Br. J. Pharmac. (1985), **85**, 639–646

Reactivity to bradykinin and potassium of the isolated duodenum from rats with genetic and renal hypertension

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1 The biphasic (relaxation-contraction) response of the isolated duodenum was used to study the reactivity of non-vascular smooth muscles in genetic (SHR) and renal hypertensive rats compared to their respective controls (WKY and Wistar).

2 For the contractile component of the response to bradykinin, the duodenum from WKY rats was more sensitive, whereas the duodenum from SHR was both more sensitive and hyperreactive, compared to that from Wistar rats.

3 The relaxant component of the response to bradykinin was present in the duodenum of both WKY rats and SHR, but was concentration-dependent only in the WKY group.

4 The relaxant response to K^+ was very small in SHR, and was not concentration-dependent.

5 The concentration-response curves for relaxant responses to adrenaline and for contractile responses to acetylcholine did not differ in the SHR and WKY groups.

6 Ca^{2+}/Mg^{2+}-ATPase activity was found to be markedly reduced in the SHR group.

7 No qualitative or quantitative differences were observed between the responses of the duodenum of renal hypertensive rats and those of their normotensive controls.

8 It is proposed that the altered reactivity of the SHR duodenum is due to changes in ion handling by the smooth muscle cell membrane.

Introduction

The vascular reactivity of rats with genetically-selected or experimentally-induced hypertension has been widely studied, and different results have been obtained depending on the kind of vascular smooth muscle used (for a review see Winquist *et al.*, 1982). In those cases in which increased reactivity was observed, it appears to be associated with instability of the vascular smooth muscle cell membrane due to deficiency of calcium handling by the cell, since inhibition of membrane Ca^{2+}-adenosine triphosphatase (ATPase) and Ca^{2+}/Mg^{2+}-ATPase, as well as inhibition of binding and uptake of calcium by intracellular structures, have been found (Moore *et al.*, 1975; Wei *et al.*, 1976; Kwan *et al.*, 1979; 1980). Changes in the electrogenic Na^+/K^+-ATPase activity detected in vascular preparations from hypertensive rats might also be involved in the increased vascular reactivity. However, both an increase and decrease in the activity of this pump have been described (for a review see Overbeck & Grissette, 1982).

The altered reactivity of the vascular smooth muscles in hypertensive animals may reflect either primary changes involved in the onset of hypertension, or secondary adaptation to the increased blood pressure. Studies with non-vascular smooth muscle, where the haemodynamic influence is excluded, may help to identify primary changes of the smooth muscle cell membrane in hypertension. A few studies of this kind have been done using the genetically hypertensive rat and the stomach fundus and vas deferens were found to exhibit alterations similar to those previously described for vascular smooth muscle (Altman *et al.*, 1977; Caulfield *et al.*, 1977; Yanagawa *et al.*, 1978; Corbett *et al.*, 1980; Kwan *et al.*, 1982).

To gain further information on this problem we have made use of the interesting properties of the rat duodenum, which gives a biphasic response to some stimulants: low concentrations of K^+ or bradykinin induce relaxation whereas higher concentrations of these agents also cause contraction (Antonio, 1968; Faber & van der Meer, 1973; Boschcov *et al.*, 1984). In the case of bradykinin, the biphasic response appears

to be due to two different receptors, and to be determined by the Na^+ permeability across the membrane (Boschcov *et al.*, 1984).

In the case of K^+, different mechanisms are involved in the two phases of the response: the relaxation induced by small increases in K^+ concentration is due to membrane hyperpolarization as a result of activation of the Na^+/K^+-ATPase, whereas the contractile response to higher K^+ concentrations is due to depolarization (Webb & Bohr, 1978; Kuriyama, 1963).

