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EVIDENCE FOR A VISCERAL SMOOTH MUSCLE ABNORMALITY IN OKAMOTO SPONTANEOUS HYPERTENSION

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1 In order to discover whether the changes in reactivity are related to the primary cause of hypertension in spontaneously hypertensive rats (SHR) or are just an adaptation induced by the high arterial blood pressure we tested the contractile response of a visceral smooth muscle from such rats.

2 Longitudinal strips of the fundus from 20 week old male and female SHR and Wistar normotensive (NW) rats were used. Dose-response curves to Ba^{2+} in SHR strips were displaced to the right as compared to NW rats. Maximal responses were identical. Male SHR fundus strips contracted much more with Sr^{2+} (SHR: $42 \pm 3\%$ of maximum response to Ba^{2+}, $n = 10$; NW: $19 \pm 4\%$, $n = 10$, $P < 0.01$) than NW strips. There was no difference in the response to both $BaCl_2$ and $SrCl_2$ between female SHR and NW fundus strips, and $MnCl_2$ and $LaCl_3$ were relaxant in all cases.

3 Dose-response curves to Ca^{2+} of depolarized SHR and NW fundus strips were obtained and the effect of diazoxide on Ca^{2+} contractions was observed. The contractile action of Ca^{2+} in depolarized preparations was enhanced in both male and female SHR strips. The effect of diazoxide was more marked in SHR strips than in NW fundus strips.

4 SHR fundus smooth muscle shows the same modification of reactivity to Ba^{2+}, Sr^{2+}, Ca^{2+} and diazoxide that was previously described in arterial smooth muscle. This indicates that the cellular modification responsible for the increase of vascular tonus in SHR is not an adaptive reaction to high blood pressure. The differences between female SHR and male SHR responses are not unexpected, considering the natural evolution of hypertension in Okamoto rats which is milder in the female.

Introduction

Okamoto & Aoki obtained in 1963 a strain of spontaneously hypertensive rats (SHR) by inbred crossing of male and female Wistar rats which had high arterial blood pressure. This Okamoto strain provides a suitable model for the study of the physiopathology of human essential hypertension. SHR show an increase in vascular resistance that could be due either to increased stimulation of the arterioles by some vasoactive substance (a fact that has not yet been demonstrated) or to a greater reactivity of vascular smooth muscle (Hausler & Finch, 1972). An alternative explanation was given by Folkow, Hallbäch, Lundgren, Sivertsson & Weiss (1972) who suggested that the changes in resistance are due to an increase in the arteriolar wall/lumen ratio. This hypothesis is refuted by the fact that changes in vascular reactivity were also observed in helical strips of SHR arteries. Shibata & Kurahashi (1972) demonstrated that there were qualitative differences in the way SHR aortae responded to some cations. La^{3+} and Mn^{2+}, which relax normotensive vascular smooth muscle, contract SHR strips. This finding was confirmed by Bohr (1974) who also showed a decreased sensitivity to Ba^{2+} in SHR carotid arteries. Janis & Triggle (1973) found that diazoxide has a greater inhibitory effect on the response to Ca^{2+} in SHR than in normal aortic strips. These results have been interpreted as indicating that spontaneous hypertension in the Okamoto strain is associated with a defect in Ca^{2+} binding or uptake by some cellular structure. This suggestion was recently confirmed by Moore, Hurwitz, Redman-Davenport & Landon (1975) who showed that Mg-ATP-dependent Ca^{2+} uptake was decreased in SHR aortic microsomes. These authors suggested that increased vascular tonus in SHR may be due to an impaired relaxation mechanism. In all cases, all the observed alterations may result from adaptive changes to an increase in blood pressure, produced by a primary increase in cardiac output (Finkielman, Worcel & Agrest, 1965) induced by various humoral factors (Guyton, Coleman & Granger, 1972). This possibility may have

been excluded by Greenberg & Bohr (1975) who observed an increased spontaneous contractility, decreased passive extensibility and a lower threshold for the response to prostaglandins A_2 and B_2 in SHR portal veins. In order to exclude any haemodynamic influence we have explored the reactivity of SHR extravascular smooth muscle. Experiments performed with SHR stomach fundus strips indicate that spontaneous hypertension in Okamoto rats is associated with a generalized alteration in smooth muscle reactivity that could hardly be explained by an adaptive change to the high blood pressure.

