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Effect of high extracellular Ca^{2+} levels in spontaneously hypertensive rat aorta

Ana Ortega Mateo, María Amaya Aleixandre de Artiñano *

Departamento de Farmacología, Facultad de Medicina, Universidad Complutense de Madrid, Ciudad Universitaria s/n 28040, Madrid, Spain

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Abstract

The release of endothelial relaxing factors has been suggested to be important in modulating the inhibition of the contractile activity caused by the increase in extracellular Ca^{2+} concentration in arterial tissue. Since the hypertensive process in spontaneously hypertensive rats (SHR) could be associated with the release of endothelial vasoconstrictor factors (mainly cyclooxygenase-dependent endoperoxides and endothelin-1), we studied the contractile responses to KCl, methoxamine and phenylephrine in different aorta ring preparations (intact, de-endothelized, 10^{-5} M indomethacin-treated, 10^{-6} M CGS-27830 [meso-1,4-dihydro-5-methoxycarbonyl-2, 6-dimethyl-4-(3-nitrophenyl)-3-pyridine carboxylic acid anhydride]-treated, and treated simultaneously with 10^{-5} M indomethacin and 10^{-6} M CGS-27830) from SHR and normotensive Wistar Kyoto rats (WKY), at various Ca^{2+} concentrations (1.25, 2.5, 5 and 10 mM) in the organ bath. In endothelium-intact preparations from WKY rats we observed a decrease in KCl, methoxamine and phenylephrine contractions with high Ca^{2+} concentrations (5 and 10 mM), but in the endothelium-intact preparations from SHR, the increase in extracellular Ca^{2+} concentration potentiated methoxamine contractions and caused no change in KCl and phenylephrine contractions. When the endothelium was disrupted in preparations from both WKY rats and SHR, we observed a decrease in KCl and methoxamine contractions with high Ca^{2+} concentrations. The decrease in phenylephrine contractions caused by high Ca^{2+} concentrations was clear in de-endothelized preparations from WKY rats but slight in de-endothelized preparations from SHR. In all indomethacin- and CGS-27830-treated preparations, and also in the preparations from WKY rats and SHR treated with both drugs, we observed a decrease in all the contractile responses with increased Ca^{2+} concentration. Besides, there was a clear reduction in the responses of the α_1-adrenoceptor agonists in the WKY and SHR preparations treated with both drugs. The results indicate that, in the hypertensive arteries, endothelium-derived contractile factors can counteract the relaxing effect of high extracellular Ca^{2+} concentrations.

Keywords: Endothelial vasoconstrictor factor; Ca^{2+} extracellular; Spontaneously hypertensive, rat (SHR)

1. Introduction

The ability of elevated levels of extracellular Ca^{2+} to reduce the contractile response of vascular smooth muscle has been long recognized. Bohr (1963) demonstrated that the fast component of the noradrenaline response in rabbit aorta strips decreased when the Ca^{2+} concentration in the organ bath increased between 0.2 and 3.2 mM. Later, this group also observed that supraphysiological Ca^{2+} concentrations relaxed rat aorta strips precontracted with noradrenaline (Webb and Bohr, 1978) and could also decrease the contraction induced by KCl in rat aorta rings (Wu and Bohr, 1991). These researchers suggested that the increase in extracellular Ca^{2+} could facilitate the synthesis of the endothelial relaxing factors. They observed that the increase in the Ca^{2+} bath concentration caused a greater relaxation in the rat aorta rings when the preparations were intact than when they were de-endothelized (Wu and Bohr, 1991). Other researchers have also established that the increase in extracellular Ca^{2+} within the physiological range favours the synthesis and/or release of endothelial relaxing factor (Luckhoff et al., 1988; White and Martin, 1989; López-Jaramillo et al., 1990). Our group has also managed to establish that the increase in Ca^{2+} bath concentration causes a decrease in the contractile responses induced by both depolarization and α_1-adrenoceptor stimulation in rabbit aorta rings (Ortega et al., 1997).