The purpose of this study is to determine whether primary changes in ion handling by smooth muscles may be responsible for the hypertension in genetically hypertensive rats (SHR). With this aim, we have studied the reactivities to bradykinin and to increased K^+ of duodenum preparations isolated from SHR and their Wistar-Kyoto normotensive controls (WKY). Control experiments were also performed with renal hypertensive rats in an attempt to exclude differences occurring secondary to hypertension.

Methods

Animals

Experiments were carried out using the Okamoto-Aoki strain of spontaneously hypertensive rats (SHR) and their Wistar-Kyoto normotensive controls (WKY), derived from an original colony supplied by the National Institutes of Health, Bethesda, MD, U.S.A. The animals were of either sex, aged 20–24 weeks, and weighed 170–190 g (SHR) and 210–230 g (WKY). For the experiments with renal hypertension, Wistar rats from the Wistar Institute, Philadelphia, PA, U.S.A. and inbred in Escola Paulista de Medicina, S.P., Brazil, were used.

'One-kidney-one-clip' renal hypertension was produced by applying a silver clip to the main left renal artery under ether anaesthesia (Schaffenburg, 1959), with simultaneous right nephrectomy. The rats were maintained on standard laboratory chow and tap water *ad libitum*, and used 15 weeks after surgery when their body weight was 280–310 g. Systolic blood pressures were determined in the conscious state by means of a cannula previously inserted into the abdominal aorta through the femoral artery.

Concentration-response curves

Rats were killed by a blow on the head. After bleeding, the abdomen was opened and the duodenum removed. The preparation was set up in a 5 ml organ bath containing Tyrode solution kept at 37°C and aerated with 95% O_2 plus 5% CO_2 (pH 7.4). The composition of the Tyrode solution was (mM): NaCl 137, KCl 2.7, $CaCl_2$ 1.36, $MgCl_2$ 0.49, NaH_2PO_4 0.36, $NaHCO_3$ 11.9, D-glucose 5.5. In the potassium-free solution KCl was omitted. After a 30 min equilibration period, isotonic recordings were made, under 1 g load, on smoked drums using frontal levers with 6 fold amplification. The concentration-response curves were obtained within the first 90 min after the end of the equilibration period. The drugs, in volumes not exceeding 0.1 ml, were added directly to the organ bath at 5 min intervals for acetylcholine and potassium, and at 15 min intervals for bradykinin and adrenaline.

Membrane Mg^{2+}-ATPase and Ca^{2+}/Mg^{2+}-ATPase assays

Female four month old SHR and Wistar-Kyoto rats were killed by a blow on the head, the duodenum quickly removed and washed with 0.32 M sucrose solution, pH 7.4. Subcellular fractions of longitudinal smooth muscle were prepared by homogenizing a 1 : 10 (w/v) suspension in cold 0.32 M sucrose for 30 s at high speed in an Ultra-Turrax homogenizer. The homogenate was centrifuged at 1,000 *g* for 15 min, and then the supernatant was centrifuged at 10,000 *g* for 30 min. The post-mitochondrial supernatant was centrifuged at 100,000 *g* for 60 min. The microsomal pellet was suspended in 0.25 M sucrose. All procedures were carried out at 4°C.

ATPase assays

The reaction medium in a final volume of 0.21 ml contained Tris-HCl buffer 95 mM (pH 7.4), $MgCl_2$ 4 mM (for Mg^{2+}-ATPase and Ca^{2+}/Mg^{2+}-ATPase), $CaCl_2$ 0.4 mM (for Ca^{2+} Mg^{2+}-ATPase), NaCl 60 mM, KCl 40 mM, ouabain 0.2 mM, EGTA 0.133 mM, ATP-Tris 10 mM and the microsomal fraction to be assayed. Incubations were carried out at 37°C for 30 min and stopped by the addition of 0.04 ml of ice-cold 30% trichloroacetic acid. After centrifugation at 1,000 *g* for 5 min, inorganic phosphate in the supernatant was determined by the method of Lowry & Lopez (1946). Protein concentration was determined by the method of Lowry *et al.* (1951).

