10^{-6}$
10^{-5}
Noradrenaline (M)
400mg
2 min
Control
Salt-loaded
$4x10^{-8}$
$1.6x10^{-7}$
$6.4x10^{-7}$
$2.5x10^{-6}$
10^{-5}
$4x10^{-5}$
5-Hydroxytryptamine (M)

Figure 9. Cumulative dose-response of aortic rings from control and salt-loaded rats to noradrenaline and 5-hydroxytryptamine.

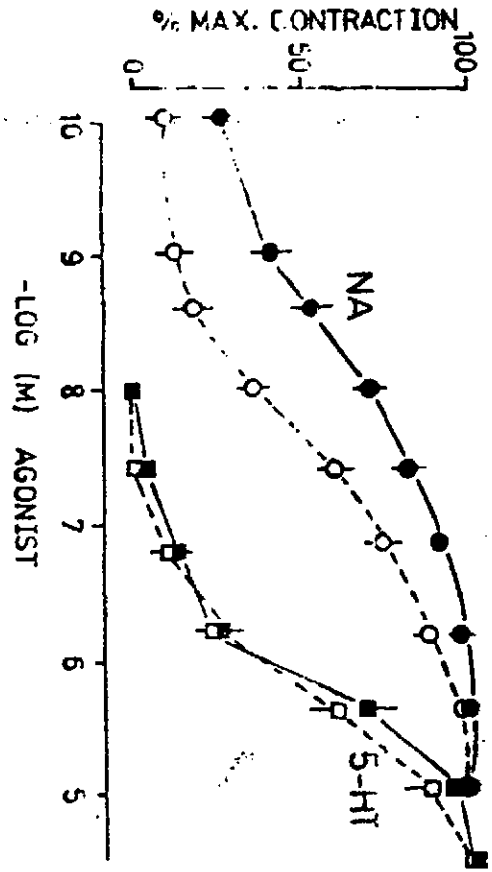

% MAX. CONTRACTION
0
50
100
-LOG (M) AGONIST
10
9
8
7
6
5
NA
5-HT

Figure 10. Concentration response curves to NA (circles) and 5-HT (squares) in aortic rings from control (open symbols) and salt- loaded (closed symbols) rats. Mean ±S.E.M.

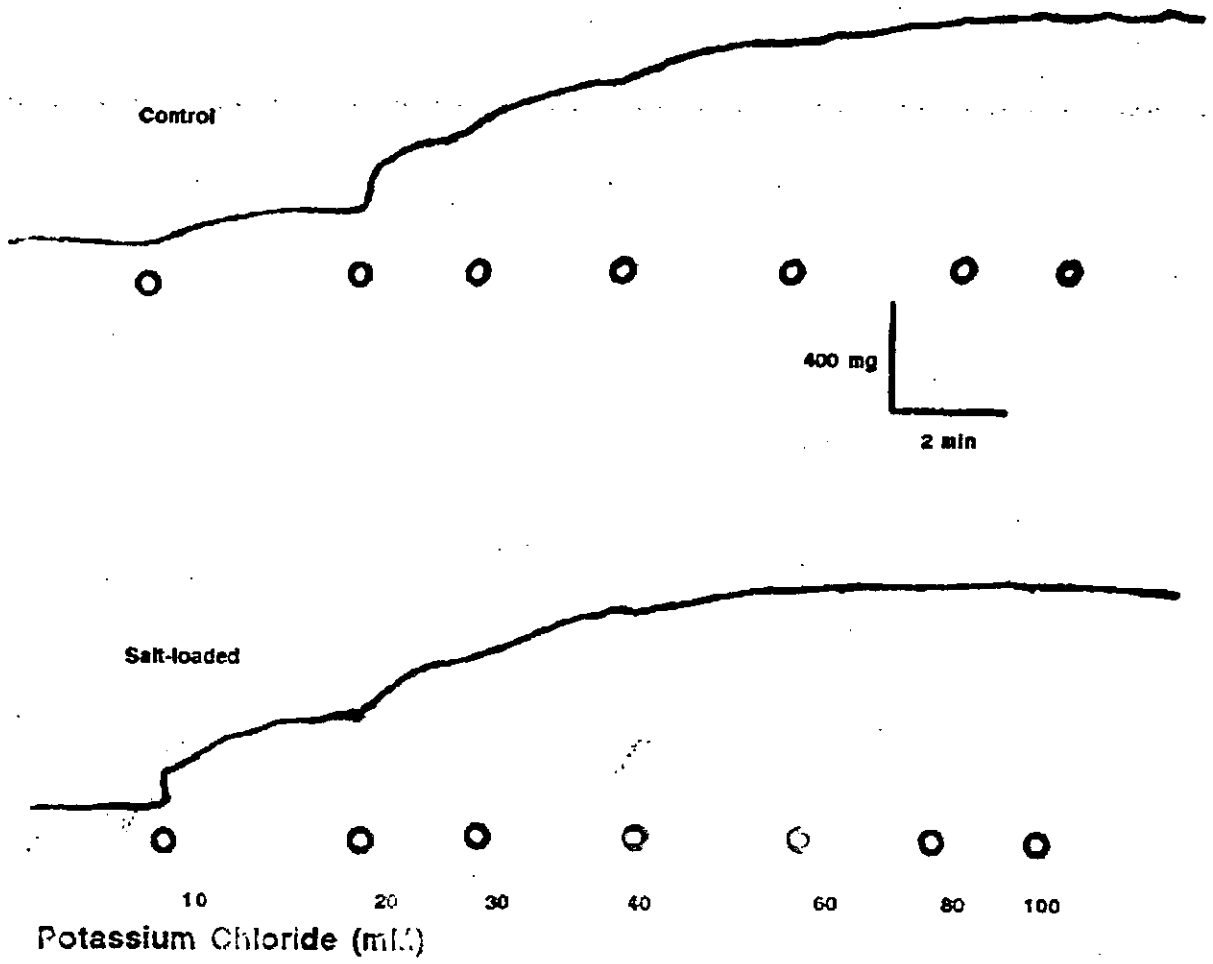

Control
400 mg
2 min
Salt-loaded
10
20
30
40
60
80
100
Potassium Chloride (mM)

Figure 11. Concentration response of aortic rings from control and salt-loaded rats to high-K^+-PSS. The various KCl concentrations were achieved non-cumulatively.

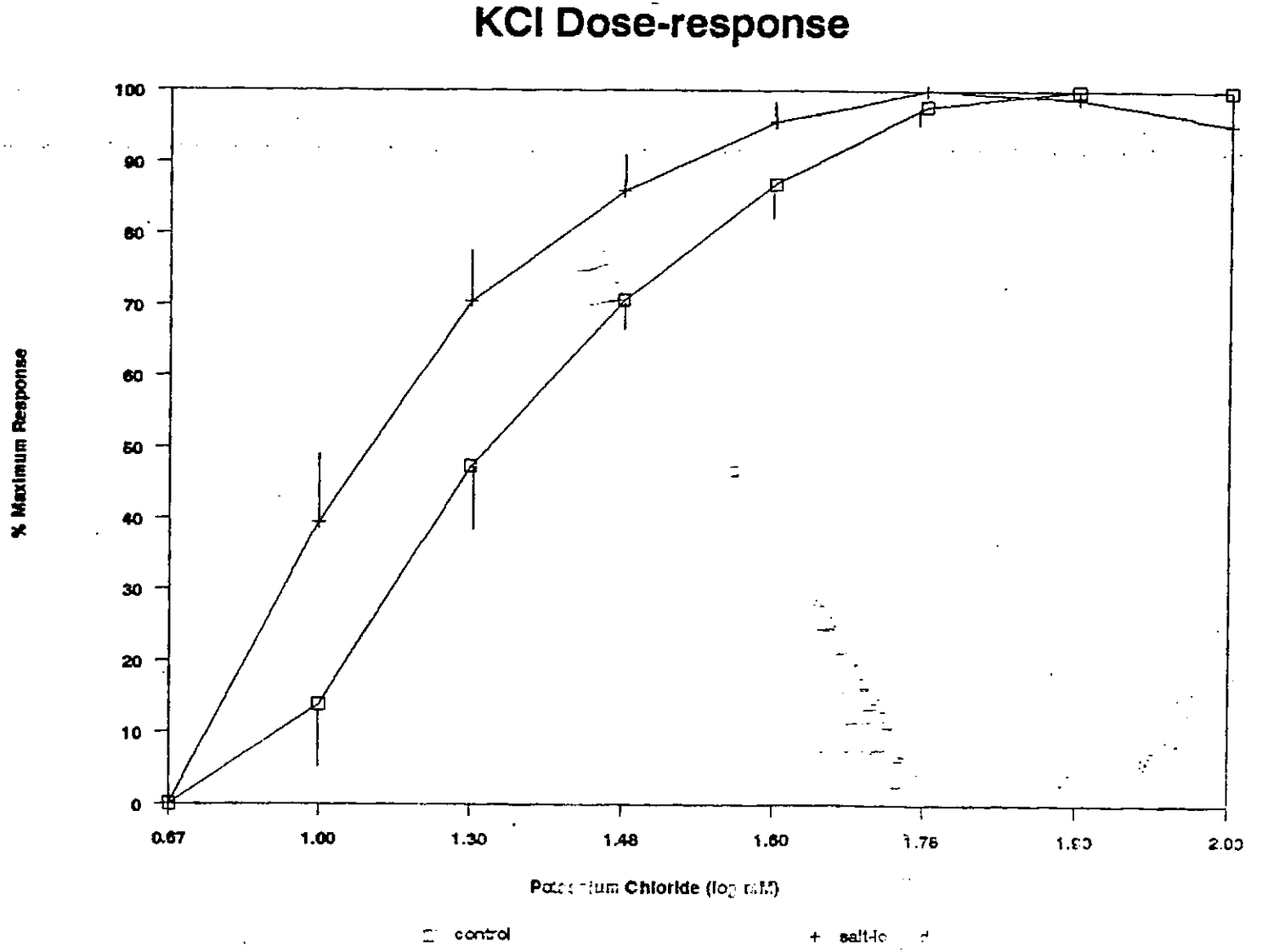

KCl Dose-response
% Maximum Response
100
90
80
70
60
50
40
30
20
10
0
0.67
1.00
1.30
1.48
1.60
1.76
1.90
2.00
Potassium Chloride (log mM)
control
+ salt-lo

Figure 12. Concentration-response of aortic rings from control and salt-loaded rats to KCl (Mean ±S.E.M.)

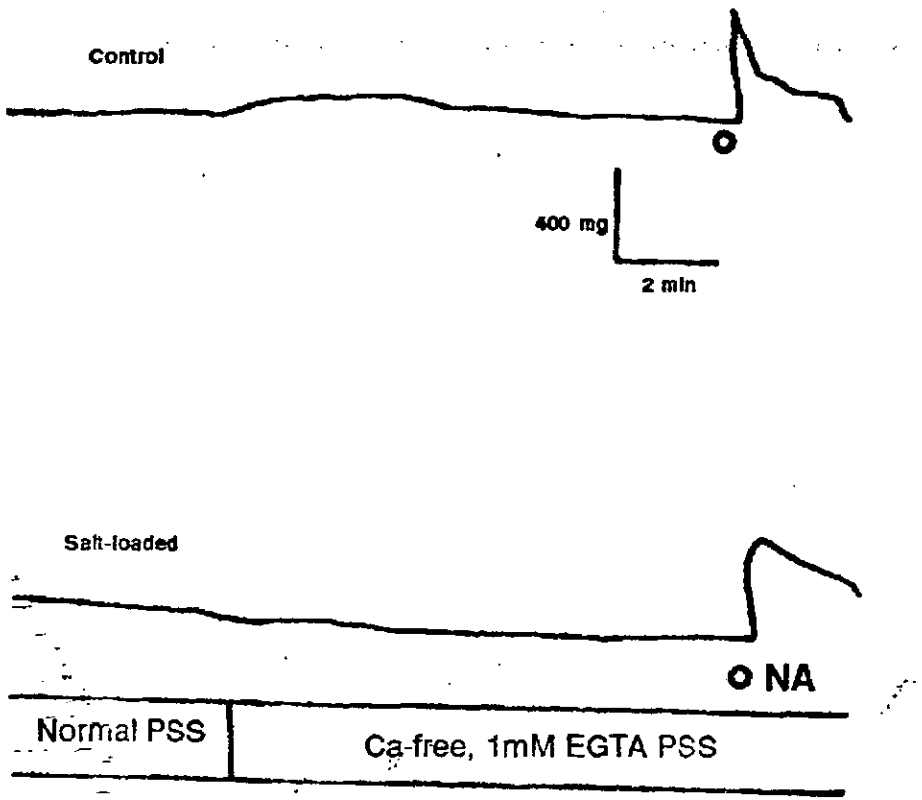

Control
400 mg
2 min
Salt-loaded
NA
Normal PSS
Ca-free, 1mM EGTA PSS

Figure 13. Phasic contraction of aortic rings from control and salt-loaded rats, in response to 10^{-5}M noradrenaline (NA).

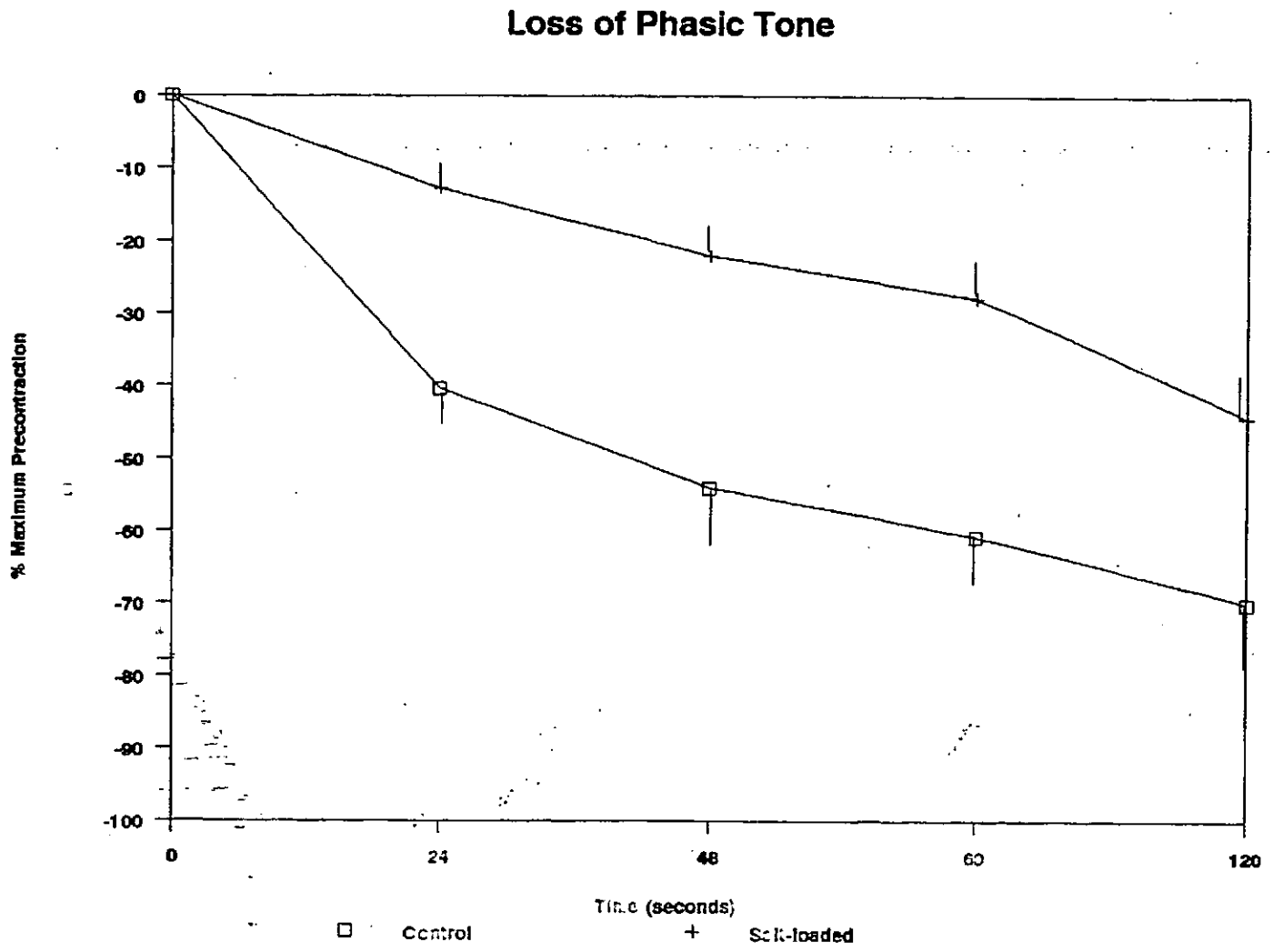

Loss of Phasic Tone
% Maximum Precontraction
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
0
24
48
60
120
Time (seconds)
Control
Salt-loaded

Figure 14. Rate of tone loss following NA-induced phasic contraction of control and salt-loaded rat aortic rings in Ca- free EGTA PSS.