Methods

We used 20 week old male (blood pressure (BP)= 212 ± 3 mmHg) and female (BP = 155 ± 5 mmHg) Okamoto Aoki Kyoto Wistar rats (SHR). Normotensive Wistar (NW) rats of either sex (male BP= 126 ± 3 mmHg; female BP = 108 ± 2 mmHg) of the same age were used as controls. Blood pressure was measured by a sphygmomanometric plethysmographic method on the tail artery. The animals were killed by a blow on the head, the stomach excised and a longitudinal strip of the fundus was prepared according to the method of Vane (1957). The strips were equilibrated for 2 h in a solution containing (mM): Na^+ 137.5, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Tris 15.5, H_2PO_4 1.2, Cl^- 134.1, glucose 11.5 aerated with 100% O_2 at 37°C. The strips were attached at one end to a stainless steel holder and at the other to a strain gauge (isometric recording). The initial tension was fixed at 2 grams. The drugs tested were introduced in the 10 ml organ bath in small volumes (0.05 ml).

Drugs

Diazoxide was a gift from the Shering Co, New Jersey, USA. All salts used were reagent grade.

Statistical analysis

The results were compared with a group *t* test and expressed in all cases as the average ± s.e. mean (Schwartz, 1960).

Results

Contractile response to Ba^{2+}, Sr^{3+}, Mn^{2+} and La^{3+}

All the cations were added in concentrated solution as the chloride salt. In contrast to previous observations on SHR vascular smooth muscles (Bohr, 1974), Mn^{2+} and La^{3+} caused relaxation of fundus strips of both SHR and control animals. Concentration-response curves to Ba^{2+} and Sr^{2+} were obtained cumulatively.

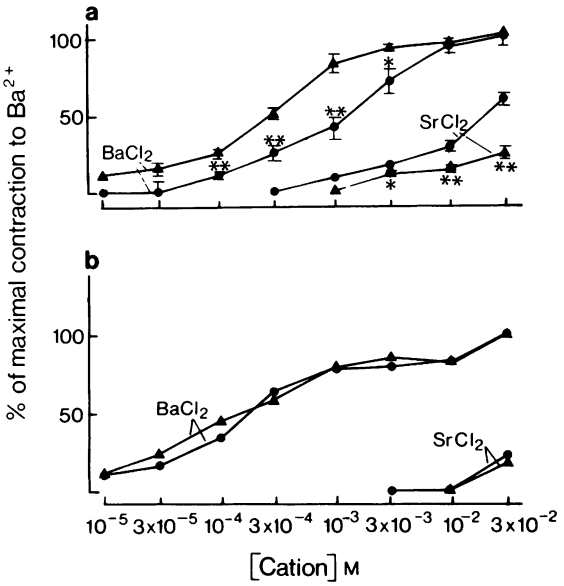

Figure 1 Mean concentration-response curves to Ba^{2+} and Sr^{2+} of rat fundus strips. Cumulative $BaCl_2$ curves were obtained first. $SrCl_2$ was added cumulatively. Responses to $SrCl_2$ are expressed as a percentage of the maximum response to Ba^{2+}. (a) Concentration-response curves in strips from male SHR. (b) Concentration-response curves in strips from female SHR. Each point is the mean of 10 experiments. SHR (●); control (▲). The bars represent the s.e. mean. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

$BaCl_2$ was always administered first. Once its maximum response was obtained the strip was washed and left for 60 min in the Krebs solution. $SrCl_2$ was later added cumulatively. Both concentration-response curves were expressed as a percentage of the $BaCl_2$ maximum response. $BaCl_2$ gives a concentration-dependent response with a maximum at 30 mM (Figure 1). $SrCl_2$ has a contractile effect at concentrations higher than 0.3 mM. The actions of both cations were different in male and female SHR. Dose-response curves to $BaCl_2$ on the fundus of male SHR are shifted to the right as compared with controls (Figure 1a); the maximum response is not changed. Responses to $SrCl_2$ are higher in strips from male SHR. On the other hand both $BaCl_2$ or $SrCl_2$ dose-response curves of the fundus of female SHR are identical to control curves (Figure 1b).