Ca^{2+}/Mg^{2+}-ATPase activity was calculated from the difference before and after addition of $CaCl_2$ in the presence of Mg^{2+}. The free Ca^{2+} concentration was 15 μM.

Drugs

The drugs used were ATP-Tris, ouabain octahydrate, adrenaline bitartrate and acetylcholine chloride from Sigma Chemical Company. Bradykinin was a synthetic product made in this laboratory (Sabia *et al.*, 1977). The inorganic salts were from Merck Darmstadt.

Table 1 pD_2 values for the relaxant and contractile components of the responses of the rat duodenum from renal hypertensive rats (RHR) and their normotensive controls (NWR)

	Relaxation		*Contraction*	
Stimulant	*NWR*	*RHR*	*NWR*	*RHR*
KCl	2.92 ± 0.07 (8)	3.05 ± 0.14 (7)	1.67 ± 0.05 (11)	1.67 ± 0.06 (12)
Bradykinin	8.44 ± 0.16 (28)	8.57 ± 0.31 (11)	6.91 ± 0.15 (16)	6.94 ± 0.16 (14)
Acetylcholine	—	—	6.59 ± 0.05 (17)	6.38 ± 0.10 (17)

Values are means ± s.e.mean; the number of experiments is given in parentheses.

Statistical analyses

Results are presented as means ± s.e.mean and the significance of the differences between values was assessed by Student's *t* test. *P* values less than 0.05 were considered significant.

Results

Responses of renal hypertensive rats and of their normotensive controls to bradykinin and KCl

The duodenum preparations of the renal hypertensive rats were studied 15 weeks after the clipping of the left renal artery, when mean arterial blood pressure averaged 152 ± 4 mmHg ($n = 46$), while the value for the control animals was 122 ± 2 mmHg ($n = 46$).

No qualitative or quantitative differences were observed in the behaviour of the duodenum of renal hypertensive rats when compared with their normotensive controls. The isolated duodenum from both groups of rats responded to low concentrations of bradykinin (below 10 nM) or KCl (below 10 mM) with a relaxation, and to higher concentrations of the two agents with relaxation followed by contraction. The relaxant component of the responses was concentration-dependent for both bradykinin (0.1–20 nM) and KCl (0.2–8 mM) and the pD_2 values are given in Table 1. Although the contractile component was present in all responses, only in about 30% of the experiments (for both the control and hypertensive animals) was it possible to obtain concentration-response curves from which pD_2 values could be estimated (Table 1).

Responses of genetically hypertensive rats (SHR) and their controls (WKY) to bradykinin

The mean arterial blood pressure of the animals used averaged 170 ± 2 mmHg ($n = 45$) in the SHR and 115 ± 2 mmHg ($n = 45$) in the WKY groups.

Duodenum preparations from SHR and WKY rats also presented biphasic responses to bradykinin, but they differed from the normotensive Wistar controls in that the contractile component of the response was present at much lower concentrations of the agonist, e.g. 2 nM (Figure 1).

The relaxant component of the response of WKY rats was concentration-dependent in the range of 10–100 nM, while that of the SHR was less marked and did not show concentration-dependence in the whole range of concentrations studied (Figure 2). Although the pD_2 values for the contractile component of the responses were similar for both groups (Table 2), larger responses were obtained from organs of SHR (Figure 3).