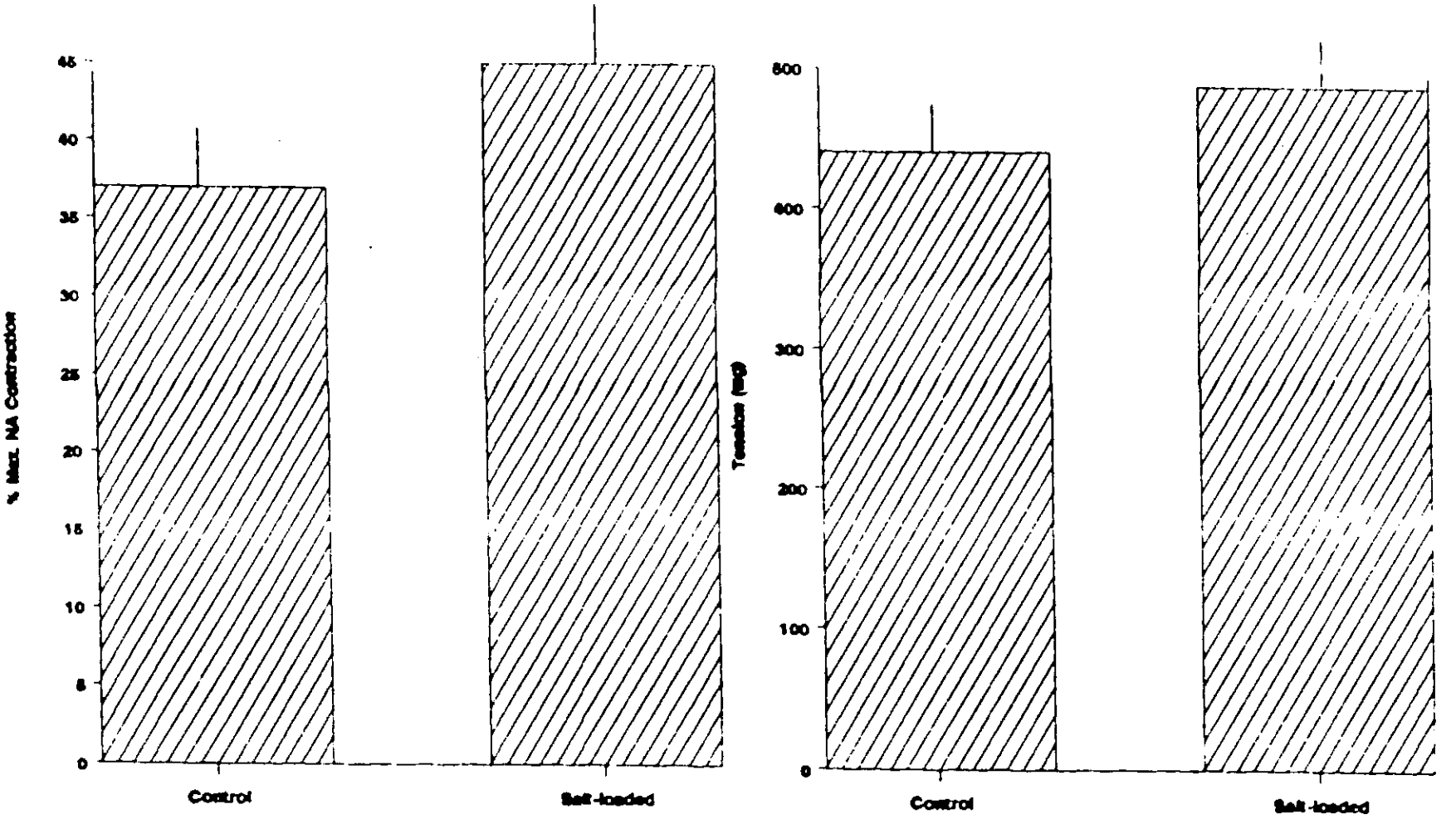

% Max. NA Contraction
0
8
10
15
20
25
30
35
40
45
Control
Salt-loaded
Tension (mg)
0
100
200
300
400
500
Control
Salt-loaded

Figure 15. Maximal phasic contraction of control and salt-loaded rat aortic rings in Ca-free EGTA PSS. There was no significant difference from control.

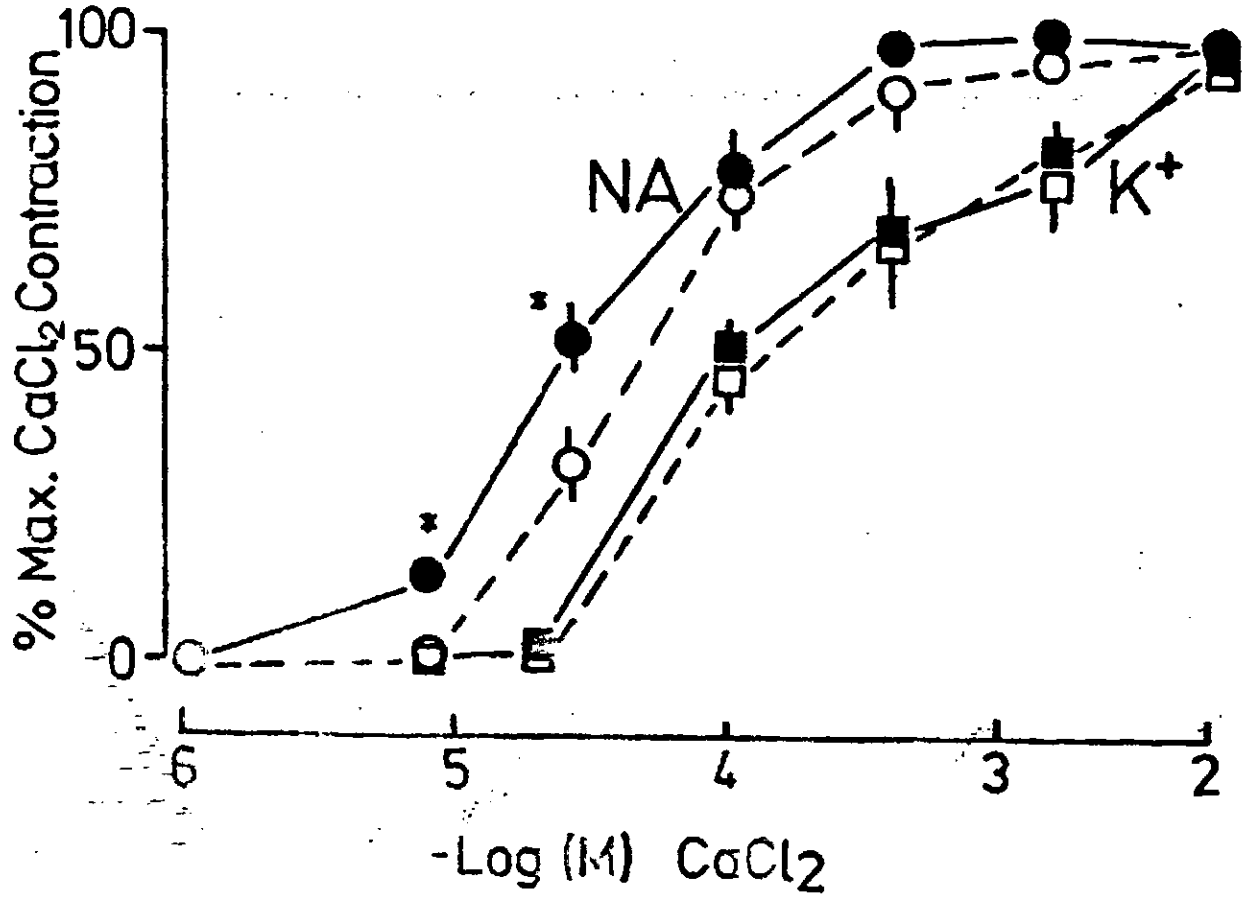

100
50
0
% Max. $CaCl_2$ Contraction
NA
K^+
*
*
6
5
4
3
2
-Log (M) $CaCl_2$

*Figure 16. Concentration-response to $CaCl_2$ of aortic rings from control (open symbols) and salt- loaded (closed symbols) rats following exposure to Ca-free PSS containing 10^{-5}M noradrenaline (circles) or 100mM K^+ (squares). *denotes significant difference.*

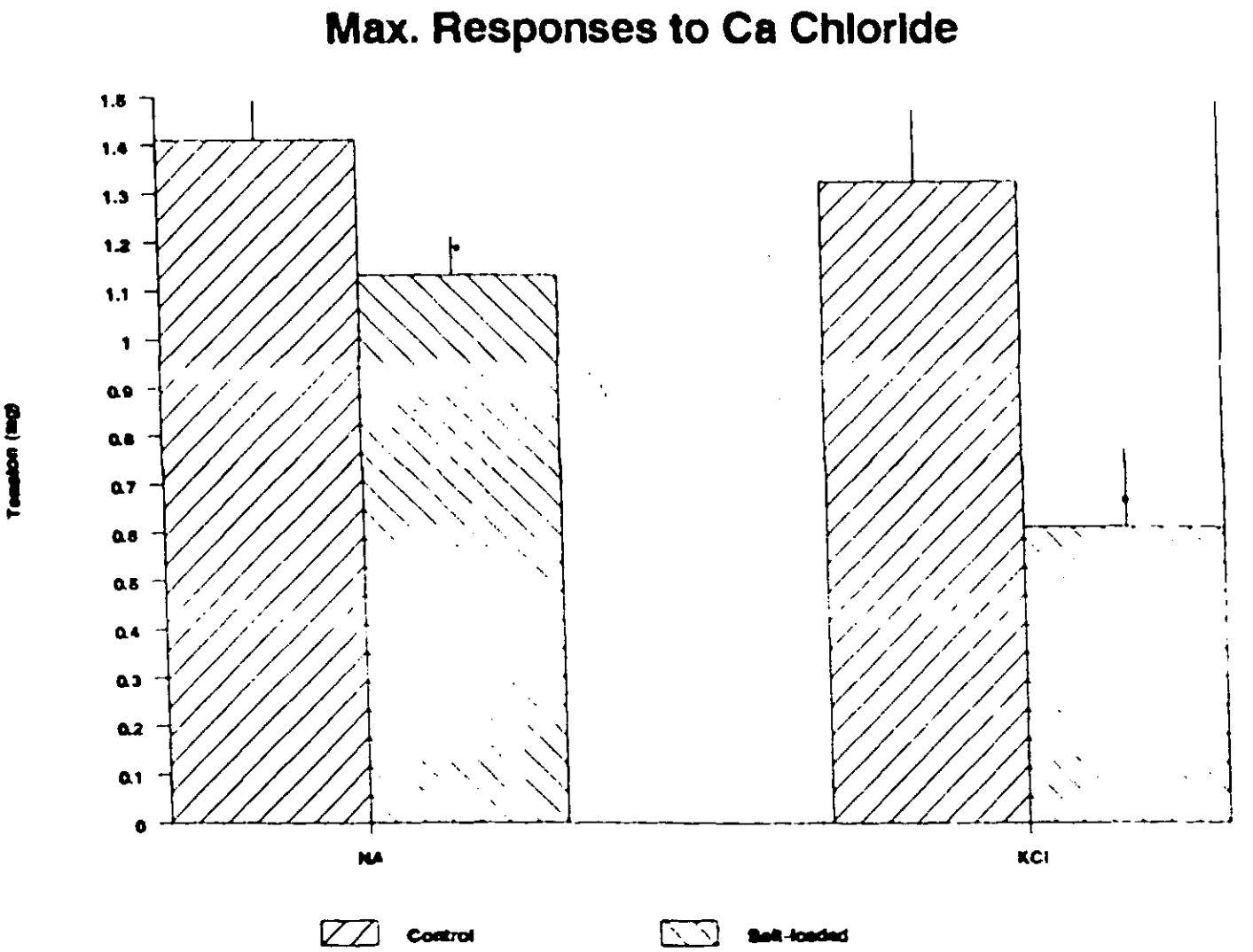

Max. Responses to Ca Chloride
Tension (mg)
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
NA
KCl
Control
Salt-loaded

Figure 17. Maximal response to $CaCl_2$ of aortic rings from control and salt-loaded rats following exposure to Ca-free PSS containing 10^{-5}M NA or 100mM KCl. *denotes significant difference from control.

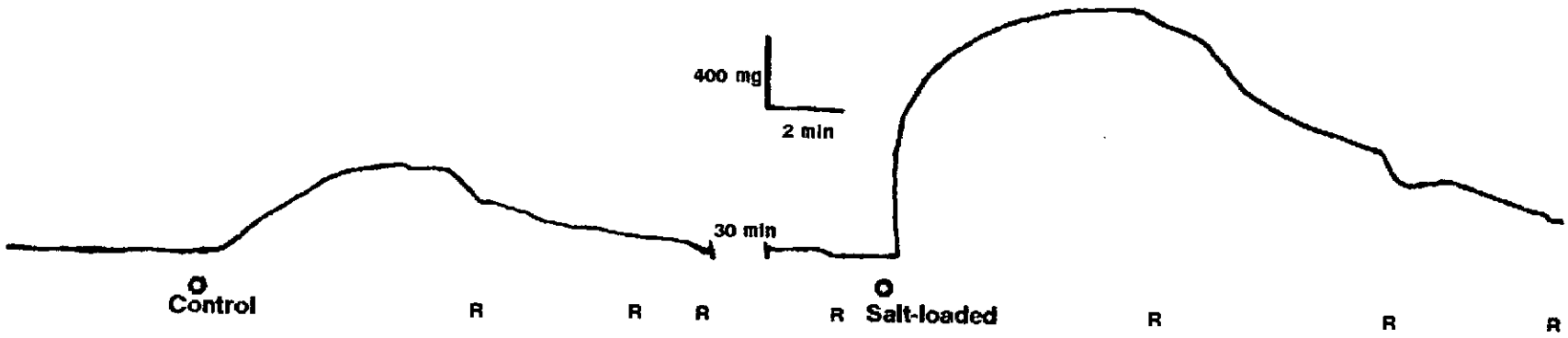

400 mg
2 min
30 min
Control
R
R
R
R
Salt-loaded
R
R
R

Figure 18. Contractile effects of serum samples from control and salt-loaded rats on an arterial ring of a normotensive rat. Serum effects were completely reversible by washout (R).

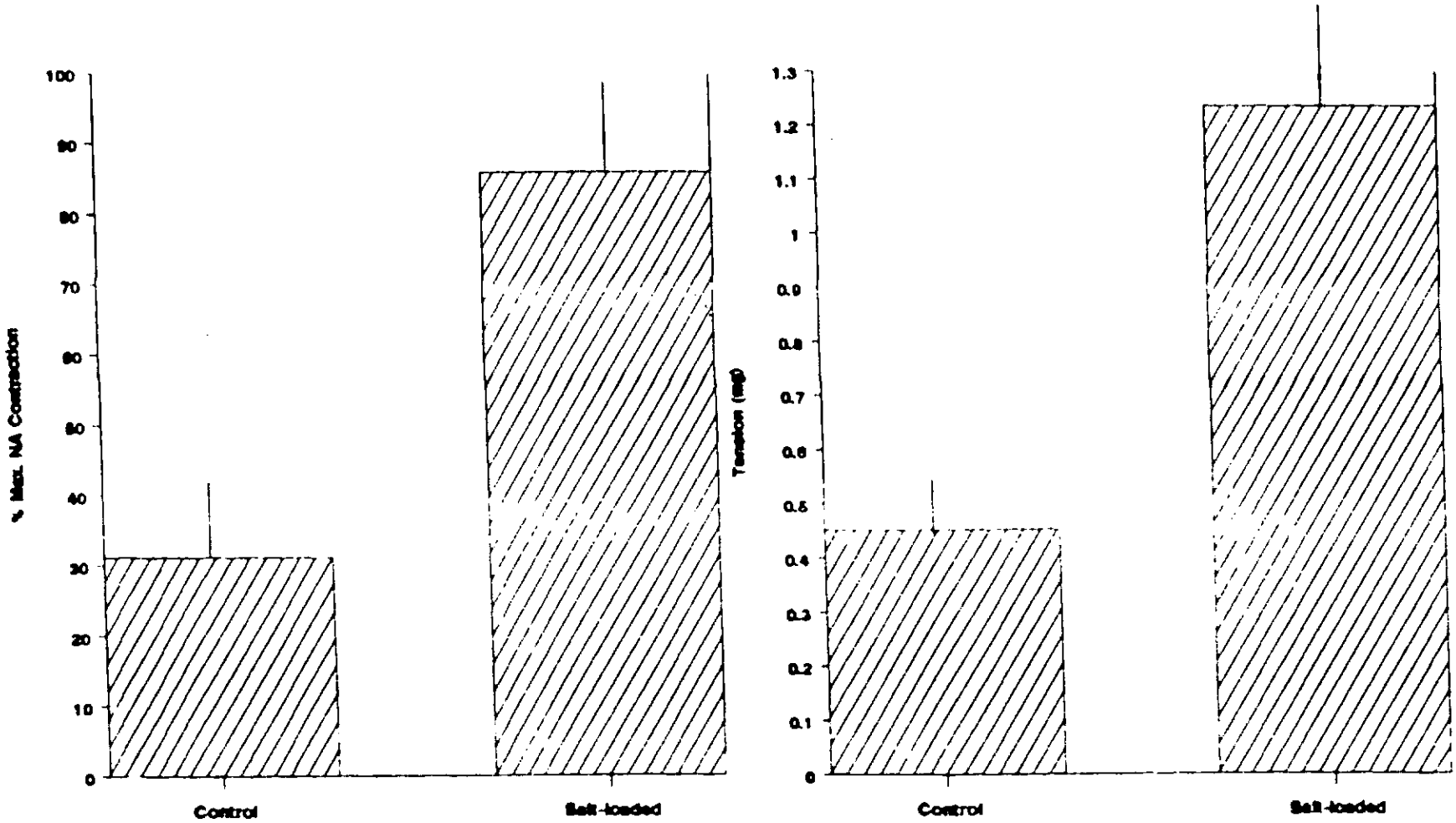

% Max. NA Contraction
100
90
80
70
60
50
40
30
20
10
0
Control
Salt-loaded
Tension (mg)
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Control
Salt-loaded

Figure 19. Maximal contractile responses of normotensive rat aortic rings to serum samples from control and salt-loaded rats.