Contractile response to $CaCl_2$ and relaxant effects of diazoxide

Ca^{2+} contractions were induced in K^+-depolarized strips (Janis & Triggle, 1973). After equilibration with

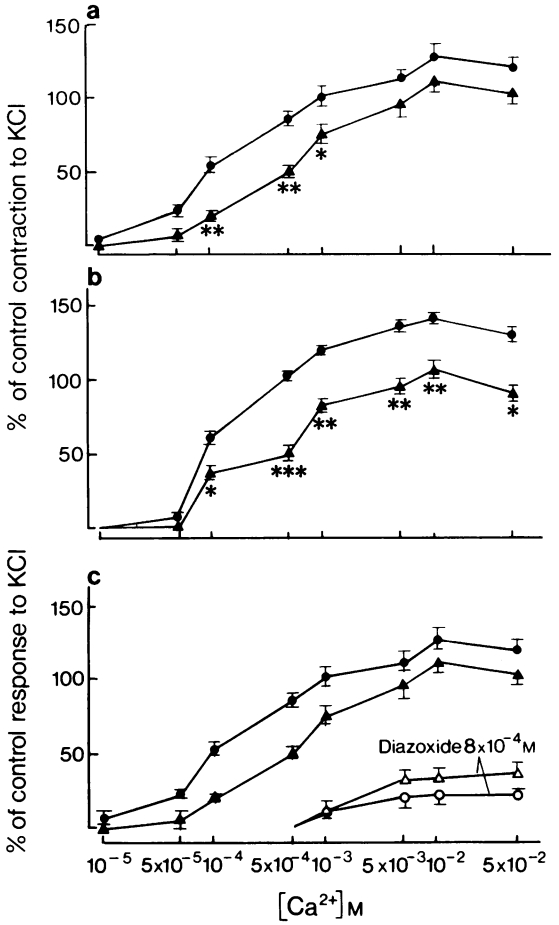

Figure 2 Cumulative concentration-response curves to Ca^{2+} of depolarized preparations obtained in the presence or in the absence of diazoxide. Contractions were recorded isometrically. A contraction was obtained with 80 mM KCl solution which served as a reference for the following responses. The tissues were then washed with a Krebs solution without HPO_3- and Ca^{2+}-free. After 100 min, 80 mM KCl was added. When the small response stabilized near the base line, cumulative dose-response curves to Ca^{2+} were obtained in the presence or in the absence of diazoxide. Diazoxide was added 10 min before the first Ca^{2+} dose and maintained throughout the experiment at a fixed concentration. (a) Ca^{2+} concentration-effect curves in male SHR depolarized fundus strips. (b) Ca^{2+} concentration-effect curves in female SHR depolarized fundus strips. (c) Comparison of the response to the cumulative addition of Ca^{2+} to male depolarized fundus strips and male control depolarized strips before and after diazoxide 8×10^{-4} M. The points are the mean of 5 to 7 experiments. Dose-response curves with and without diazoxide were performed simultaneously on two strips of the same stomach immersed in separate chambers of the same temperature-controlled bath. SHR (●); control (▲); diazoxide SHR (○), diazoxide control (△).

the normal Krebs solution a contraction was obtained by changing the solution in the organ bath to a 80 mM KCl solution (composition (mM): Na^+ 57.2, K^+ 80.0, Ca^{2+} 2.5, Mg^{2+} 1.2, Tris 15.5, H_2PO_4 1.2, Cl^- 134.1, glucose 11.5, gassed with 100% O_2 at 37°C). This contraction served as a reference for the following responses. The tissues were then washed with a Krebs solution without HPO_3 or Ca^{2+} (composition (mM): Na^+ 141.2, K^+ 5.9, Mg^{2+} 1.2, Tris 15.5, Cl^- 135.3, glucose 11.5, aerated with 100% O_2 at 37°C). After 100 min it was replaced by a 80 mM KCl, Ca^{2+} and HPO_3-free solution containing (mM): Na^+ 66.3, K^+ 80.0, Mg^{2+} 1.2, Tris 15.5, Cl^- 135.3, glucose 11.5, aerated with 100% O_2 at 37°C. A small contraction was obtained which faded in about 5 minutes. When the tension stabilized near the base line, cumulative dose-response curves to Ca^{2+} were obtained in the presence or in the absence of diazoxide added 10 min before the first $CaCl_2$ dose and maintained throughout the experiment. Diazoxide was dissolved in 0.9% w/v NaCl solution (saline) by a dropwise addition of NaOH solution. The tissues used as controls were treated with the same volume of NaOH-saline solution of the same pH. No effects were detected after the addition of NaOH-saline alone to the tissues. In the fundus of either sex of SHR (Figure 2) there was a significant shift of the Ca^{2+} dose-response curve to the left which indicates that there is an enhancement of Ca^{2+} contractile effects in depolarized SHR preparations. Diazoxide behaved as a non-competitive antagonist of Ca^{2+} in these depolarized strips. In both sexes the antagonist effect was more marked in SHR. Nevertheless strips from female rats were more relaxed by diazoxide than those from males. The drug was tested at concentrations of 1, 2, 4, 6 and 8×10^{-4} M. In female strips the antagonist started to be effective at 4×10^{-4} M, in male rats at 6×10^{-4} M (Table 1).