It is interesting to note that organs from WKY and SHR differed from those of the normotensive Wistar

Table 2 pD_2 values for the relaxant and contractile components of the responses of the duodenum from spontaneously hypertensive (SHR) and normotensive (WKY) rats

	Relaxation		*Contraction*	
Stimulant	*WKY*	*SHR*	*WKY*	*SHR*
Bradykinin	7.79 ± 0.14 (7)	ND	8.30 ± 0.38 (12)	8.48 ± 0.18 (7)
Adrenaline	4.21 ± 0.07 (5)	4.26 ± 0.05 (6)	—	—
Acetylcholine	—	—	6.57 ± 0.06 (17)	6.42 ± 0.06 (17)
KCl	2.95 ± 0.08 (8)	2.41 ± 0.11 (7)*	1.62 ± 0.06 (11)	1.54 ± 0.03 (12)

Values are means ± s.e.mean; the number of experiments is given in parentheses. ND indicates that pD_2 was not determined because the effect was not concentration-dependent. * $P < 0.01$, significantly different from value in WKY rats.

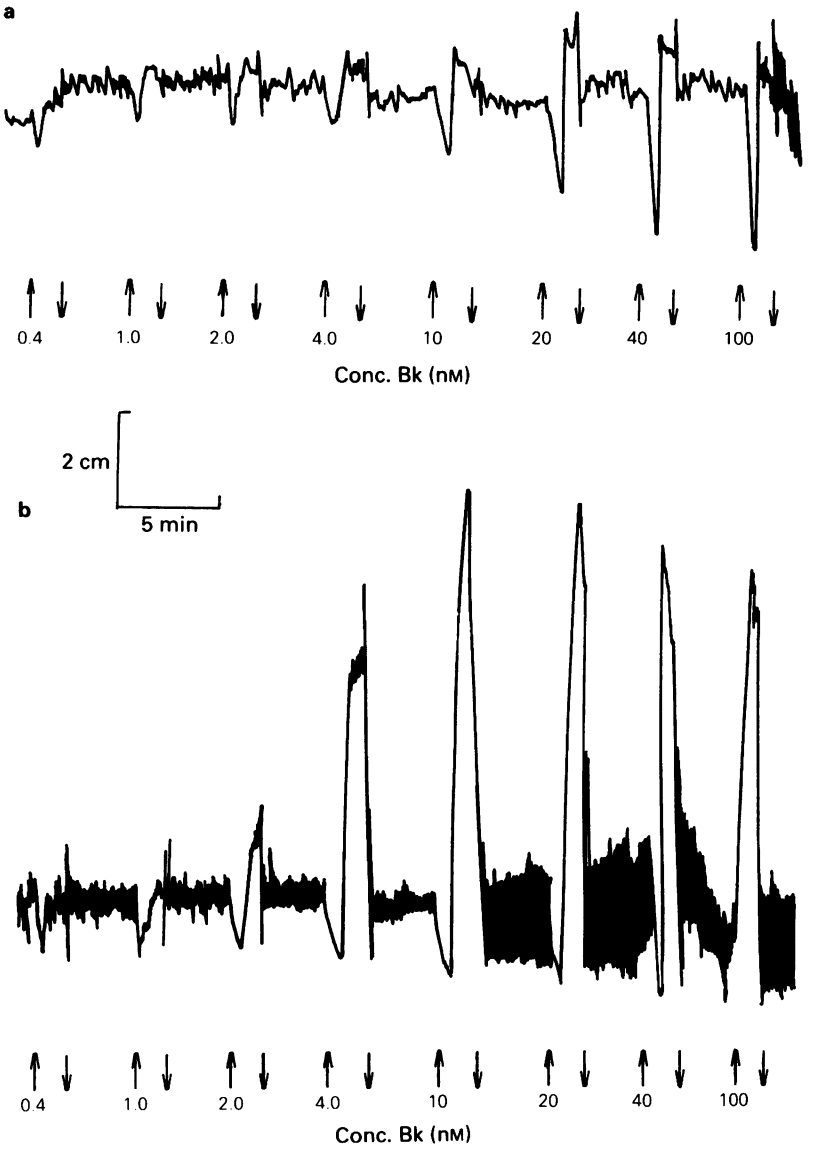

Figure 1 Responses of the rat duodenum to increasing concentrations of bradykinin (Bk) in preparations isolated from normotensive WKY rats (a) and genetically hypertensive (SHR) rats (b). Upward arrows indicate addition of the peptide. Downward arrows indicate washing with fresh medium and interruption of chart movement.

rats in that their pD_2 values were lower for the relaxation and higher for the contraction (compare Tables 1 and 2).