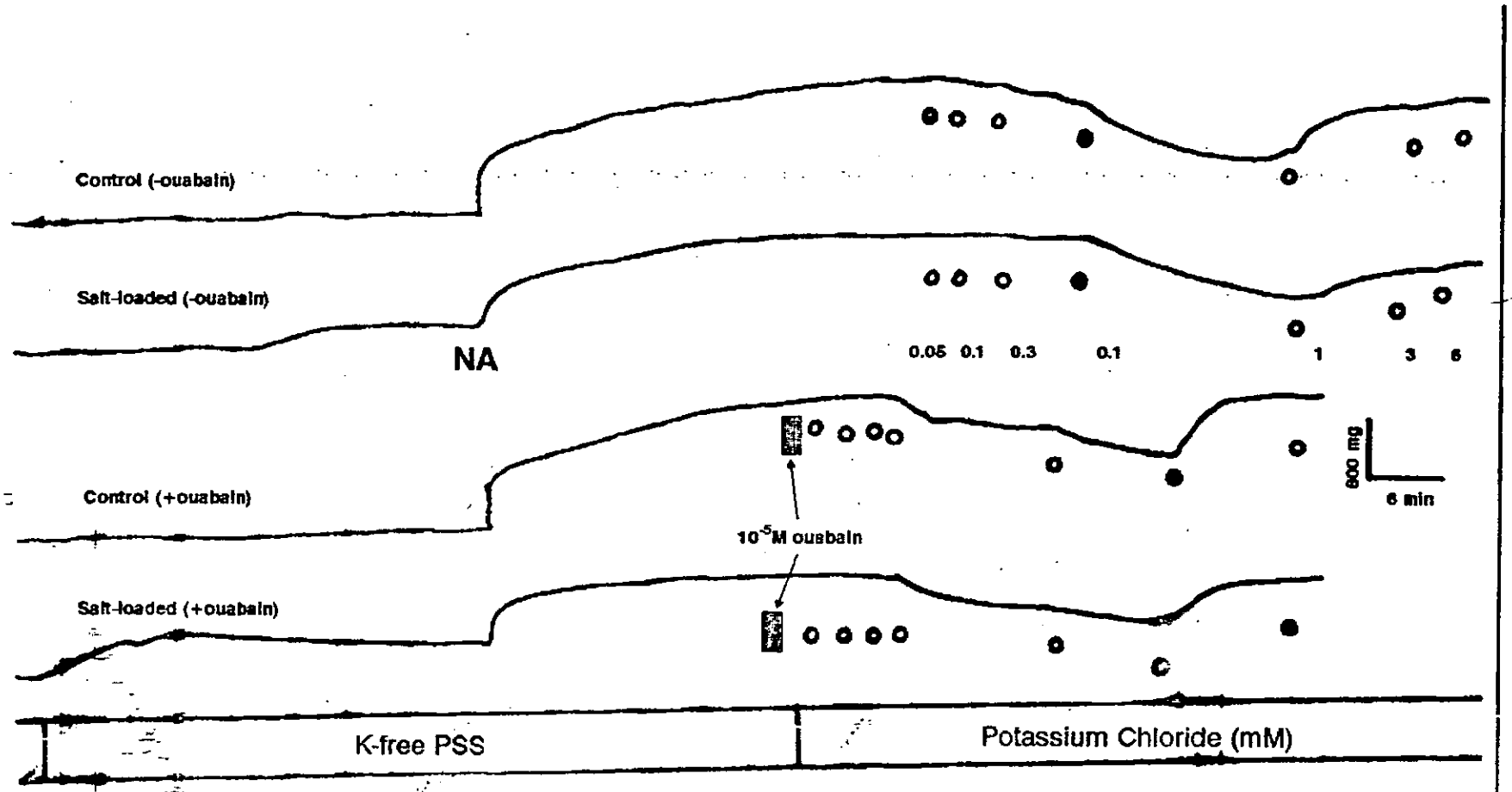

Control (-ouabain)
Salt-loaded (-ouabain)
NA
0.05 0.1 0.3 0.1 1 3 6
Control (+ouabain)
10^{-5}M ousbain
800 mg
6 min
Salt-loaded (+ouabain)
K-free PSS
Potassium Chloride (mM)

Figure 20. Relaxation responses of aortic rings from control and salt-loaded rats to added KCl following contraction with 10^{-7}M NA in K- free PSS. Experiments were performed with or without 10^{-5}M ouabain. Rings from salt- loaded rats developed tension in K-free PSS. R denotes washout.

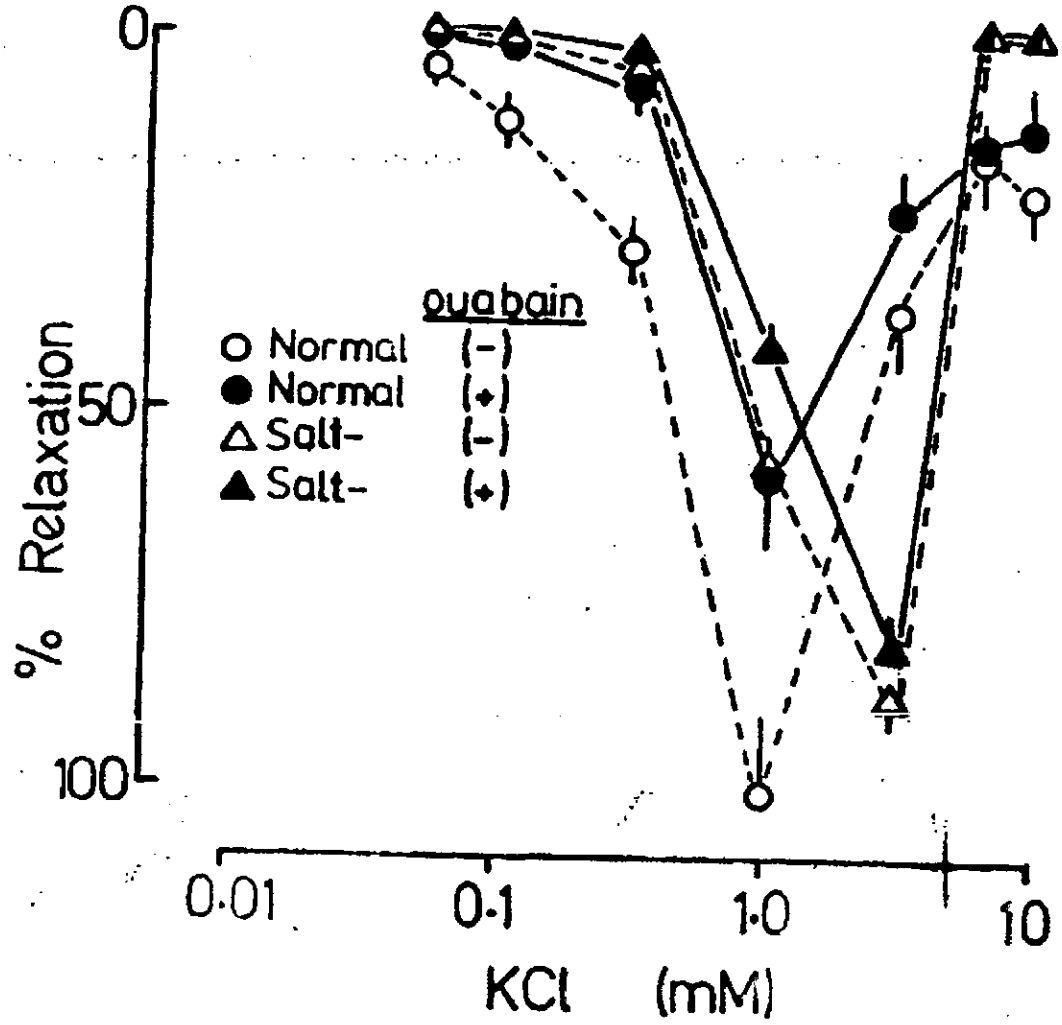

0
50
100
% Relaxation
ouabain
Normal (-)
Normal (+)
Salt- (-)
Salt- (+)
0.01
0.1
1.0
10
KCl (mM)

Figure 21. K^+-induced relaxation following cumulative addition of KCl to aortic rings from control and salt-loaded rats exposed to K-free medium, and in the presence (+) or absence (-) of 10^{-5}M ouabain.

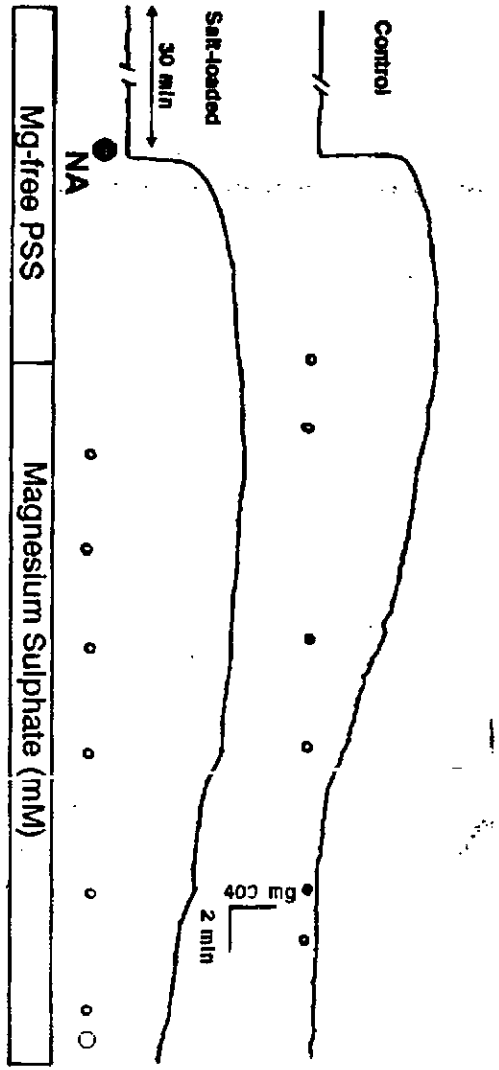
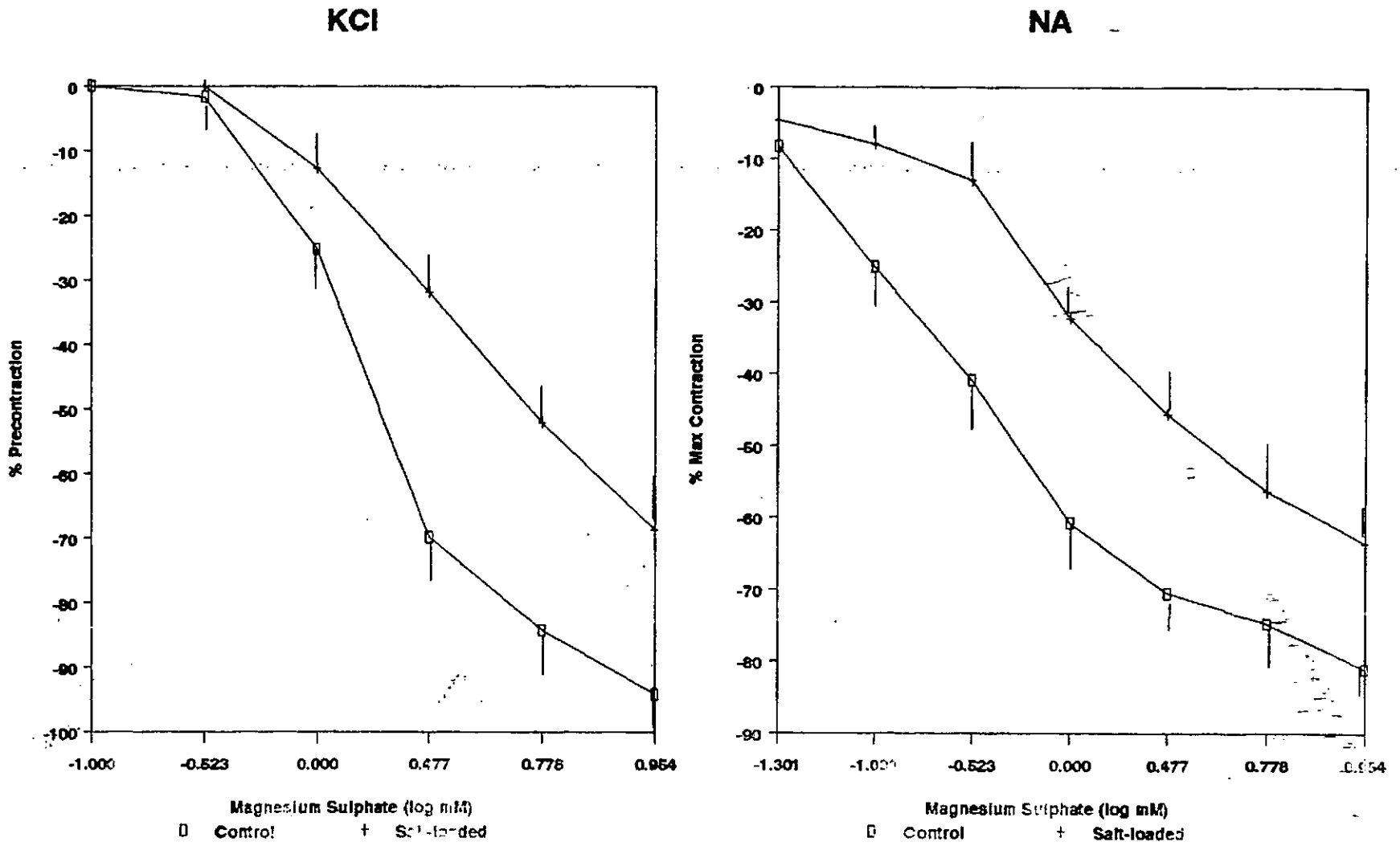

Control
Salt-loaded
30 min
NA
Mg-free PSS
Magnesium Sulphate (mM)
400 mg
2 min

Figure 22. Relaxation responses of aortic rings from control and salt-loaded rats to added $MgSO_4$ following contraction to 10^{-7}M noradrenaline.


KCl
% Precontraction
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-1.000
-0.523
0.000
0.477
0.778
0.954
Magnesium Sulphate (log mM)
Control
Salt-loaded
NA
% Max Contraction
-1.301
-0.523
0.000
0.477
0.778
Magnesium Sulphate (log mM)
Control
Salt-loaded

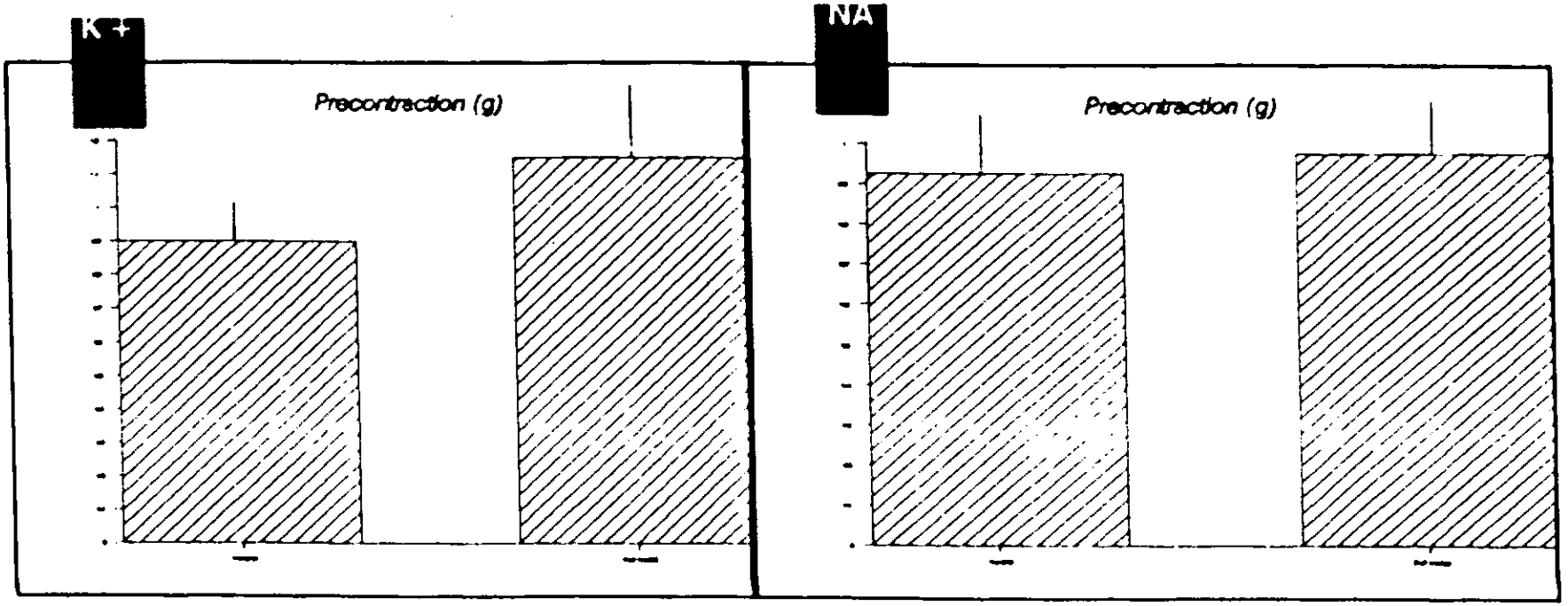
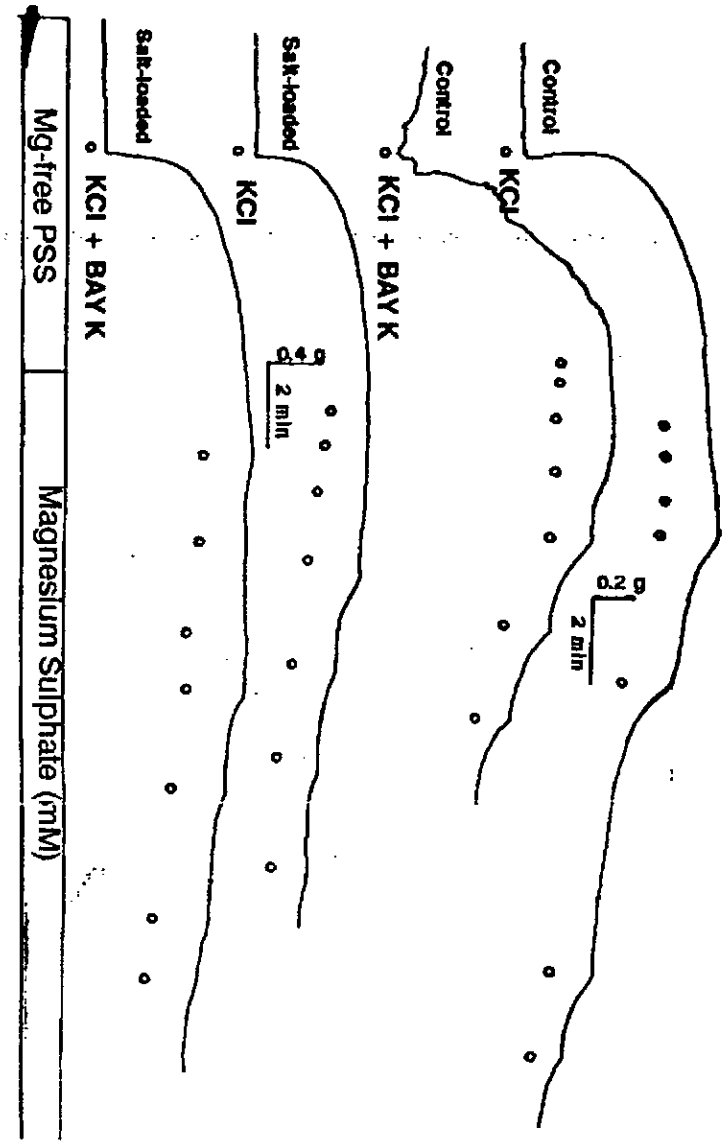


Figure 23. Mg^{2+}-induced relaxation of 40mM K^+- of 10^{-7}M NA-induced contractions, following cumulative addition of $MgSO_4$ to aortic rings from control and salt-loaded rats exposed to Mg-free medium. Precontractions (bars) are shown as insets.



MgSO4 (mM) = 0.05, 0.1, 0.3, 1, 3, 6, 9

Figure 24. Attenuation of magnesium-induced relaxation of $[K^+]_o$-contractions by 4 x 10^{-8}M Bay K8644 in control and salt-loaded rat aortic rings.