The endothelial cells become dysfunctional in pathological situations and many studies have described abnormal endothelium-dependent responses in hypertensive blood

* Corresponding author. Tel.: +91/3941475; fax: +91/3941463.
E-mail address: Amaya@eucmax.sim.ucm.es (M.A. Aleixandre de Artiñano).

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vessels (Lüscher et al., 1992; Vanhoutte and Boulanger, 1995; Vanhoutte, 1996; Ferro and Webb, 1997). The spontaneously hypertensive rat (SHR) has proved to be particularly useful for studying the mechanisms involved in the endothelial dysfunction conditioned by hypertensive vasoconstriction. In these animals, the hypertensive process may result from a greater production of vasoconstrictor cyclooxygenase-dependent endoperoxides and endothelin (Vanhoutte, 1996).

It is possible that the effect of a high extracellular Ca^{2+} concentration on vascular smooth muscle responses is different under normotensive and under hypertensive conditions, and more specifically, it is possible that in hypertensive subjects, hyperproduction of endothelial contractile factors may counteract the vasodilator effect of Ca^{2+}. Therefore, we now studied the contraction induced by both depolarization and α_1-adrenoceptor stimulation in rat aorta rings from SHR and their normotensive control, the Wistar Kyoto rats (WKY), with different Ca^{2+} concentrations in the organ bath, including physiological and supraphysiological levels, and we also carried out experiments on de-endothelized preparations, on preparations treated with the cyclooxygenase inhibitor, indomethacin, on preparations treated with the potent nonpeptide endothelin receptor antagonist, CGS-27830 [meso-1,4-dihydro-5-methoxycarbonyl-2,6-dimethyl-4-(3-nitrophenyl)-3-pyridine carboxylic acid anhydride] (Mugrage et al., 1993), and on preparations treated simultaneously with both compounds.

2. Materials and methods

2.1. Preparations

Adult 20-week-old male SHR and WKY were decapitated. The thorax was opened and the aorta from the aortic arch to the diaphragm was rapidly excised and transferred to a beaker containing a low-bicarbonate physiological salt solution of the following composition (mM): 118.2 NaCl; 4.7 KCl; 2.5 $CaCl_2$; 6.25 $NaHCO_3$ and 10.0 glucose. The low bicarbonate concentration allows the Ca^{2+} concentration to increase without precipitation. A portion of the extracted aorta was cleaned of surrounding connective and fat tissue and cut into rings about 1.5 to 2 mm in width. The de-endothelized preparations used in this study were prepared by gently rubbing the tissue before it was cut into rings. For the experiments the aorta rings were always suspended between two stainless steel hooks immersed in organ baths that also contained the same medium kept at 37 °C, and constantly bubbled with 95% O_2 and 5% CO_2 (pH = 7.3). The preparations were mounted with a resting tension of 2 g and were left to equilibrate for 90 min. During this period the bathing solution was changed every 15 min.

2.2. Experimental protocols

Experiments were carried out with KCl and with selective α_1-adrenoceptor agonists (methoxamine and phenylephrine). There was a different design for the experiments with KCl and for those with α_1-adrenoceptor agonists, and they are both described below. This was necessary because of the different tissue responses to repeated administration of KCl on one hand, and the α_1-adrenoceptor agonists on the other. Identical successive doses of KCl in the tissue produce similar responses but repeated doses of α_1-adrenoceptor agonists to a single aorta preparation do not produce the same response and there is a clear potentiation of the effect, particularly with the first administrations.