The concentration-response curves for adrenaline, which is a purely relaxant drug for the rat duodenum, and for acetylcholine, which elicits only contraction, did not differ in the SHR and WKY groups, yielding similar pD_2 values (Table 2).

Responses of duodenum from SHR and WKY rats to increased potassium

Duodenum preparations from WKY rats presented a pattern of responses to increased K^+ which was similar to that previously observed with normal Wistar rats (Boschcov *et al.*, 1984). Small increases in K^+ (below 10 mM) evoked only relaxation, while

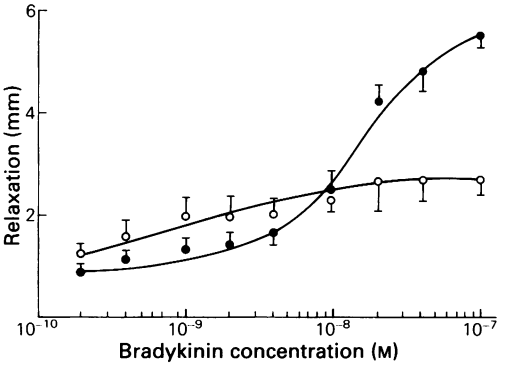

Figure 2 Concentration-response curves for the relaxant component of the response of the duodenum to bradykinin. The curves were obtained within the first 90 min after mounting of the duodenum isolated from normotensive WKY rats (●) and genetically hypertensive rats (○). Each point represents the mean and vertical lines show s.e.mean.

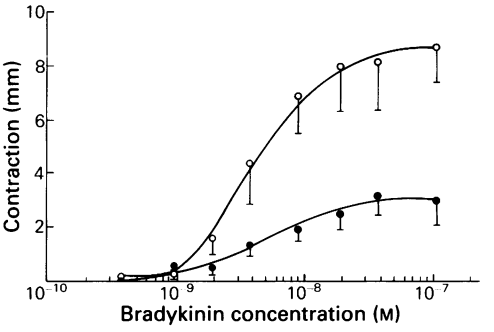

Figure 3 Concentration-response curves for the contractile component of the response of the duodenum to bradykinin. The curves were obtained within the first 90 min after mounting of the duodenum isolated from normotensive WKY rats (●) and genetically hypertensive rats (○). Each point represents the mean and vertical lines show s.e.mean.

higher concentrations (above 40 mM) elicited predominantly contractile responses (Figure 4a).

Most of the organs from SHR responded to the smaller increases in K^+ with weak relaxation responses, that were not concentration-dependent, and to the larger increases in K^+ (above 10 mM) with concentration-dependent contractions (Figure 4b). However, in about one third of the organs, a good concentration-response curve for the relaxant component of the response could also be obtained, from which pD_2 values were estimated. These values were significantly lower than those of the WKY group (Table 2). No significant difference was observed between the pD_2 values for the contractile components of the responses in the SHR and those in the WKY groups (Table 2).

The diminished relaxant response of the SHR duodenum to increased K^+ cannot be ascribed to an impaired relaxation mechanism since both SHR and WKY preparations responded equally well to the relaxant effect of adrenaline (Table 2). Since the relaxation induced by small increases in the extracellular K^+ concentration is attributed to hyperpolarization, due to stimulation of the Na^+/K^+-dependent ATPase (Na^+/K^+-pump), the specific decrease of K^+-induced relaxation in the SHR duodenum may indicate that this pump activity is either inhibited or already maximally enhanced in the spontaneously hypertensive rat. To investigate this problem we have used the K^+-induced relaxation as a functional indicator of the Na^+/K^+-ATPase activity in smooth muscle (method described by Webb & Bohr, 1978). After the duodenum was equilibrated for 15 min in K^+-free medium (to inhibit the Na^+/K^+-pump), it was exposed to different concentrations of K^+ in the range 0.2–15 mM for periods of 90 s at 10 min intervals. The organs from the WKY group exhibited concentration-dependent relaxations to these stimuli of the Na^+/K^+-pump, while those from the SHR group responded much less, in a concentration-independent manner, indicating that the pump is inhibited in genetically hypertensive animals. A typical example of these results is shown in Figure 5.