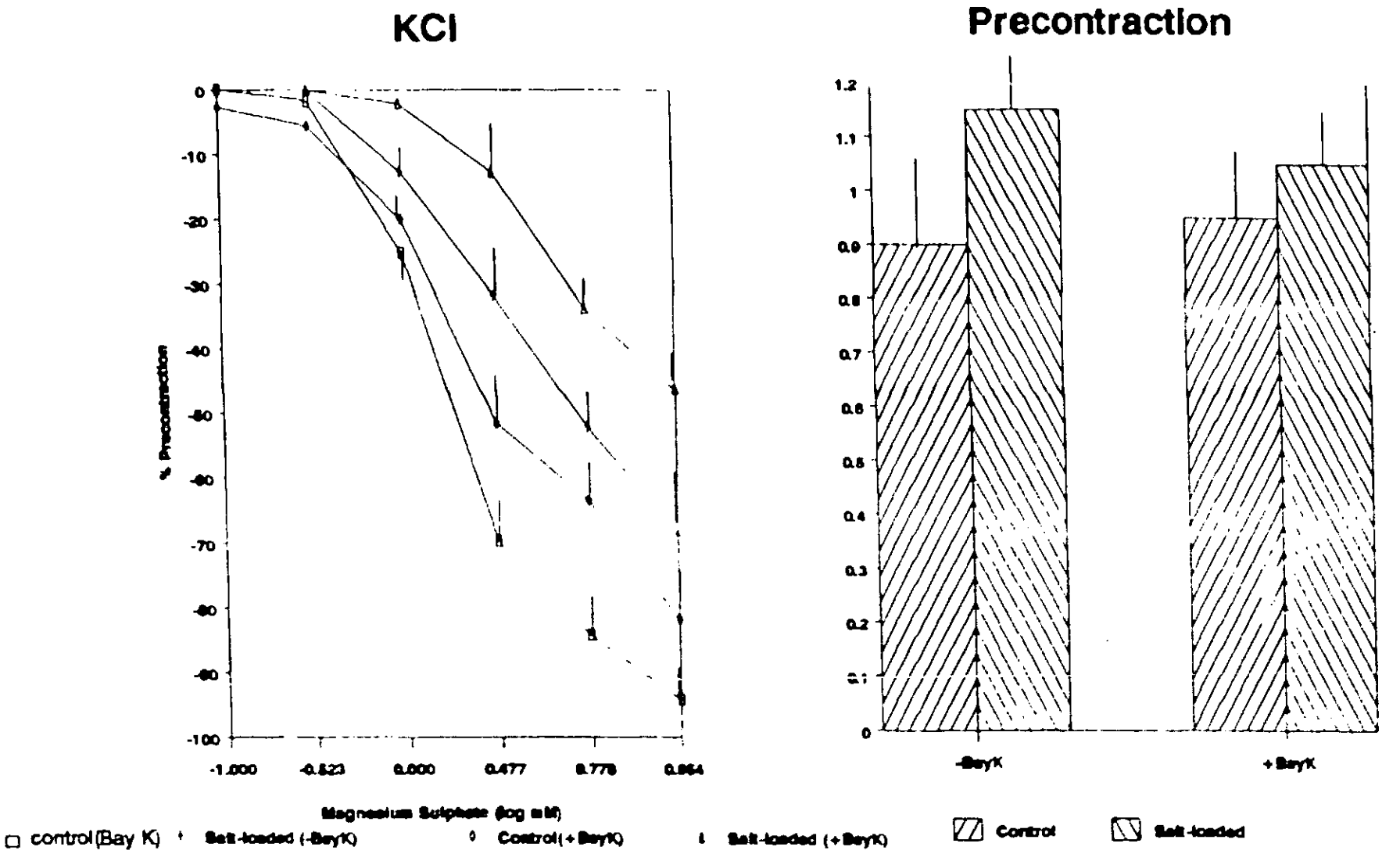

KCl
Precontraction
% Precontraction
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-1.000
-0.523
0.000
0.477
0.778
0.954
Magnesium Sulphate (log mM)
control (Bay K)
Salt-loaded (-Bay K)
Control (+ Bay K)
Salt-loaded (+ Bay K)
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-Bay K
+ Bay K
Control
Salt-loaded

Figure 25. Effect of 4 x 10^{-8}M Bay K8644 on $[Mg^{2+}]_o$- induced relaxation of 40mM KCl contractions of aortic rings from control and salt- loaded rats. Note that addition of Bay K8644 did not affect the magnitude of $[K^+]_o$- induced precontractions (bars, ANOVA).

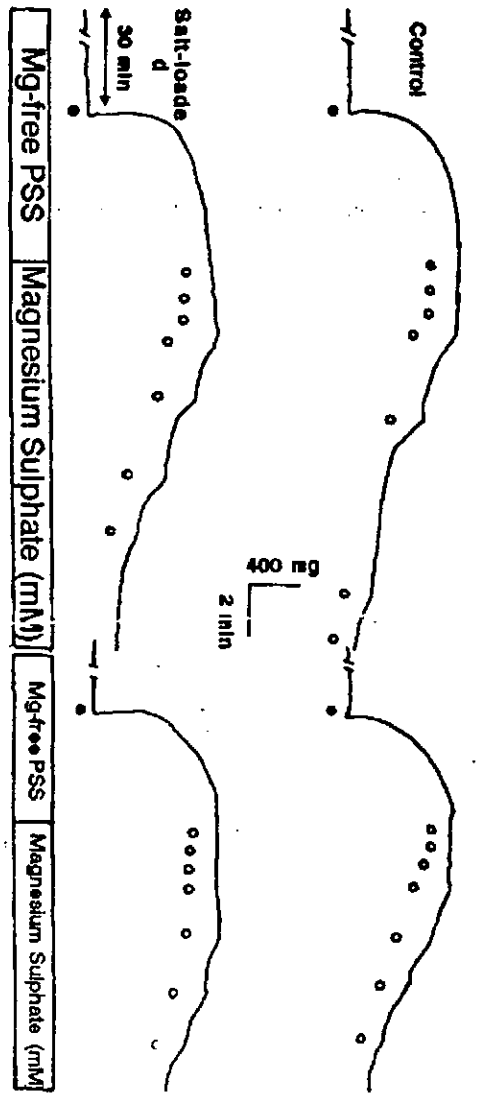

Control
Salt-loaded
30 min
400 mg
2 min
Mg-free PSS
Magnesium Sulphate (mM)
Mg-free PSS
Magnesium Sulphate (mM)
MgSO4 (mM) = 0.05, 0.1, 0.3, 1, 3, 6, 9

Figure 26. Attenuation of magnesium-induced relaxation of $[K^+]_o$-contraction by 4 x 10^{-5}M CGP 28392 in control and salt-loaded rat aortic rings.

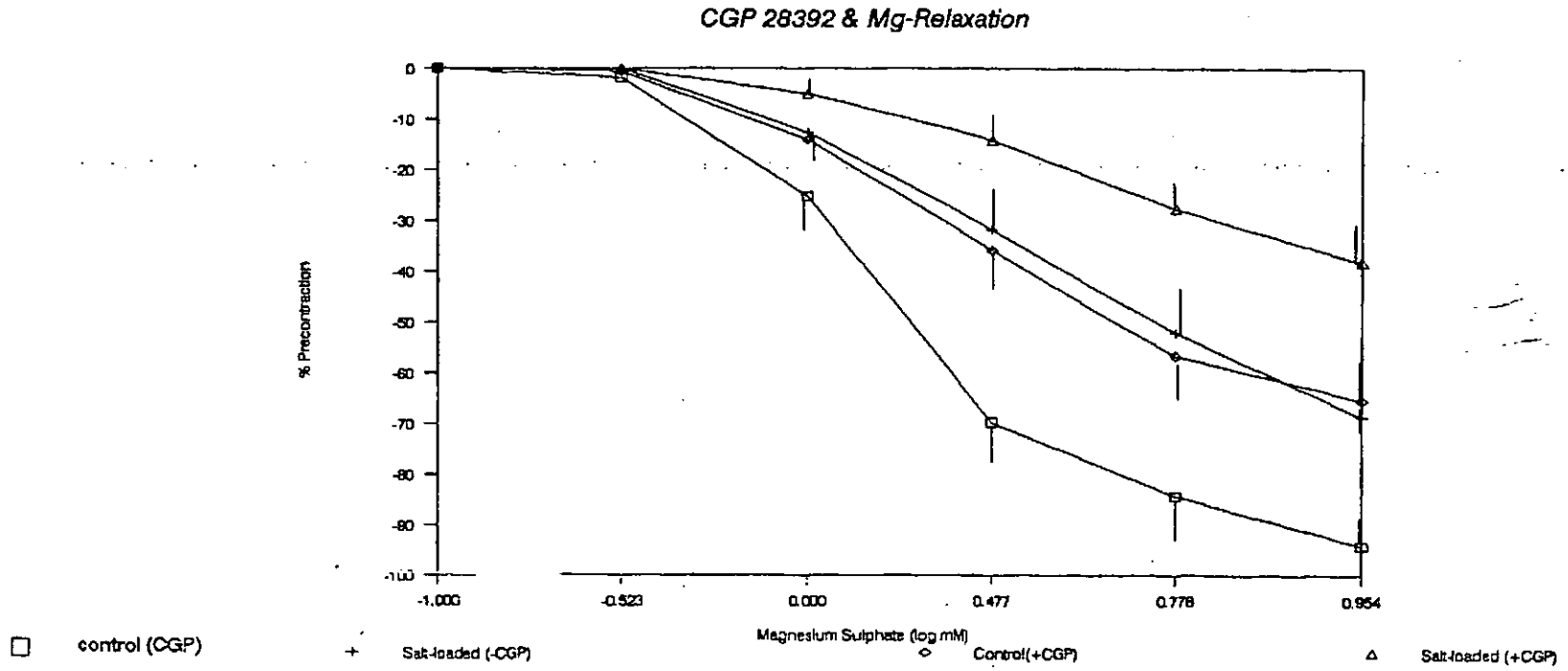


Figure 27. Effect of 4 x 10^{-5}M CGP 28392 on $[Mg^{2+}]_o$- induced relaxation of 40mM KCl contractions of aortic rings from control and salt- loaded rats (Legends are as in Figure 28).

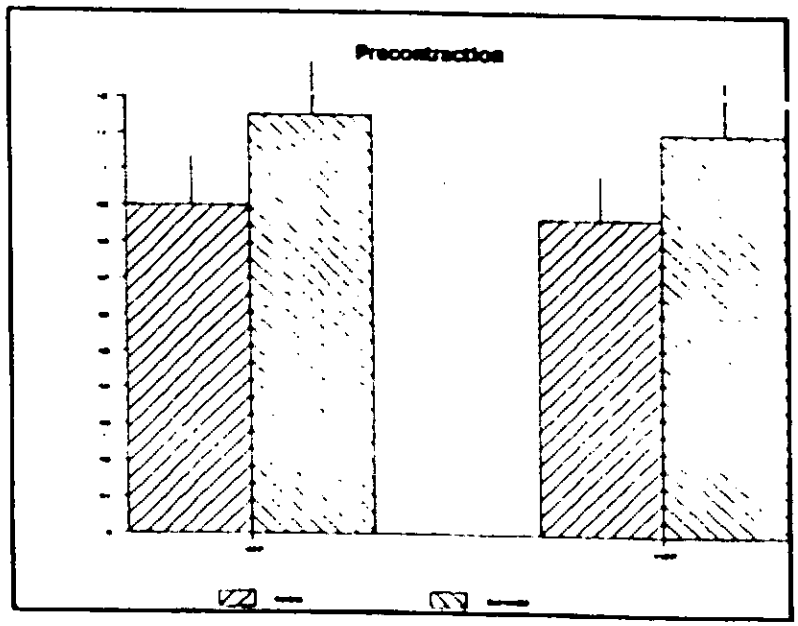
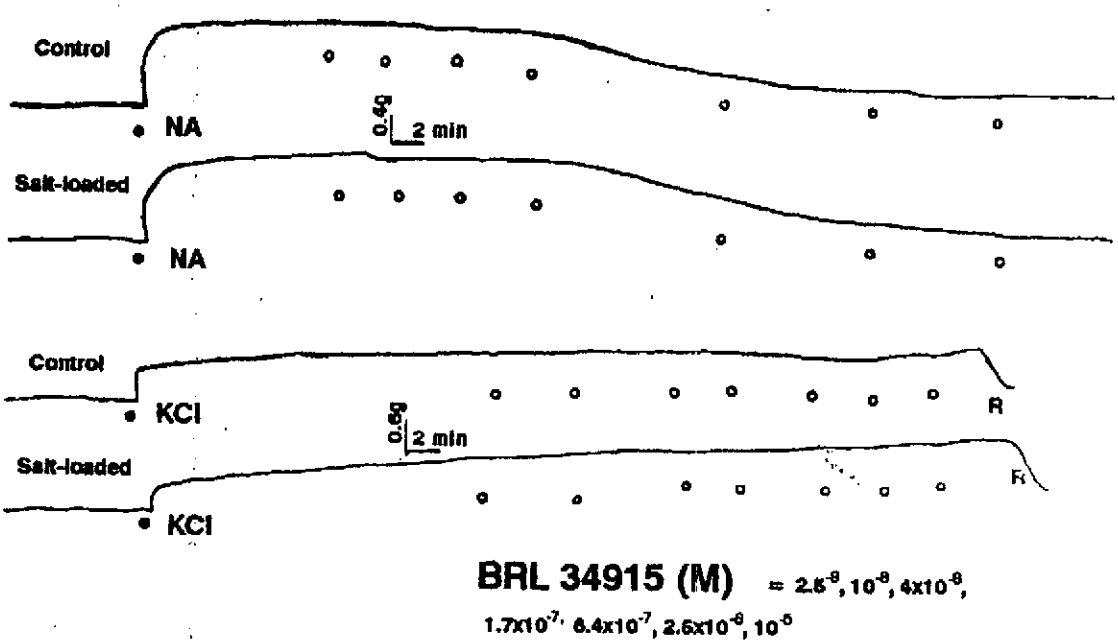
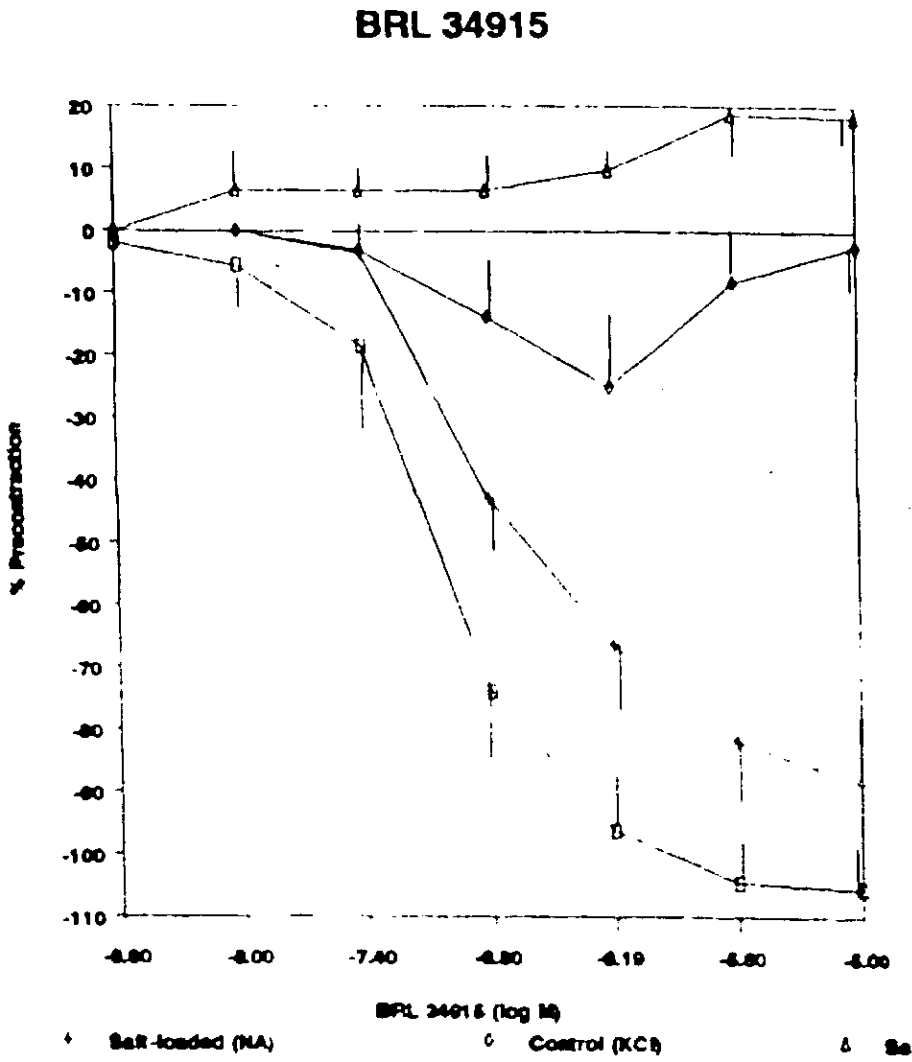


Figure 28. $[K^+]_o$-induced contractions of aortic rings from control and salt-loaded rat aortic rings in the absence and presence of 4×10^{-5}M CGP 28392. Note that addition of CGP 28392 did not significantly significantly alter the magnitude of $[K^+]_o$-induced contractions (ANOVA).



BRL 34915 (M) = 2.5^{-9}, 10^{-8}, $4x10^{-8}$, $1.7x10^{-7}$, $6.4x10^{-7}$, $2.5x10^{-6}$, 10^{-5}

Figure 29. Relaxation responses, of aortic rings from control and salt-loaded rats, to BRL 34915 following 10^{-7}M NA- and 60mM K^+_o-induced contractions. R indicates washout.