Table 1 Reduction of the maximal response to Ca^{2+} of depolarized control and SHR strips produced by diazoxide (% of control response to KCl)

Diazoxide	*Male*			*Female*		
(M)	*SHR*	*Control*	P	*SHR*	*Control*	P
4×10^{-4}	8.71 ± 1.3*	10.8 ± 2.4	NS	36.3 ± 2.8	16.8 ± 2.1	0.01
6×10^{-4}	12.7 ± 1.5	12.0 ± 3.3	NS	42.3 ± 3.3	19.1 ± 4.0	0.001
8×10^{-4}	101.0 ± 3.8	67.5 ± 5.4	0.01			

* Values obtained by averaging the differences of response at Ca^{2+} concentrations of 10^{-3}, 5×10^{-3}, 10^{-2} and 5×10^{-2}M, which give maximum contractions. Each value is the average of 5 to 7 experiments.

Discussion

The present experiments indicate that the changes in reactivity observed in SHR visceral smooth muscle are very similar to the variations in SHR arterial smooth muscle responsiveness reported previously by Holloway, Sitrin & Bohr (1972), Shibata & Kurahashi (1972), Janis & Triggle (1973) and Bohr (1974). However, some differences should be stressed: the decreased response to $BaCl_2$ and the increased effect of Sr^{2+} was observed only in male SHR fundus. Diazoxide was more effective in female tissues. Unfortunately these findings cannot be compared with the results in the literature on vascular smooth muscle, because of the lack of a systematic comparison between male and female specimens. The disparity in Ba^{2+}, Sr^{2+} and diazoxide effects is not unexpected considering the fact that the natural history of spontaneous hypertension is rather different in the two sexes. Blood pressure levels in female SHR are lower than in male SHR at all ages. The reason for this feature could be related to the effect of sexual hormones on smooth muscle or to some sex-linked cellular characteristics. We do not think that the haemodynamic changes associated with the lower blood pressure in females is the cause of these findings.

It is difficult to interpret the present results from a physiological point of view, because of the absence of data concerning the mode of action of the cations and diazoxide. Both Ba^{2+} and Sr^{2+} have been shown to compete with Ca^{2+} for binding in vascular smooth muscle (Hudgins & Weiss, 1969), but it is not known whether these cations displace Ca^{2+} from storage sites or troponin. Bohr (1974) observed that in vascular smooth muscle Ba^{2+} contraction is dependent on extracellular Ca^{2+}. This suggests that Ba^{2+} contraction is due, at least in part, to a displacement of Ca^{2+} bound to the cell membrane. Possibly there is a decrease of Ca^{2+} binding properties of smooth muscle membranes or other superficial storage sites in SHR rats. Similarly the increased contractile effect of Ca^{2+} in SHR depolarized smooth muscles may be caused by a defect in muscle relaxation. Faulty relaxation may be produced by a decrease of the activity of Ca^{2+} pump located either in the sarcoplasmic reticulum (Moore *et al.*, 1975) or in the cell membrane (Schatzmann, 1973). Alternatively Ca^{2+} efflux from the cell through the Ca^{2+}: Na^+ exchange mechanism (Brading & Widdicombe, 1976) could be slowed down because of an increase in intracellular Na^+ (Jones, 1974). Any of these alterations could be a primary defect of smooth muscle or secondary to modifications in hormonal secretion (Kojima, Kubota, Sato, Yamada, Yamori & Ikamoto, 1975) or to an increase in prostaglandin synthesis as recently suggested by Greenberg (1976).

In conclusion it is suggested that in SHR there is a generalized alteration of the excitation-contraction coupling mechanism in both extravascular and vascular smooth muscles that could hardly be considered as an adaptation to the haemodynamic changes associated with the increase in blood pressure. Hypertension could still play a primary role if it induces humoral disturbances which affect smooth muscle function secondarily, even in extravascular tissues but this hypothesis is far from being demonstrated.

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References

BOHR, D.F. (1974). Reactivity of vascular smooth muscle from normal and hypertensive rats: effects of several cations. *Fed Proc.*, **33**, 127–132.