Ca^{2+}/Mg^{2+}-ATPase activity in duodena from SHR and WKY rats

The Ca^{2+}/Mg^{2+}-ATPase activity was determined in the microsomal fraction obtained from the duodenum smooth muscle of SHR and WKY rats. The activity in the WKY group was found to be 4.8 ± 0.8 μmol P_i mg^{-1} protein h^{-1} ($n = 3$); whereas, in the SHR group, the Ca^{2+}/Mg^{2+}-ATPase activity was found to be much lower (0.5 ± 0.5 μmol P_i mg^{-1} protein h^{-1}, $n = 4$, $P < 0.05$).

Discussion

In view of the biphasic nature of the responses of the duodenum, in which the same dose of bradykinin or K^+ may sequentially induce relaxation and contraction, it is not possible to dissociate unequivocally the components of the response. Thus, the enhancement of the contractile component observed in the responses of the SHR group to bradykinin may have masked the relaxant component, resulting in the flat log concentration-relaxation response curve seen in Figure 2. Nevertheless, Figure 3 clearly shows

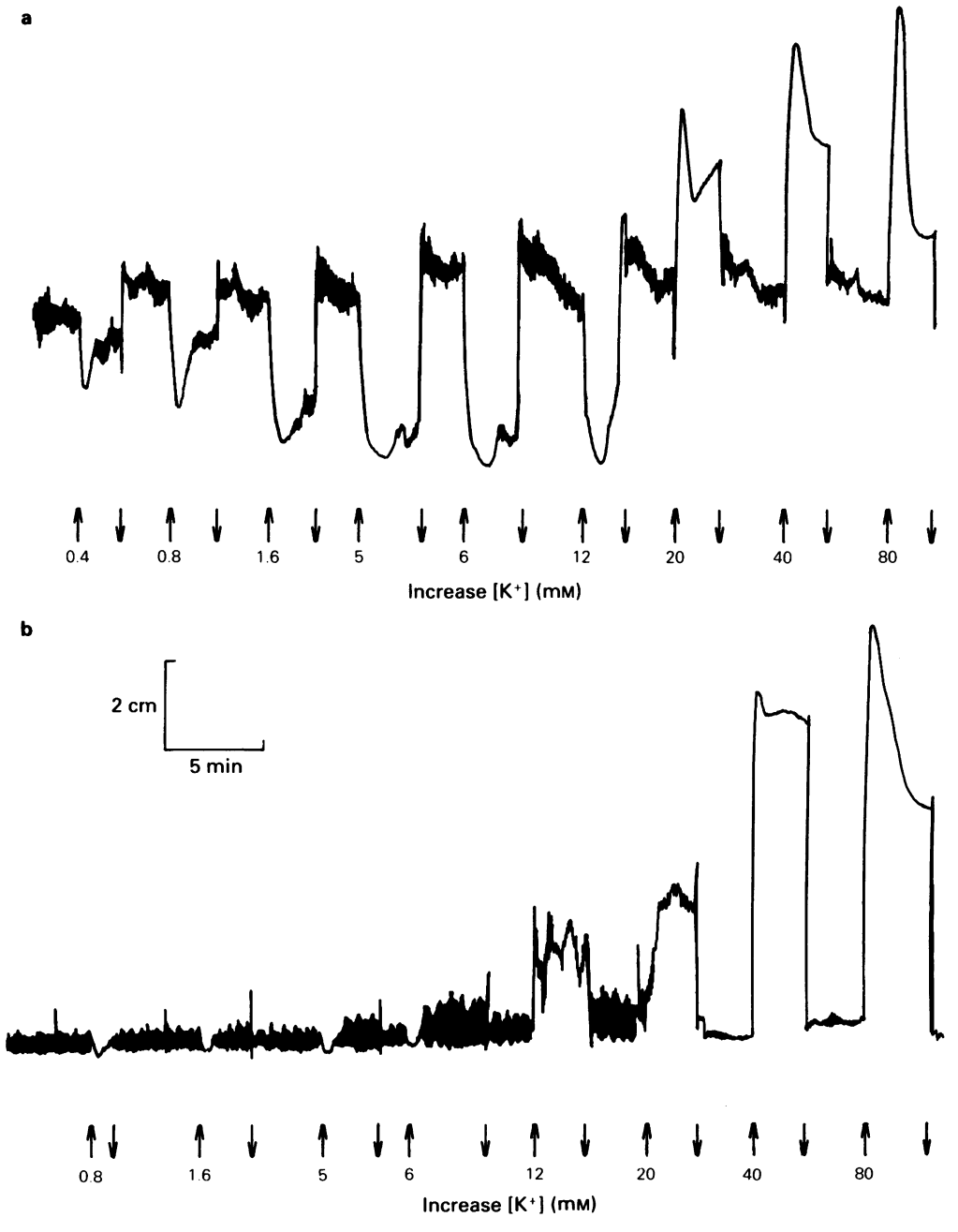

Figure 4 Effect of varying the K^+ concentration of the bathing medium on the responses of rat duodenum from normotensive WKY (a) and genetically hypertensive rats (b). Upward arrows indicate addition of the K^+ and downward arrows indicate washing with fresh medium and interruption of chart movement. Numbers indicate the increase in K^+ concentration (in mM) above the normal medium value (2.7 mM). Osmolarity was not corrected.

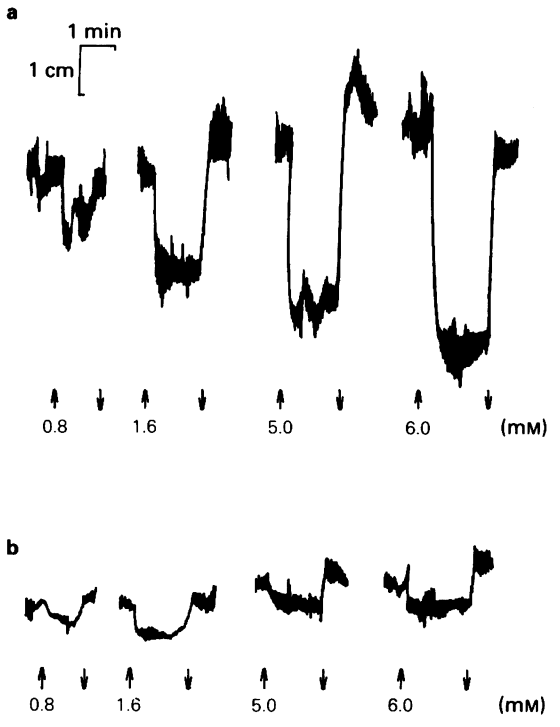

Figure 5 Responses of the duodenum, previously equilibrated in K^+-free medium, to different concentrations of added KCl (indicated in mM). (a) Preparations from WKY rats; (b) from genetically hypertensive rats. Upward arrows indicate addition of KCl to the K^+-free solution. Downward arrows indicate reintroduction of the K^+-free medium. Interval between additions, 10 min.