BRL 34915
% Precontraction
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-8.80
-8.00
-7.40
-6.80
-6.19
-5.60
-5.00
BRL 34915 (log M)
Control (NA)
Salt-loaded (NA)
Control (KCl)
Salt-loaded (KCl)

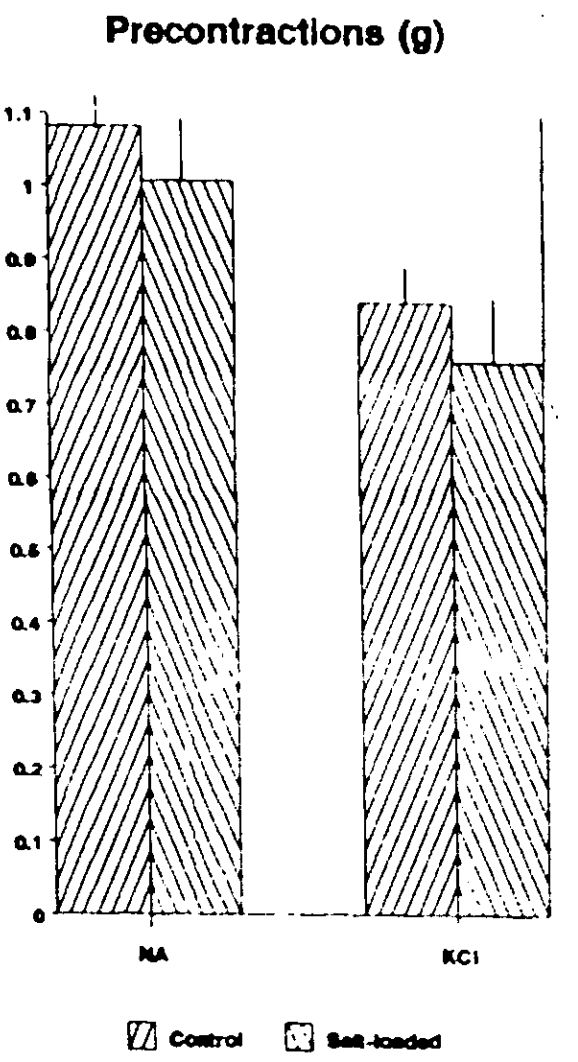
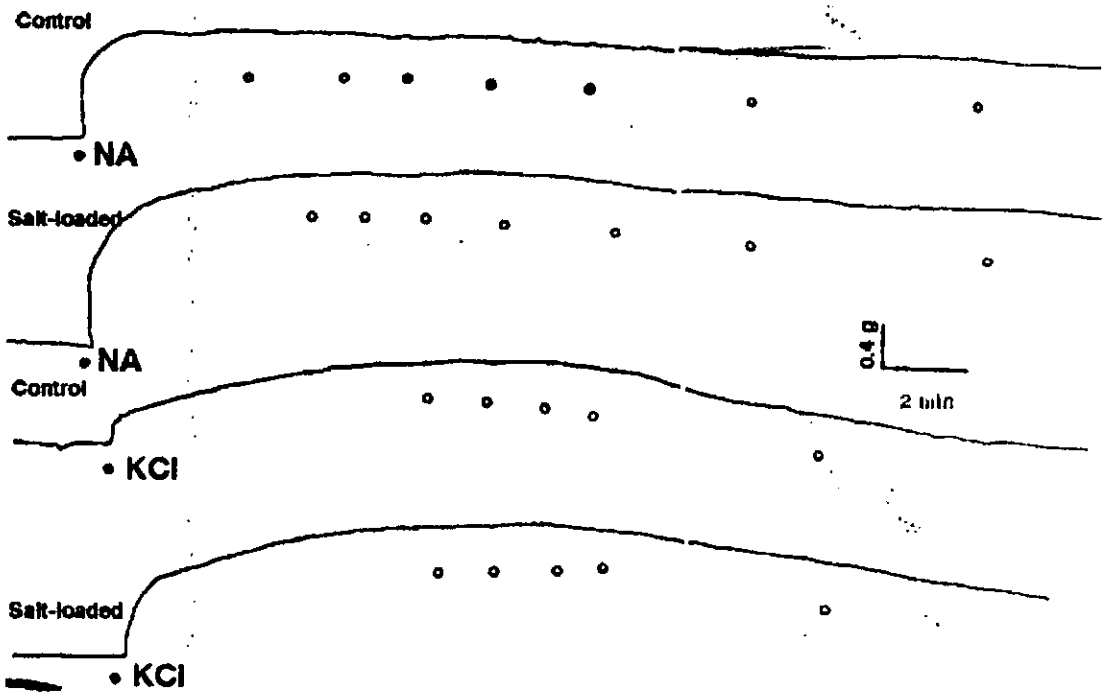

Precontractions (g)
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
NA
KCl
Control
Salt-loaded

Figure 30. Responses of 10^{-7}M NA- and 60mM K^+_o- contracted aortic rings from control and salt-loaded rats to cumulative addition of BRL34915. Precontractions are inset as bars.



DILTIAZEM (M) = 2.5^{-9}, 10^{-8}, $4x10^{-8}$, $1.7x10^{-7}$, $6.4x10^{-7}$, $2.5x10^{-6}$, 10^{-5}

Figure 31. Relaxation responses of aortic rings from control and salt-loaded rats to diltiazem following 10^{-7}M NA- and 60mM K^+-induced contractions.

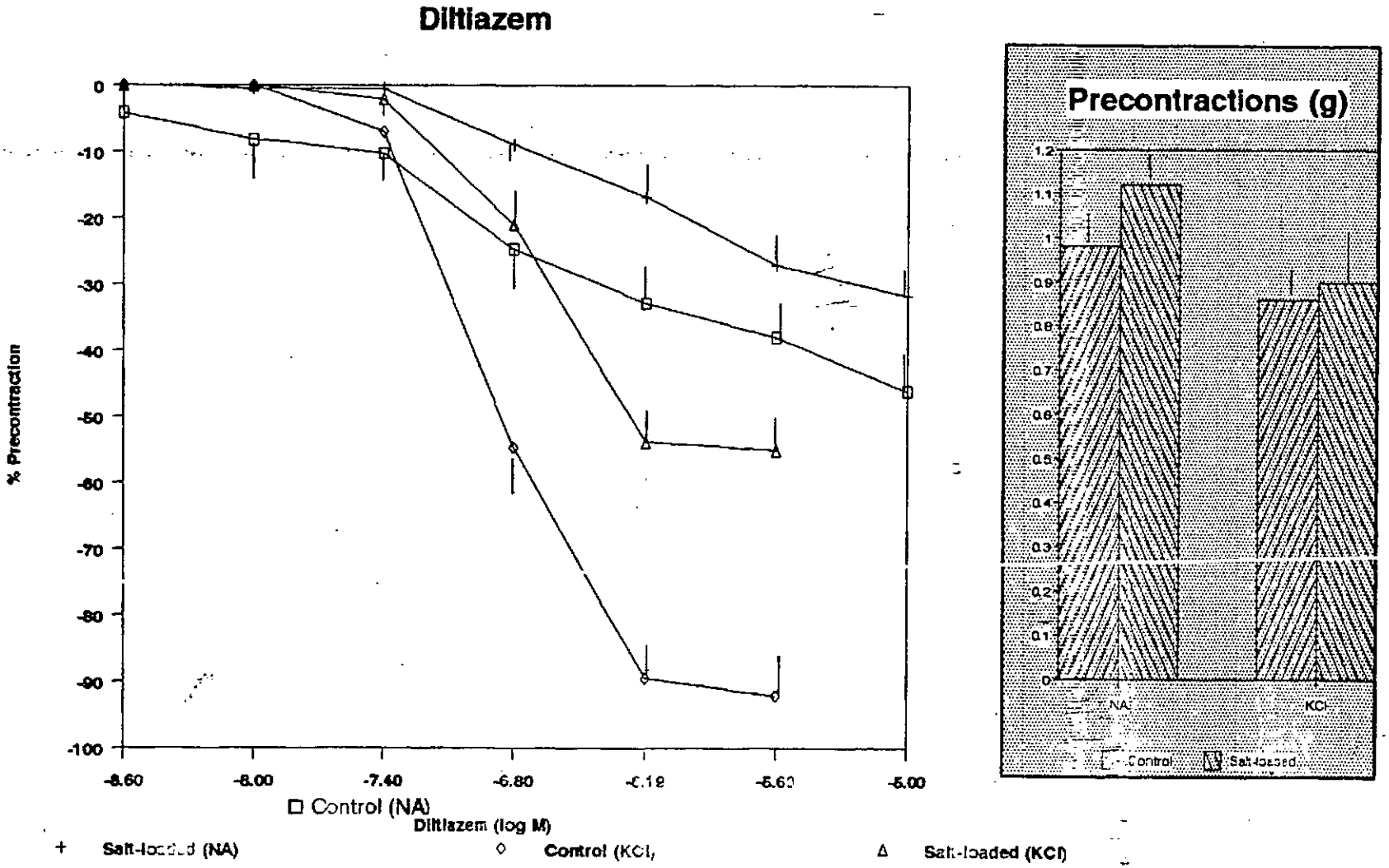

Diltiazem
% Precontraction
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-8.60
-8.00
-7.40
-6.80
-6.20
-5.60
-5.00
Diltiazem (log M)
□ Control (NA)
+ Salt-loaded (NA)
◇ Control (KCl)
△ Salt-loaded (KCl)
Precontractions (g)
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
NA
KCl
Control
Salt-loaded

Figure 32. Relaxation responses of 10^{-7}M NA- and 60mM K^+-contracted aortic rings from control and salt-loaded rats to cumulative addition of diltiazem. Precontractions are inset as bars.

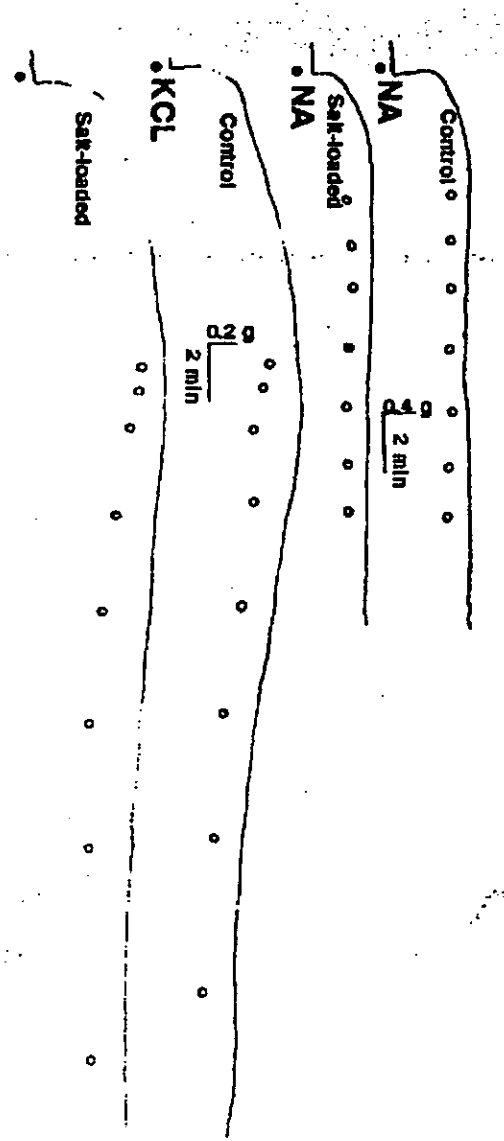
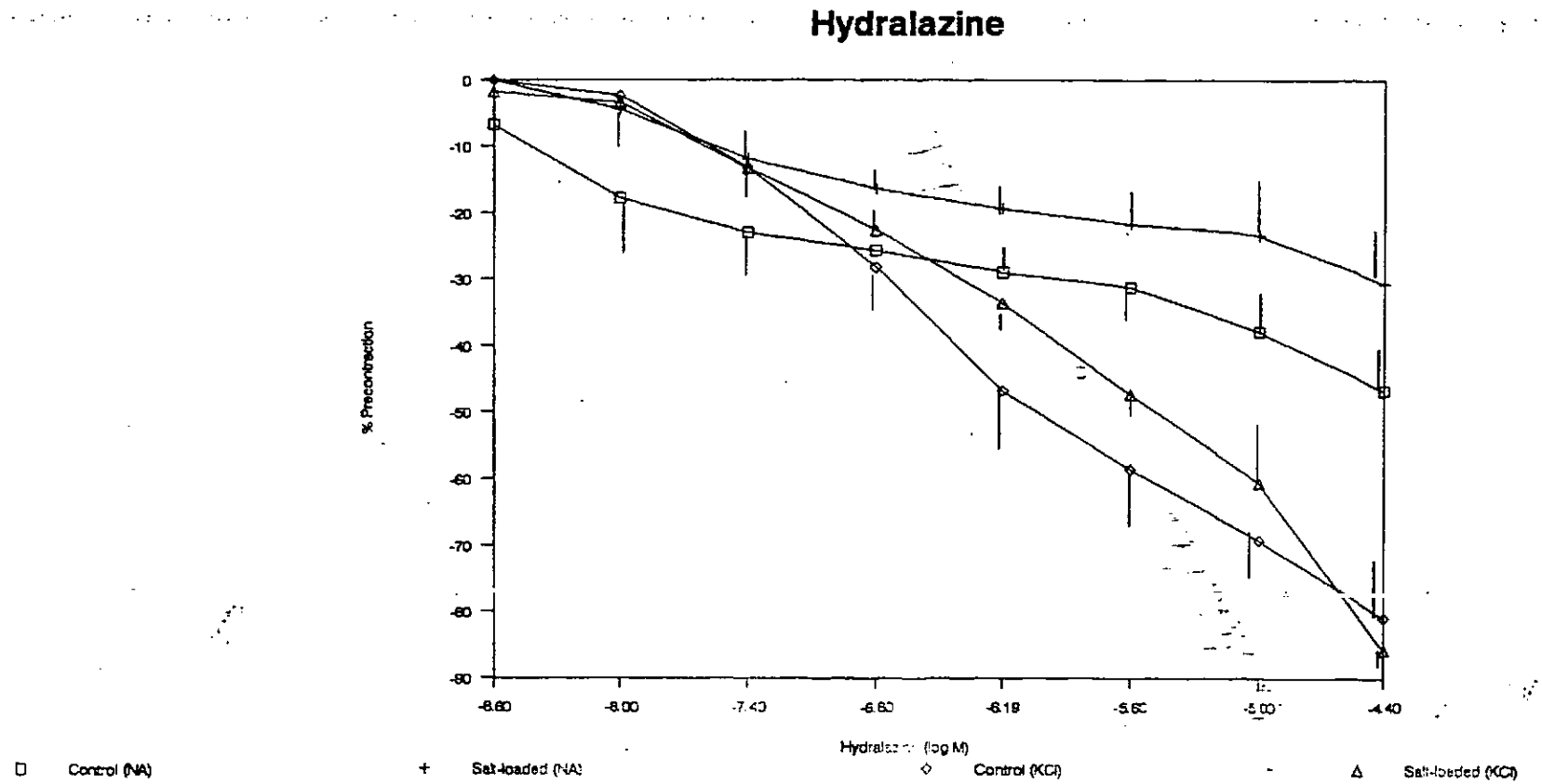

Control
NA
d1
2 min
Salt-loaded
NA
d2
2 min
Control
KCL
Salt-loaded
HYDRALAZINE (M) = 2.5^{-9}, 10^{-8}, 4x10^{-8},
1.7x10^{-7}, 6.4x10^{-7}, 2.5x10^{-6}, 10^{-5}, 4x1-$^{-5}$

Figure 33. Relaxation responses of aortic rings from control and salt-loaded rats to hydralazine following 10^{-7}M NA- and 60mM K^+-induced contractions.


Hydralazine
% Precontraction
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-8.60
-8.00
-7.40
-6.80
-6.19
-5.60
-5.00
-4.40
Hydralazine (log M)
Control (NA)
Salt-loaded (NA)
Control (KCl)
Salt-loaded (KCl)

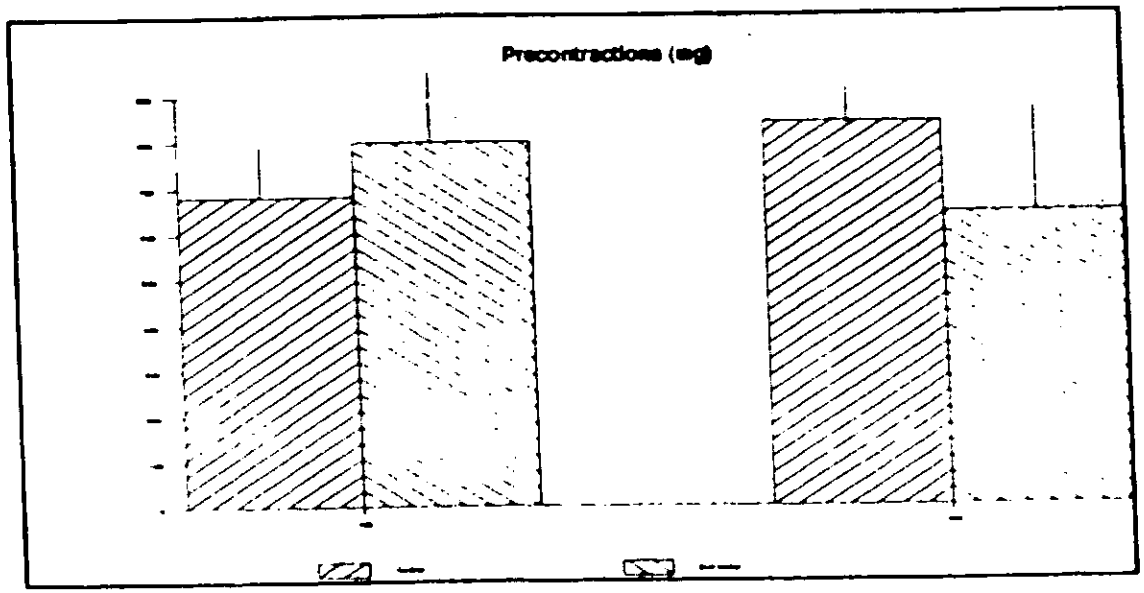
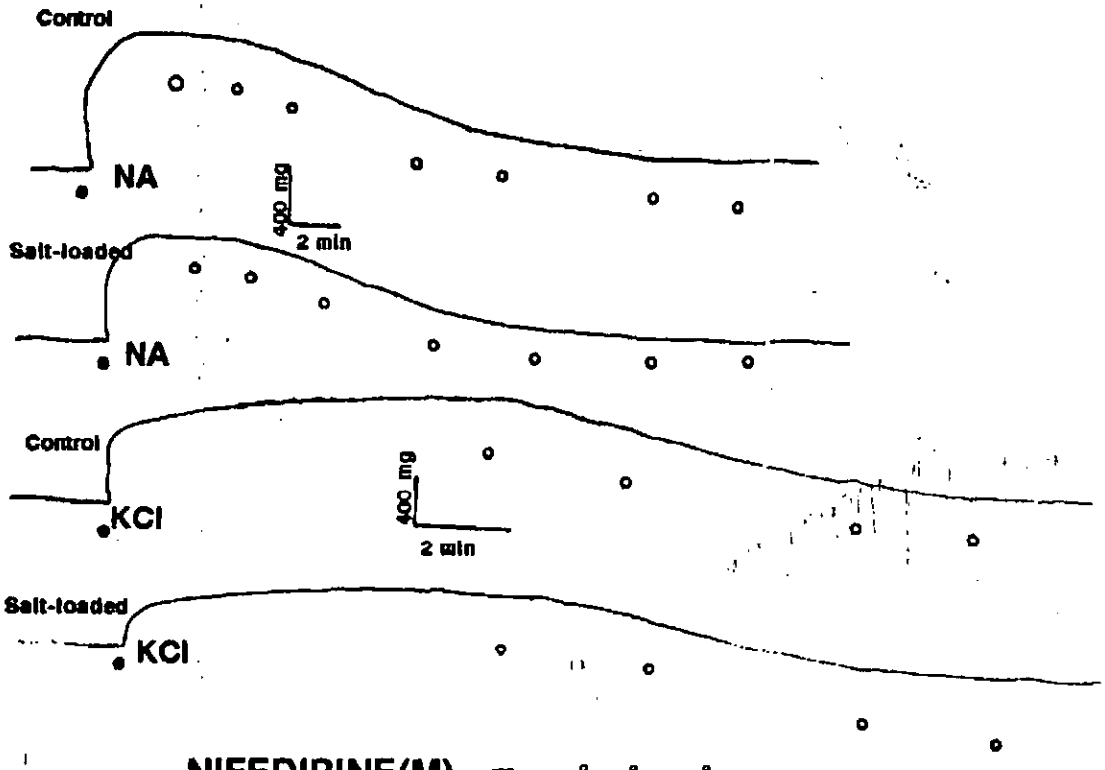


Figure 34. Relaxation responses of 10^{-7}M NA- and 60mM K^{+}-contracted aortic rings from control and salt-loaded rats to cumulative addition of hydralazine. Precontractions are inset as bars.