BRADING, A.F. & WIDDICOMBE, J.H. (1976). Interaction between sodium and calcium movements in smooth muscle. In *Smooth Muscle Pharmacology & Physiology*. ed. Worcel, M. & Vassort, G. pp. 235–246. Paris: INSERM.

FINKIELMAN, S., WORCEL, M. & AGREST, A. (1965). Hemodynamic patterns in essential hypertension. *Circulation*, **31**, 356–368.

FOLKOW, B., HALLBÄCH, M., LUNDGREN, G., SIVERTSSON, R. & WEISS, L. (1972). The importance of adaptive changes in vascular design for the establishment and maintenance of primary hypertension, as studied in man and in spontaneously hypertensive rats. In

Spontaneous Hypertension. ed. Okamoto, K. pp. 103–114. Igaku Shoin Tokyo, Springer-Verlag, Berlin, Heidelberg, New York.

GUYTON, C., COLEMAN, T.G. & GRANGER, H.J. (1972). Circulation: overall regulation. *Ann. Rev. Physiol.*, **34**, 13–46.

GREENBERG, G. (1976). Evidence for enhanced venous smooth muscle turnover of prostaglandin-like substance in portal veins from spontaneously hypertensive rats. *Prostaglandins*, **11**, 163–193.

GREENBERG, S. & BOHR, D.F. (1975). Venous smooth muscle in hypertension. Enhanced contractility of portal veins from spontaneously hypertensive rats. *Circulation Res.*, **36–37** (suppl. I), 208–215.

HAUSLER, G. & FINCH, L. (1972). Vascular resistance and reactivity to various vasoconstrictor agents in hypertensive rats. In *Spontaneous Hypertension.* ed. Okamoto, K. pp. 97–102. Tokyo: Igaku Shoin. Berlin, Heidelberg, New York: Springer-Verlag.

HOLLOWAY, E.T., SITRIN, M.D. & BOHR, D.F. (1972). Calcium dependence of vascular smooth muscle from normotensive and hypertensive rats. In *Hypertension '72.* ed. Genest, J. & Koin, E. pp. 400–408. Berlin, Heidelberg: Springer Verlag.

HUDGINS, P.M. & WEISS, G.B. (1969). Characteristics of ^{45}Ca binding in vascular smooth muscle. *Am. J. Physiol.*, **217**, 1310–1315.

JANIS, R.A. & TRIGGLE, D.J. (1973). Effect of diazoxide on aortic reactivity to calcium in spontaneously hypertensive rats. *Canad. J. Physiol. Pharmac.*, **51**, 621–626.

JONES, A. (1974). Reactivity of ion fluxes in rat aorta during hypertension and circulatory control. *Fedn Proc.*, **33**, 133–137.

KOJIMA, A., KUBOTA, T., SATO, A., YAMADA, T., YAMORI, Y. & IKAMOTO, K. (1975). Congenital abnormality of pituitary-thyroid axis in spontaneously hypertensive rats (SHR) and stroke-prone rats (SPR). *Proc. Soc. exp. Biol. Med.*, **150**, 571–573.

MOORE, L., HURWITZ, L., REDMAN-DAVENPORT, G. & LANDON, E.J. (1975). Energy-dependent calcium uptake activity of microsomes from the aorta of normal and hypertensive rats. *Biochim. biophys. Acta*, **413**, 434–443.

OKAMOTO, K. & AOKI, K. (1963). Development of a strain of spontaneously hypertensive rats. *Jap. Circulat. J.*, **27**, 282–293.

SCHATZMAN, H.J. (1973). Dependence on calcium concentration and stoichiometry of the calcium pump in human red cells. *J. Physiol., Lond.*, **235**, 551–563.

SCHWARTZ, D. (1960). *Méthodes Statistiques à l'Usage des Médecins et des Biologistes.* pp. 151–162. Paris: Flammarion.

SHIBATA, S. & KURAHASHI, K. (1972). Possible mechanisms of vascular reactivity differences in spontaneously hypertensive and normotensive rat aortae. In *Spontaneous Hypertension.* ed. Okamoto, K. pp. 115–120. Tokyo: Igaku Shoin. Berlin, Heidelberg, New York: Springer-Verlag.

VANE, J.R. (1957). A sensitive method for the assay of 5-hydroxytryptamine. *Br. J. Pharmac. Chemother.*, **12**, 344–349.

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