predominance of the contractile component in the SHR response when compared to that of the WKY group. Although the pD_2 values for the contractile effect of bradykinin did not differ between the two groups, the duodenum from both WKY and SHR was significantly more sensitive (lower pD_2) than that of the Wistar controls (compare Tables 1 and 2). This indicates that a tendency for a predominantly contractile response is already present in the WKY strain from which the genetically hypertensive rats were originally selected. This tendency cannot be ascribed to a general increase in sensitivity to agonists, since the contractile response to acetylcholine did not differ in the normotensive Wistar, WKY and SHR groups. Since the biphasic effect of bradykinin is the result of the activation of two receptor sites, the predominance of the contractile component in the responses of the SHR duodenum could be due to these animals having a larger proportion of the receptors responsible for the contraction. Alternatively, the stimulus-contraction coupling mechanism for bradykinin might be more affected than that for acetylcholine by changes in Na^+, K^+ and Ca^{2+} handling by the SHR smooth muscle cell membrane.

In contrast to the responses to bradykinin, those to K^+ were not different in the WKY as compared to the normal Wistar group, whereas the SHR duodenum showed a marked inhibition of the relaxant component. This inhibition was specific for K^+, since the relaxation in response to adrenaline was the same for the two groups (Table 2). Since relaxation induced by K^+ is caused by stimulation of the Na^+/K^+-pump, depression of this response might be due either to a depressed or to a maximally stimulated state of that pump in the SHR duodenum. To choose between these two alternatives we used the method described by Webb & Bohr (1978) to determine the state of the Na^+/K^+-pump in our preparations. The inhibition of the response of the SHR duodenum to K^+ added to a K^+-free medium (Figure 5) showed that the Na^+/K^+-pump is inhibited in the SHR group. Although most of the studies done with vascular smooth muscle have indicated an increased activity of the Na^+/K^+-pump in the SHR (Webb & Bohr, 1979), Sowers *et al.* (1983) have found it to be depressed in the erythrocyte membrane and in the myocardium. The finding that it is also inhibited in the SHR duodenum suggests that the stimulated state in some vascular preparations might reflect compensatory mechanisms.

Our findings lead us to conclude that the supersensitivity to bradykinin observed in the duodenum of SHR and WKY animals was of genetic origin and that the hyperreactivity found in the SHR organs might be due to partial inhibition of the Na^+/K^+-pump and to the instability of the membrane in consequence of the inhibition of the Ca^{2+}/Mg^{2+}-ATPase. Our finding that the Ca^{2+}/Mg^{2+}-ATPase is inhibited in the SHR agrees with previous observations made with vascular tissues (Kwan *et al.*, 1982). Although the physiological function of Ca^{2+}/Mg^{2+}-ATPase is not well understood, it is generally accepted that this enzyme is involved in Ca^{2+} binding to the membrane (Moore *et al.*, 1975). The deficiency in Ca^{2+} handling can be compared to the condition described by Antonio (1968), in which the isolated duodenum equilibrated in low calcium medium presented similar contractile responses. In both conditions the Na^+ conductance is increased (Brading *et al.*, 1969; Postnov *et al.*, 1979), which favours the contractile component of the response induced by bradykinin (Boschcov *et al.*, 1984).

Duodenum preparations isolated from renal hypertensive rats, with the same average arterial blood pressure as in the SHR group, exhibited no changes in the parameters studied, indicating that the alterations shown in the genetically hypertensive rats are not a consequence of the hypertensive state.

Our results, as well as those of other studies done

with various non-vascular smooth muscle preparations, (for a review see Kwan *et al.*, 1982) give support to the idea that genetic hypertension is associated with a general alteration in smooth muscle function probably due to altered ion handling by the cell membrane.

The authors are indebted to Prof. A.C.M. Paiva for helpful discussion and careful review of the manuscript. The technical assistance of Nelson A. Mora is gratefully acknowledged. This work was supported by grants from FINEP, CNPq, FIPEC and FAPESP.

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(Received December 7, 1984.
Revised February 20, 1985.
Accepted February 26, 1985.)