NIFEDIPINE(M) = $2.5^{-9}, 10^{-8}, 4x10^{-8}, 1.7x10^{-7}, 6.4x10^{-7}, 2.5x10^{-6}, 10^{-5}$

Figure 35. Relaxation of aortic rings from control and salt-loaded rats to nifedipine following 10^{-7}M NA- and 60mM K^+-induced contractions.

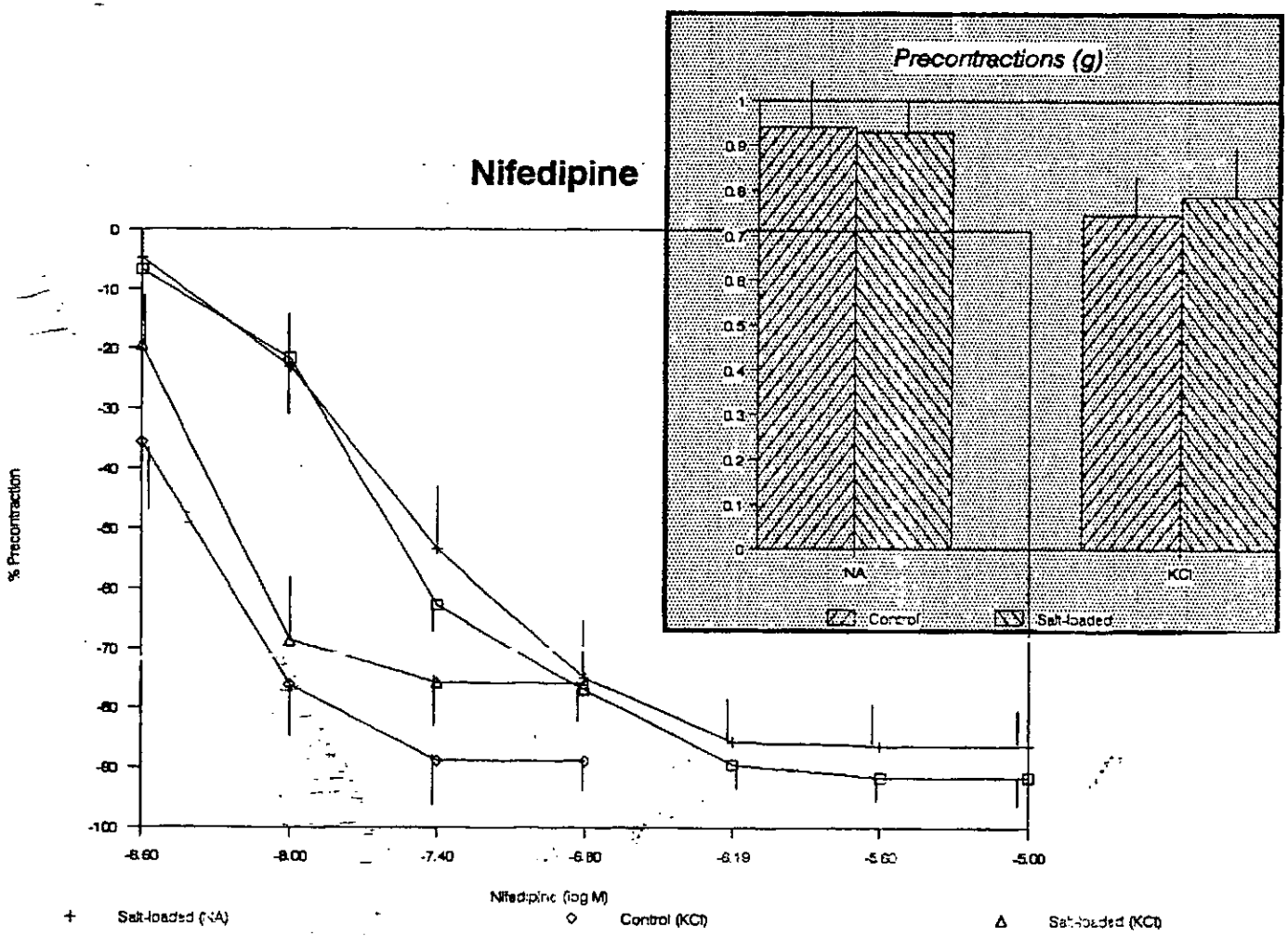

Nifedipine
Precontractions (g)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
NA
KCl
Control
Salt-loaded
% Precontraction
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-8.60
-8.00
-7.40
-6.80
-6.19
-5.60
-5.00
Nifedipine (log M)
Control (NA)
Salt-loaded (NA)
Control (KCl)
Salt-loaded (KCl)

Figure 36. Relaxation responses of 10^{-7}M NA- and 60mM K^{+}-contracted aortic rings from control and salt-loaded rats to cumulative addition of nifedipine. Precontractions are inset as bars.

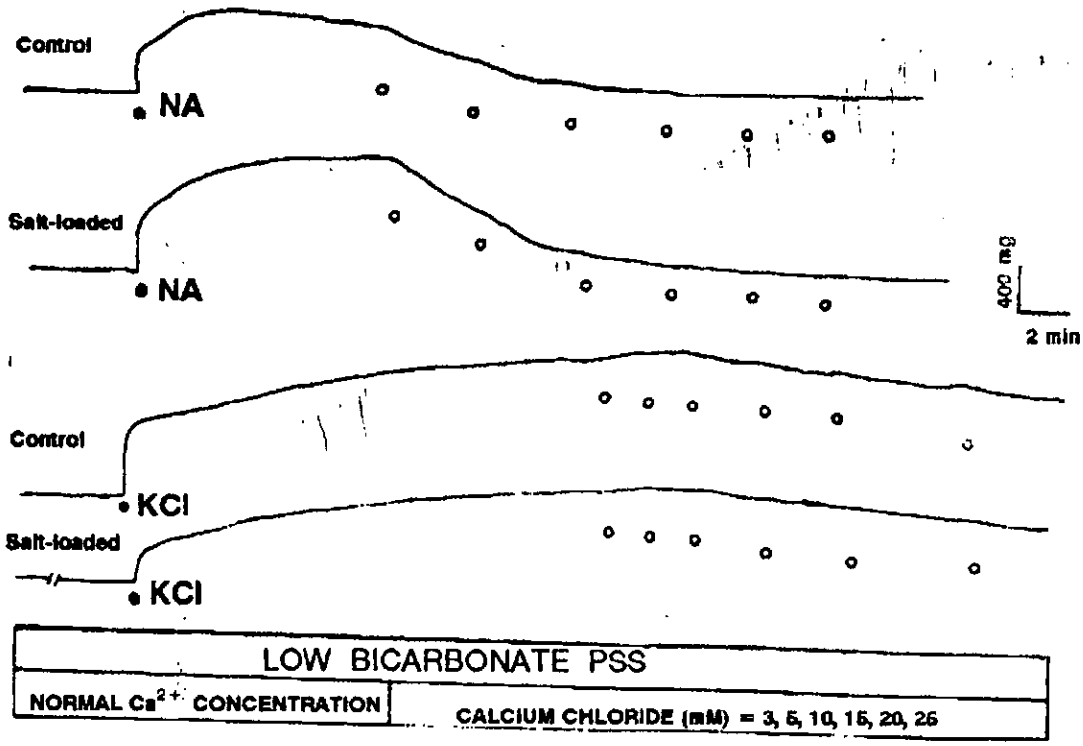

Control
NA
Salt-loaded
NA
400 mg
2 min
Control
KCl
Salt-loaded
KCl
LOW BICARBONATE PSS
NORMAL Ca2+ CONCENTRATION
CALCIUM CHLORIDE (mM) = 3, 5, 10, 15, 20, 26

Figure 37. Relaxant effect of raised $[Ca^{2+}]_o$ on 10^{-7}M NA- and 60mM K^+-induced contractions of aortic rings from control and salt-loaded rats.

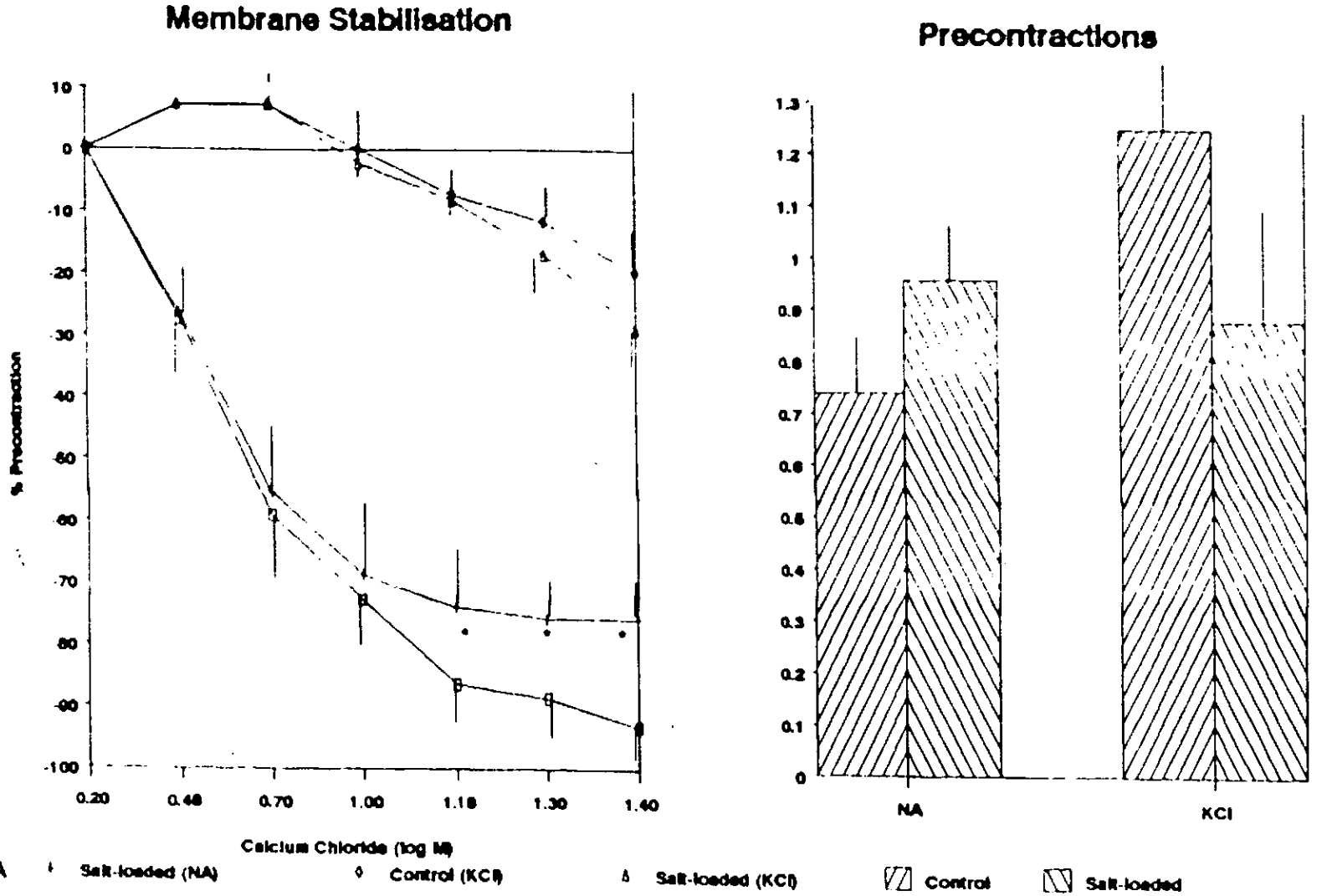

Membrane Stabilisation
% Precontraction
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
0.20
0.46
0.70
1.00
1.18
1.30
1.40
Calcium Chloride (log M)
[] control NA
Salt-loaded (NA)
Control (KCl)
Salt-loaded (KCl)
Precontractions
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
NA
KCl
Control
Salt-loaded

*Figure 38. Responses of 10^{-7}M NA- and 60mM K^+- contracted aortic rings from control and salt-loaded rats to increased $[Ca^{2+}]_o$ in a low-bicarbonate medium. Precontractions are inset as bars. *denote significant difference from control.*

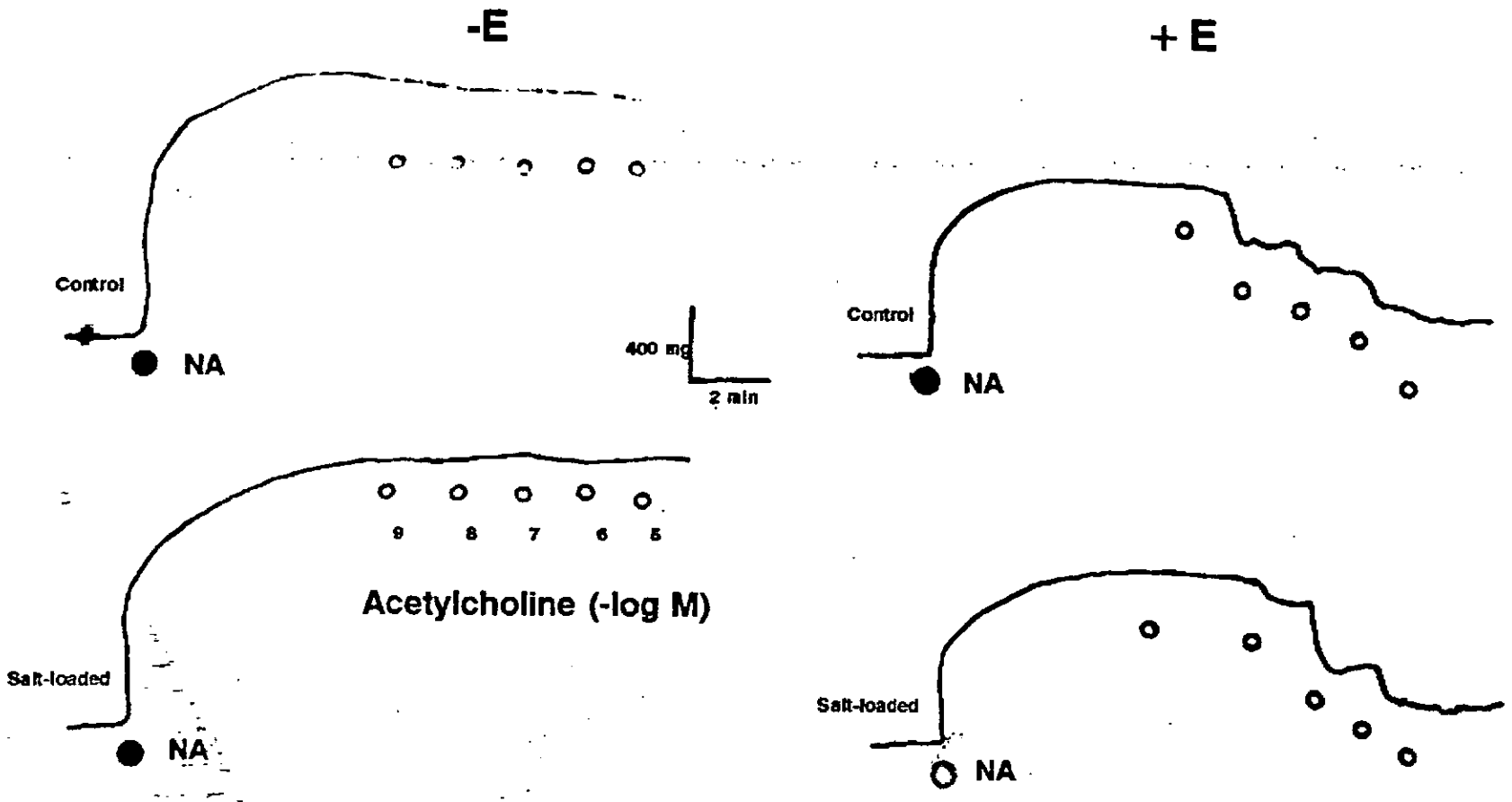

-E
+E
Control
NA
400 mg
2 min
Control
NA
9
8
7
6
5
Acetylcholine (-log M)
Salt-loaded
NA
Salt-loaded
NA

Figure 39. Endothelium-dependent relaxation, to acetylcholine, of 10^{-7}M NA contractions of control and salt-loaded rat aortic rings. +E and -E denote intact and rubbed rings respectively.

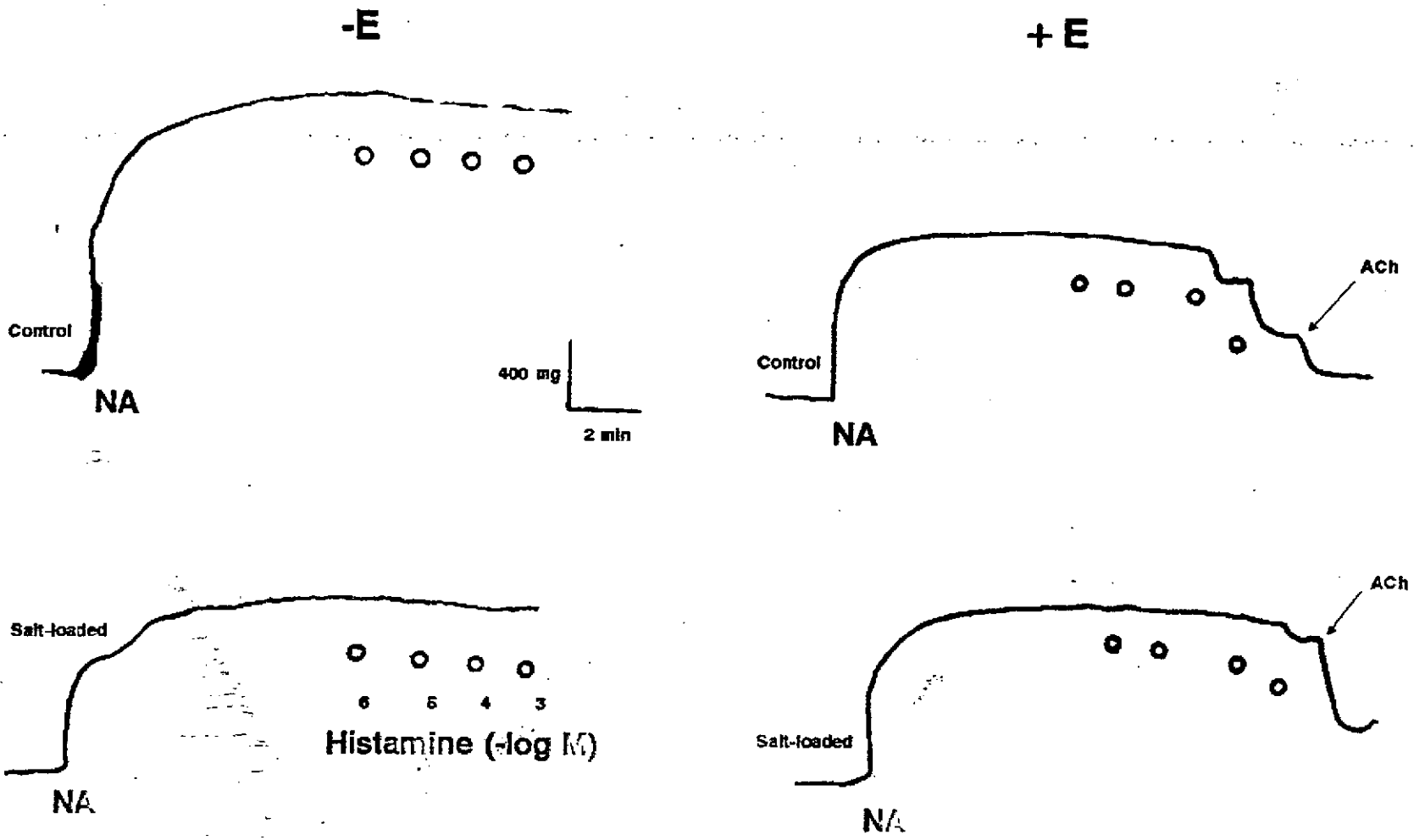

-E
+E
Control
NA
400 mg
2 min
ACh
Control
NA
Salt-loaded
6
5
4
3
Histamine (-log M)
NA
ACh
Salt-loaded
NA

Figure 40. Endothelium-dependent relaxation, to histamine, of 10^{-7}M NA contractions of control and salt-loaded rat aortic rings. +E and -E denote intact and rubbed rings respectively. ACh was applied to tissues which responded poorly to histamine to confirm that the endothelium was not mechanically damaged.

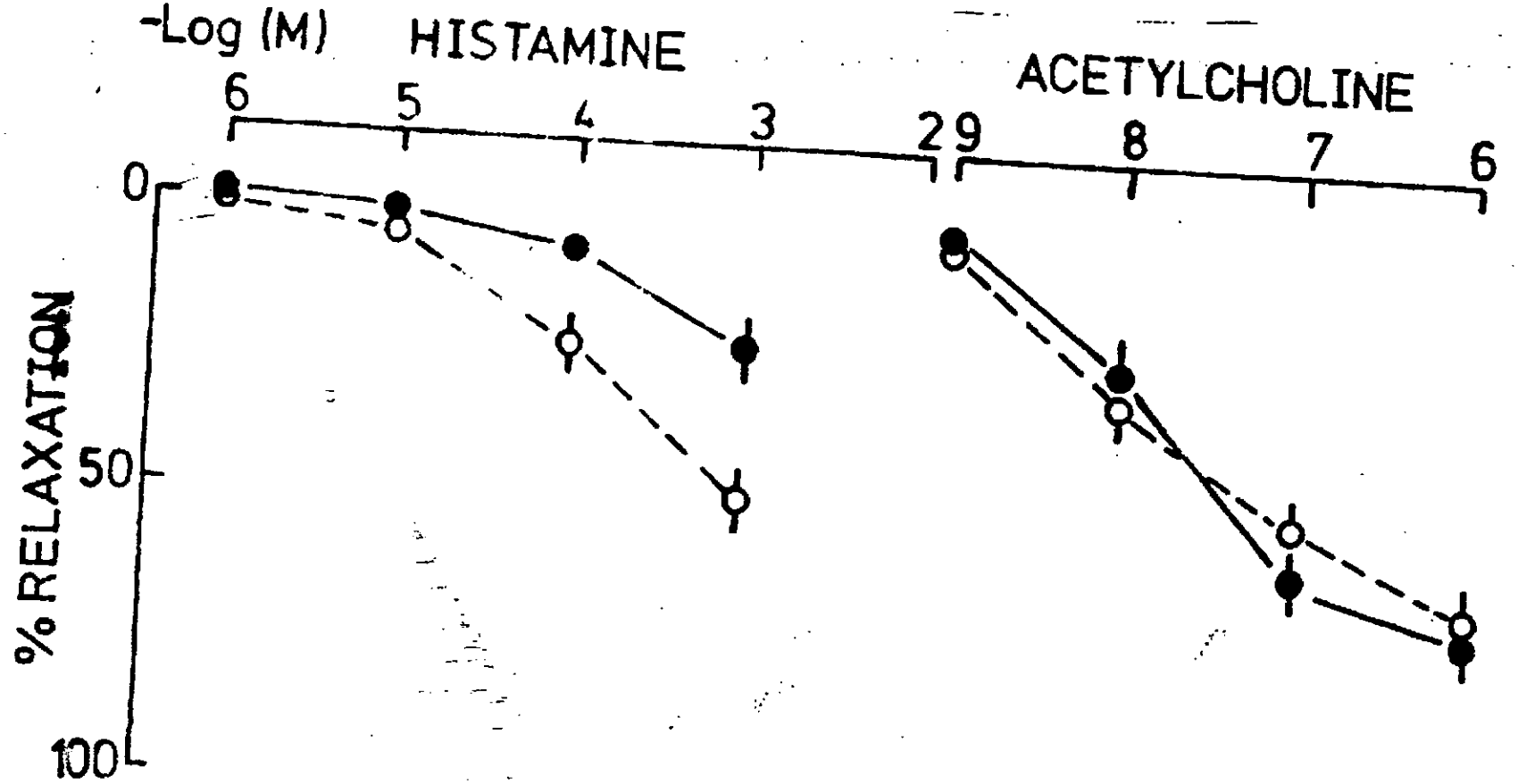

-Log (M)
HISTAMINE
ACETYLCHOLINE
6
5
4
3
2
9
8
7
6
0
50
100
% RELAXATION

Figure 41. Magnitude of relaxation in response to cumulative increases in concentration of histamine (left) and ACh (right) in aortic rings from control (open circles) and salt- loaded (filled circles) rats. Note the attenuated histamine-relaxation in the salt group.

CHAPTER 4
DISCUSSION

4.1. VALIDATION OF METHODOLOGY

4.1.1. SALT LOADING

Sprague-Dawley rats of either sex were used in our experiments. Various strains of rat have been used in studies relating salt-loading with hypertension. The most commonly used in recent times is the Dahl rat (Luft, 1989). Other commonly used strains are the Lyon, the Wistar and the Sprague-Dawley rats (Miyajima & Bunag, 1985; Dina *et al.*, 1986; Adigun & Akinyanjuola, 1989; Julien, Barres, Sacquet, Kandza, Cuisinaud, Vincent & Sassard, 1989; Nwaigwe & Sofola, 1989; Obiefuna *et al.*, 1991).

The Wistar and Sprague-Dawley rats have been most commonly used in our laboratories, mainly because of the ease of obtaining and maintaining them. The preference for Spraque-Dawley rats, in this study, is on the basis of reports that the Sprague-Dawley rats are more susceptible to salt- induced hypertension than Wistar rats (Torii, 1980; Miyajima & Bunag, 1985). Six-week-old rats were used because it is

easier to produce salt-induced hypertension in young rats than in adult ones (Luft, 1989).

The relative amount of salt required to induce hypertension in animals is, admittedly, far in excess of the usual content of human diets. The 8% salt diet fed to our rats and commonly used in salt- loading experiments (Maxwell & Waks, 1987), is equivalent to 40g/day sodium diet given to humans if compared on the basis of surface area or glomerular filtration rate (Luft, 1989). Although experimental salt-induced hypertension does not necessarily support a primary role for sodium in the pathogenesis of essential hypertension, the model may be applicable to a subset of genetically susceptible individuals.

The high NaCl content of the salt-diet did not appear to affect food consumption since both rat groups consumed similar quantities of food per unit body weight. The reduced quantity of chow consumed by rats in the salt group at the sixth week is likely to be due to significant weight loss. Miyajima & Bunag (1985) observed that salt-loaded Sprague- Dawley rats consumed less food and drank more water than the controls. This conclusion was made on the basis of food and water intake measured for each rat by these workers. Similar observations were made in these experiments but when food intake was corrected for body weight differences, consumption rate per unit weight was found to be similar for both control and salt-loaded rats.

The cause of the significant weight loss observed in the salt-rats in the sixth week of salt loading is not known. It may be related to the excessive diuresis observed in the salt-loaded rats (Miyajima & Bunag, 1985; Sofola, Obiefuna & Adesanya, 1991)

4.1.2. ANAESTHESIA

An anaesthetic mixture containing 1%(w/v) alpha- chloralose and 25% (w/v) urethane was used in this study. Urethane or chloralose when given separately could produce long-lasting anaesthesia (Hall, 1977; Ito & Feigl, 1985). However, chloralose when given alone causes spontaneous muscular contraction, which is suppressible by urethane (Hall, 1977). Also, alpha- chloralose has been shown to elevate heart rate (Hall, 1977) while urethane reduces both arterial pressure and heart rate (Hall, 1977; Osunkwo, Eferakeya & Moneke, 1989). A combination of both agents, on the other hand, has been shown to have a little or no effect on resting blood pressure and cardiovascular reflexes for about the first three hours of experimentation (Adigun & Fentem, 1984). However, during investigations that require prolonged anaesthesia and surgical trauma, acidaemia may result (Linden & Norman, 1969). All blood pressure measurements in our experiments were completed within the first hour of administration of the anaesthetic agent.

4.1.3. ISOLATED VESSEL EXPERIMENTS

Isolated blood vessels, either in the form of a whole or part of a vascular bed,or of ring or helical strip preparations, have been used in vascular smooth muscle studies for many years (MacWilliam, 1902; Cow, 1911; Cruickshank & Rau, 1927; Kohn, Levitsky, Strauss, Strauss & Neckeles, 1936; Furchgott & Bhadrakom, 1953; Waugh, 1962; Bevan, 1962; Rogers, Atkinson & Long, 1965; Uchida, Bohr & Hoobler, 1967; Detar & Bohr, 1968; Ebeigbe, 1979; Aloamaka, 1987; Obiefuna *et al.*, 1991).

Most *in vivo* vascular responses to vasoactive agents are modulated by one or more factors like temperature, and others. Experiments with isolated blood vessels, on the other hand, enable the identification of the precise effect of a particular agent without the overriding effects of numerous other variables.

The rat thoracic aorta was used in this study because of the ease of isolating and mounting it, and because a large number of similar experiments have been done using this tissue (Wright, 1981; Van De Vorde & Leusen, 1983; Mecca & Webb, 1984; Dong & Wadsworth, 1986; Bradlaugh, Bing, Swales & Thurston, 1987; Luscher, Vanhoutte & Raij, 1987b; Sim & Singh, 1987).

4.2. DISCUSSION OF RESULTS

4.2.1. BLOOD PRESSURE AND SERUM ELECTROLYTES

Sprague-Dawley rats are generally more susceptible to salt-induced hypertension than Wistar rats (Torii, 1980). In this study, six-week salt-loading induced a mild hypertension as well as hypokalaemia in the rats. The high-salt diet did not have any effect on terminal plasma sodium ion concentration nor on heart rate of the anaesthetised rats. These observations are comparable to the recent reports, on Wistar rats, of Nwaigwe & Sofola (1989): the observed hypokalaemia was corrected by adding 20mM KCl to the drinking water during 8% NaCl loading. Similar observations have been made in recent reports by Charlton & Armstrong (1989). It has also been possible to achieve a considerable reduction in the degree of hypokalaemia and hypertension, by treating rats with a small dose of chloroquine during salt loading (Sofola, Obiefuna & Adesanya, 1991). The mechanism by which NaCl loading induces hypokalaemia, which could be reversed by potassium supplementation, is still uncertain. A recent report by Edmonds & Willis (1990) suggested a relationship between dietary sodium and potassium secretion in the colon.

4.2.2. AGONIST- AND DEPOLARISATION-INDUCED CONTRACTIONS

The similarity in rapidity of contraction of rings from both rat groups to either agonist-induced (NA) or depolarisation-induced (KCl) stimulation suggests a common mode of calcium ion delivery to the myofilaments. Rings from salt-loaded rats appeared to contract more slowly and achieve lower mean maximal tension than controls. Other experiments (see section 3.5, page 74) have shown that the rate of Ca^{2+} entry into smooth muscle cells during agonist- or depolarisation-induced contraction is not reduced in salt-loaded rats. It is known that abnormalities in muscle fibre integrity usually affects smooth muscle reactivity (maximal tension), but may have no effects on sensitivity or threshold response (Johansson, 1974). It is possible that salt-loading induces some anomalies in myofilament integrity, which resulted in the slow rate of contraction as well as lower maximal tone in salt-loaded rat aorta.

Related observations have been reported by Konishi & Su (1983) and by Sim & Singh (1987). Konishi & Su (1983) found that the intact aortae of spontaneously hypertensive rats showed decreased responsiveness to various doses of NA. Sim & Singh (1987) reported a decrease in the maximal response of the aorta of both the spontaneous

ly hypertensive rat and the left-renal-artery stenosed hypertensive rat to various vasoactive agents.

The present results of increased sensitivity to NA and KCl suggest salt-induced enhancement of dose-dependent vascular sensitivity to agonists and to depolarisation. These results are comparable to numerous other observations on various human (Aoki, Kawaguchi, Sato, Kondo & Yamamoto, 1982; Aalkjaer, Danielsen, Johannesen, Pedersen, Rasmussen & Mulvany, 1985; Ebeigbe & Ezimokhai, 1988) and animal (Webb & Bohr, 1981) models of hypertension. The increased sensitivity to agonists as reported here appears to be selective for NA, but not 5-HT. However, Mecca & Webb (1984) have reported increased sensitivity of aortic helical strips from steroid hypertensive SD rats to 5-HT.

The increased sensitivity observed in these experiments may be explained as either due to (i) an increase in the number of both potential-sensitive Ca^{2+}-entry channels and NA-sensitive receptor-operated channels (Rusch & Hermsmeyer, 1988), (ii) an increase in the number or affinity of adrenoceptors, or (iii) an increase in efficiency of excitation-contraction coupling (Bolton, 1979) during salt loading. For instance, the efficiency of excitation- contraction coupling could be improved by an increase in the transmembrane movement of

Ca^{2+} following receptor activation (Mecca & Webb, 1984), or intracellular mobilisation of activator calcium (see below).

The technique used to determine the role of a cellular store of Ca mobilised by contractile agonists was developed by Karaki, Kubota & Urakawa (1979). This technique examines the contribution of a cellular pool of Ca in an agonist-induced contraction. The phasic contractile response produced by an agonist in Ca-free solution containing EGTA is not blocked by agents that decrease the transmembrane movement of calcium and so, is not affected by Ca^{2+}_o (Karaki *et al.*, 1979). Our result with this technique suggests that this cellular pool of calcium may not play a greater role in the development of NA-mediated contractions in arteries from salt-loaded rats than in those from normal rats.

Since calcium-entry studies (see section 3.5, page 74) indicate salt-induced, increased calcium entry through receptor-operated channels, it would appear that the difference in responsiveness to NA between salt-hypertensive and normotensive rats is related to a greater influx of calcium from the extracellular environment in arteries from salt-loaded rats. Comparative studies of calcium channel subtypes, by Rusch & Hermsmeyer (1988), in spontaneously hypertensive rats and their normotensive controls have shown a fundamental difference in the proportion of calcium channels in hypertensives versus normoten-

suggesting that increased calcium influx would occur with depolarisation in spontaneously hypertensive rats.

Salt-induced attenuation in the decay of the phasic contraction (Figure 13 and 14, pages 126 & 128) may suggest an alteration in intracellular handling of calcium (Bolton, 1979). This observation may appear to have an obvious significance but its importance in calcium dynamics that follow contractions in the presence of extracellular Ca^{2+} is not quite as obvious: Established NA or high-K contractions in normal Ca^{2+}_{o} concentrations, of salt-hypertensive and normotensive aortae, were found to relax in a similar pattern upon Ca^{2+} withdrawal (Figure 8).

4.2.3. CONTRACTILE RESPONSES TO SERUM

Increased vasoactivity observed in the serum of salt-loaded rats in this study is consistent with the result of a similar study on spontaneously hypertensive rats (Wright, 1981). Elevated plasma catecholamine concentration has been reported in patients suffering from essential hypertension (DeChamplain, Farley, Consineau & Van Ameringen, 1976) and in healthy humans after salt loading (Nicholls, *et al.*, 1980). Julien *et al.*, (1989), however, found a decrease in urinary catecholamines in genetically hypertensive Lyon rats.

The nature of the circulating vasoactive substance responsible for the increased contractile response in salt-loading is not known. Although catecholamines may be involved, serotonin has for long been shown to contribute largely to the vasoactivity of serum (Allen, Henderson, Chou & French, 1974). The results of this study suggest that salt-loading could induce the accumulation of vasoactive agents in the blood which could increase vascular tone and consequently, blood pressure in Sprague-Dawley rats.

4.2.4. IONIC INTERACTIONS

4.2.4a. SODIUM, POTASSIUM-ATPASE ACTIVITY

When the extracellular potassium concentration is reduced below physiological levels, the reduction in activity of this active transport system results in membrane depolarisation (Bohr, 1978). This decrease in the electrogenic pumping leads to an accumulation of intracellular Na^+. The later stimulates pump activity, so that if the concentration of extracellular potassium is now experimentally returned to normal, the pump will be overactive and hyperpolarisation will result (Webb *et al.*, 1981). Hendrickx & Casteels (1974) showed that ouabain, which blocks the sodium-potassium dependent ATPase, prevents hyperpolarisation resulting from the return of potassium to the extracellular fluid. Thus, the magnitude of K^+-induced relaxation

Current evidence exists of Na, K-ATPase pump inhibition in the erythrocyte of normotensive men fed a high salt diet (Quintanilla, Weffer, Koh, Rahman, Molteni & DelGreco, 1988).

4.2.4b. CALCIUM - MAGNESIUM INTERACTIONS

Lowered or raised extracellular magnesium concentration resulted in increased or decreased contractile responses, respectively, in both salt- hypertensive and normotensive rats. The mechanism by which Mg^{2+} modulates contractile responses in vascular smooth muscle is related to interference with calcium permeability, binding and translocation (Altura & Altura, 1971; Turlapaty & Altura, 1978; Turlapaty *et al.*, 1982).

The present results showed that Mg^{2+}o-induced relaxations were attenuated by salt-loading. Further experiments were designed to investigate the possible role of calcium in this observation. Mg^{2+}o-induced relaxation of high-K contractions was repeated in the presence of either Bay K8644 or CGP 28392, both dihydropyridines which augment Ca^{2+} entry into cells, chiefly by interacting with potential-sensitive channels (Schramm, Thomas, Towart, Frankowiak, 1983; Spedding & Berg, 1984). It was found that Mg- induced relaxation was similarly attenuated by increased calcium entry. The B/A or C/A ra

of Na contraction in K^+ -free PSS is a conventional estimate of vascular activity of Na, K-ATPase enzyme.

In the present study, Na, K-ATPase pump activity was found to be significantly attenuated by salt- loading and by exposure of the tissues to ouabain. Chen, Brace, Scott, Anderson & Haddy (1972) have long presented evidence indicating that the activity of the electrogenic pump and, hence, the level of the resting membrane potential may play an important role in the local regulation of blood flow. More recently, Haberey, Kloss, Buse & Beckmann (1988) demonstrated increased contractility of rabbit mesenteric arterial segments to transmural stimulation following Na, K-ATPase inhibition.

Attenuated vascular Na, K-ATPase activity has been shown in volume-expanded hypertension (Haddy & Overbeck, 1976) and in pregnancy-induced hypertension (Ebeigbe & Ezimokhai, 1988).

The precise role of the Na, K-ATPase pump in salt-hypertension has, over the years, been very controversial (Blaustein, 1984; Bing, Heagerty, Thurston & Swales, 1986; Adigun & Akinyanjuola, 1989); the major problems were the difficulty in consistently demonstrating inhibition of sodium pump during sodium loading and the lack of information on the vascular Na, K-ATPase pump status in salt- induced hypertension.

(Table 11, page 93) is an estimate of the relative effect of Ca- channel agonists on Mg^{2+}_{o}-induced responses. These ratios were similar for normotensive and hypertensive rats.

The above results suggest that attenuation of Mg^{2+}-induced relaxation in salt-hypertension may not be related to calcium movement through potential-sensitive channels.This result further supports our earlier observation that calcium ion entry through potential sensitive channels is not affected by salt-loading (see section 3.5 page 74).

It is possible that Mg^{2+}_{o}-induced relaxation in salt-hypertension is attenuated, at least in part, by increased calcium ion entry through receptor-operated channels (*cf* section 3.5, page 74).

Ebeigbe & Aloamaka (1987) have shown that Mg^{2+}_{o}- induced relaxations are dependent on endothelium integrity. It is, therefore, also possible that salt-induced alteration in endothelium-dependent relaxation to histamine, as was observed (see section 3.11, page 82), is related to the observed Mg^{2+o}-induced responses.

4.2.4c. MEMBRANE STABILISATION

The relaxation responses of the tissues from both rat groups to excessively increased Ca^{2+}_{o} was as a result of membrane-stabilisation (Bohr, 1963; Hurwitz, VonHagen & Joinder, 1967; Webb & Bohr, 1978a). The relaxation is presumed to be due to changes in conduc-

tance of major ions across the cell membrane (Hurwitz, 1965; Jones & Hart, 1975). By binding to specific loci on the cell membrane, Ca^{2+} are presumed to reduce the permeability to monovalent ions; thus by altering membrane potential, Ca^{2+} also influences its own permeability or intracellular release (Hurwitz, 1965).

The smooth muscle membrane of aortae from salt- hypertensive rats appeared to be more difficult to stabilise than the mormotensives, particularly when initial tone was induced by receptor stimulation. This may result from the low Na,K-ATPase pump activity observed in the salt-hypertensives: it is known that agents which inhibit the activity of the electrogenic Na pump, or low potassium, prevent the membrane- stabilising effect of Ca^{2+}(Webb & Bohr, 1978a).

It appears that increased Ca^{2+}_o is more effective in blocking Ca^{2+}-entry through receptor-operated channels than through potential-sensitive channels, since membrane stabilisation was minimal during high-K stimulation. It may also mean that high-K stimulation causes such changes on the cell membrane that make it difficult to stabilise. The initial small, but consistent increase in tone that followed addition of up to 5mM $CaCl_2$ during high-K stimulation (Figures 37 and 38) may arise from further Ca^{2+} entry through potential sensitive channels which were likely to be open under such conditions.

Membrane stabilisation of K^+-contracted tissue by $Ca^{2+}{}_o$ was not affected by salt loading. It would appear that membrane-stabilisation is related to the rate of Ca^{2+} entry; Ca^{2+} entry in hypertensives through PSCs does not differ from normotensives.

4.2.5. RESPONSES TO POTASSIUM CHANNEL OPENING AND CALCIUM CHANNEL BLOCKADE

4.2.5a. POTASSIUM CHANNEL OPENING

Antihypertensive agents whose modes of action are fairly well known were used in this study to help in the understanding of the various aberrations of smooth muscle activity that accompany salt-loading.

BRL 34915, now known as cromakalim, is a relatively new benzopyran derivative whose antihypertensive action derives from its ability to open K channels (Hamilton & Weston, 1989). The resulting K^+ efflux causes hyperpolarisation, reduces Ca-channel opening and ultimately, induces relaxation and inhibition of excitation (Weston, 1990).

BRL 34915 relaxed established NA contractions in both hypertensive and normotensive tissues in the present experiments. Hamilton, Weir & Weston (1986) observed that the amplitude of spontaneous contractions in the rat portal vein is progressively reduced

by BRL 34915. In contrast, Hof, Quast, Cook & Blarer (1988) reported that tension amplitude was essentially unchanged by cromakalim while frequency of contraction was reduced. Generally, reduced spontaneous mechanical activity of mammalian portal vein is one of the characteristic actions of BRL 34915 (Weston, 1990).

The results of this study are related to those reviewed above and are consistent with those of Hamilton *et al.* (1986) and Weir & Weston (1986) who found reduced maximal response to noradrenaline in rat aorta and portal vein with some right-ward shift in the agonist dose-response curves in the presence of BRL 34915.

Furthermore, it was found that salt-loading reduced the ability of BRL 34915 to relax established contractions to either NA or KCl. The reason for this is not known but it is a further indication of possible salt-induced derangement in cell K^+ metabolism.

The inability of BRL 34915 to cause appreciable relaxation of established K^+-contractions, reported here, has also been reported by many others (Hamilton *et al.*, 1986; Weir & Weston, 1986; Clapham & Wilson, 1987; Cain & Nicholson, 1988; Cook, Quast, Hof, Baumlin & Pally, 1988a; Cook, Quast & Weir, 1988b). The contraction observed in salt-hypertensive rat tissue may suggest that potassium ions could move rather freely in both directions when the channels are opened by BRL 34915. For instance, potassium influx is increased by activation

of the acetylcholine receptor, if the muscle is already depolarised (Devine, Somlyo & Somlyo, 1972; Hamon & Worcel, 1974; Worcel & Hamon, 1976) but sometimes, application of cholinergic agonists to polarised muscles decreases their total potassium content (Banerjee & Lewis, 1964; Banerjee, 1972; Joiner, 1973), suggesting that K^+ could move in both directions of the acetylcholine ROC.

4.2.5b. AGENTS THAT INTERFERE WITH CALCIUM HANDLING

The agents used in this design were diltiazem, which regulates calcium entry by allosteric modification of the dihydropyridine binding site (Spedding, 1984), hydralazine, which regulates calcium entry by interfering with both PSCs and ROCs (Ebeigbe & Aloamaka, 1985) and nifedipine, a dihydropyridine which essentially inhibits Ca^{2+} entry through PSCs. The dose range of the drugs used were not high enough to influence intracellular calcium stores (Saida & Van-Breemen, 1983).

Each of these three agents relaxed established NA contractions in both rat groups in a similar fashion. The fact that these agents do not act principally via ROCs may account partly for this.

Of all three calcium ion entry blockers, only diltiazem showed a significant difference in its action, at higher concentrations, on K^+-contraction between normotensive and hypertensive rat aortae (Figures 32 - 36). It appears that salt-induced alteration of PSC is ab

minute that it could only be detected either by small amounts of antagonists or after allosteric modification of the dihydropyridine binding sites. This may explain why large doses of hydralazine and nifedipine, which ordinarily block PSCs, did not show any difference between the two rat groups. This may also further explain why salt-loading was observed to paradoxically increase tissue response to KCl dose-dependently but not Ca^{2+}-entry through PSCs.

A recent study (Ebata, Natsume, Mitsuhashi & Yaginuma, 1991) using radio-labelled dihydropyridine ([^{3}H]PN200-110) binding to heart, brain and skeletal muscle microsomes of 4-, 8- and 15-week-old spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats showed reduced calcium sensitivity of dihydropyridine binding to calcium channels in SHR rats. Ebata *et al.* (1991), however, did not use purified calcium channels. The possible involvement of microsomal proteases and phospholipases (Glossman & Ferry, 1983; Kanngiesser & Pongs, 1989) is likely to affect their results.

It is suggested that calcium-dependent contractions as employed in the present study cannot account for all forms of anomalies of the PSCs or the binding sites of calcium.

4.2.6. ENDOTHELIUM - DEPENDENT RELAXATIONS

Acetylcholine is a classical example of an endothelium-dependent vasorelaxant (Furchgott & Zawadzki, 1980). Thus its ability to cause relaxation of precontracted vascular tissue has been generally employed to assess the integrity of the endothelium in vascular disease (Luscher, 1987). Previous studies on endothelium-dependent ACh responses in Dahl salt-sensitive rats (Luscher *et al.*, 1987a) reported depressed ACh-induced relaxation, which is contrary to the unaltered responses in SD rats observed in this study. The reason for this difference is not clear but it is noteworthy that endothelium-dependent relaxation exhibits considerable variability. For example, ACh-induced relaxation in spontaneously hypertensive rats may be attenuated (Luscher, 1987) or enhanced (Konishi & Su, 1983).

In the present study endothelium-dependent histamine-elicited relaxation was observed in NA-precontracted rat aortic rings. This is consistent with other reports (Van de Vorde & Leusen, 1983; Aloamaka, Evbuomwan, Ighoroje & Ebeigbe, 1990). The maximal relaxation caused by histamine (40%) found here in SD rats is much higher than the 21% maximal relaxation reported by Aloamaka *et al.* (1990) in Wistar rats, and comparable to the 46% reported by Van de Vorde & Leusen (1983) in an unnamed strain.

The attenuated endothelium-dependent histamine- induced relaxation in salt-loaded rats observed here suggests an impairment of EDRF release via histamine-receptor activation. It is possible that different EDRFs mediate histamine-induced and ACh- induced relaxation in salt-loaded aortic rings; however, we have no evidence for this. The possible existence of numerous EDRFs has been suggested (Vanhoutte, 1987). Histamine has been reported to play a role in the regulation of vascular tone in normal and diseased states (Ginsberg, Bristow, Kan trowitz, Baim & Harrison, 1981; Cabanie & Godfraind, 1988) but its direct involvement in the pathophysiology of hypertension has not been established.

4.3. CONCLUSION

The results of the present investigations suggest that salt-loading may cause increased blood pressure in the following ways.

(i) Adrenoceptor activity may be enhanced either by receptor modification or by increased calcium entry through ROCs. Responses to K^+ depolarisation may also be enhanced.

(ii) The Na,K-ATPase pump may be inhibited, leading to vasoconstriction that usually arises from intracellular accumulation of Na^+.

(iii) Endothelium-dependent relaxation to some substances, particularly histamine, may be attenuated.

(iv) Other membrane defects which would reduce the relaxant, modulatory roles played by increased levels of extracellular calcium and magnesium ions may arise.

(v) Vasoactive substances may accumulate in serum.

(vi) Hypokalaemia may also result which could cause vasoconstriction either by direct stimulation or by inhibition of Na,K-ATPase pump.

(vii) Calcium sequestration may be inhibited, leading to sustained contraction or prolonged retention of tone following contractions.

These mechanisms are summarised in Figure 42 (page 203) as a hypothetical scheme.

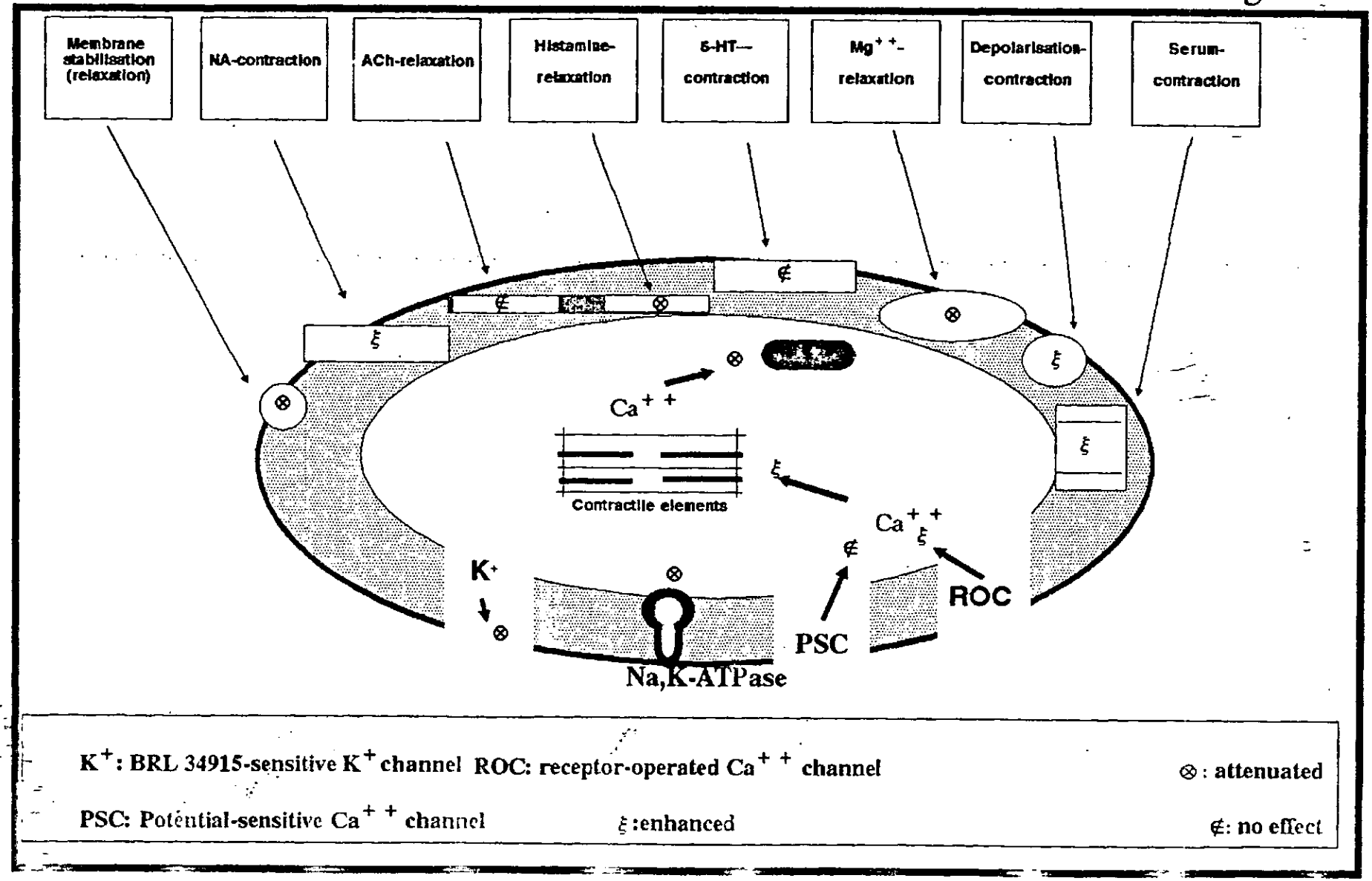

Membrane stabilisation (relaxation)
NA-contraction
ACh-relaxation
Histamine-relaxation
5-HT--contraction
Mg++-relaxation
Depolarisation-contraction
Serum-contraction
Ca++
Contractile elements
Ca++
K+
PSC
ROC
Na,K-ATPase
K+: BRL 34915-sensitive K+ channel
ROC: receptor-operated Ca++ channel
PSC: Potential-sensitive Ca++ channel
⊗ : attenuated
ξ :enhanced
∉: no effect

Figure 42. A hypothetical aortic smooth muscle cell from a salt-loaded rat drawn on the basis of the findings from this study.

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NOTES

PUBLICATIONS ARISING FROM PRESENT STUDY

i. FULL PAPERS

1. OBIEFUNA PCM, SOFOLA, OA & EBEIGBE, AB (1991) Dietary salt-loading attenuates endothelium-dependent relaxation in response to histamine but not to acetylcholine in the rat aortic rings. *Experimental Physiology* **76**, 135-138.

2. SOFOLA, OA, OBIEFUNA, PCM & ADESANYA, OP (1991) Chloroquine or captopril reduces blood pressure in salt-loaded rats. *Medical Science Research* **19**, 379-380.

3. OBIEFUNA, PCM, SOFOLA, OA & EBEIGBE, AB (1991) Depressed magnesium-induced relaxation in aortic rings from salt-loaded rats. *Nigerian Journal of Physiological Sciences* **7**, 59-62.

4. OBIEFUNA, PCM, EBEIGBE, AB, SOFOLA, OA & ALOAMAKA, CP (1992) Altered aortic smooth muscle responses in salt-loaded Sprague- Dawley rats. *Clinical and Experimental Pharmacology and Physiology* 18(1):(in press).

ii. ABSTRACTS

5. OBIEFUNA, PCM, SOFOLA, OA & EBEIGBE, AB (1991) Attenuated endothelium-dependent histamine-induced rat aortic relaxation following salt loading. *Nigerian Journal of Physiological Sciences* 7, 76.

6. OBIEFUNA, PCM, ALOAMAKA, CP, EBEIGBE, AB & SOFOLA, OA (1991) Altered rat aortic smooth muscle responses in salt-induced hypertension. *Nigerian Journal of Physiological Sciences* 7, 79.

7. OGUOGHO, A, OBIEFUNA, PCM, ALOAMAKA, CP & EBEIGBE, AB (1991) Membrane stabilizing effect of calcium in the rat aorta involves K channel activation. *Nigerian Journal of Physiological Sciences* 7, 78.