2.2.1. KCl experiments

At the end of the equilibration period, successive contractions were induced by KCl (cumulative doses of 30 and

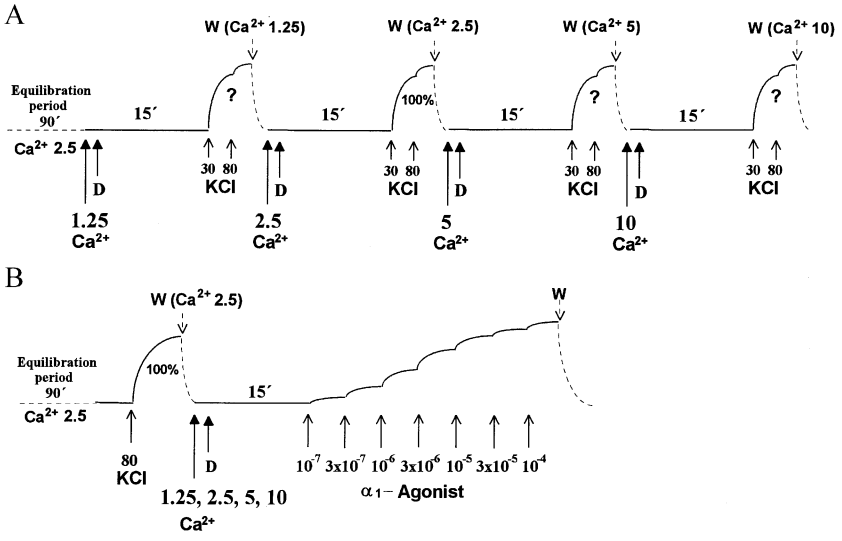

Fig. 1. Diagram of the protocol for the experiments with KC1 (A) and with α_1-adrenoceptor agonists (B). We indicate the moments when the drug(s) (D) was administered in the experiments carried out with 10^{-5} M indomethacin or 10^{-6} M CGS-27830. W = washing.

80 mM) in solutions with increasing Ca^{2+} (mM) concentrations of 1.25, 2.5, 5 and 10. The preparations were incubated for 15 min in the salt solution in which the effect of KCl was to be evaluated before the cumulative administration of this agent, and the contracted tissue was always relaxed by washing with a solution having the same Ca^{2+} concentration as used to generate this contraction. The response to 80 mM KCl in the 2.5 mM Ca^{2+} solution was always taken as the 100% response serving to compare the remaining responses.

2.2.2. α_1-Adrenoceptor agonist experiments

At the end of the equilibration period, the aorta rings were first contracted with 80 mM KCl. When the contraction had reached steady state, the preparations were washed with the same Ca^{2+} 2.5 mM solution as used for the equilibration period until the basal tension was re-established. Then the preparations were incubated for 15 min in the solution in which the α_1-adrenoceptor agonist responses were to be evaluated. Saline solutions with Ca^{2+} (mM) concentrations of 1.25, 2.5, 5 and 10 were used for these evaluations. After the 15-min incubation period, the α_1-adrenoceptor agonist was added in increasing cumulative doses and we made only one methoxamine or phenylephrine dose-response curve for each aorta ring. The initial contraction produced by 80 mM KCl in 2.5 mM Ca^{2+} was taken as 100% response and served to quantify the subsequent α_1-adrenoceptor agonist responses.

The previously described experiments with KCl and α_1-adrenoceptor agonists were done with intact and endothelium-disrupted preparations. They were also done with three other groups of intact preparations, the first with the addition of indomethacin (10^{-5} M), the second with the addition of CGS-27830 (10^{-6} M), and the third with the addition of both drugs to the incubation salt solutions in which the effect of KCl or the α_1-adrenoceptor agonists was to be evaluated.

Fig. 1 shows a diagram of the protocol followed for the experiments both with KCl and with α_1-adrenoceptor agonists. We indicate the moments when the drug(s) (indomethacin, CGS-27830, or both compounds) were added.

2.3. Statistical procedure

The results are always expressed as mean values ± S.E.M. for a minimum of five to seven experiments. Student's *t*-test was used to compare mean values and $P \leq 0.05$ indicates statistical significance.

2.4. Drugs

The following drugs were used: methoxamine HCl (Sigma), L-phenylephrine HCl (Sigma), indomethacin (Sigma) and CGS-27830 (Ciba-Geigy Pharmaceuticals). The solutions for the experiments were prepared daily. Methoxamine and phenylephrine were dissolved in distilled

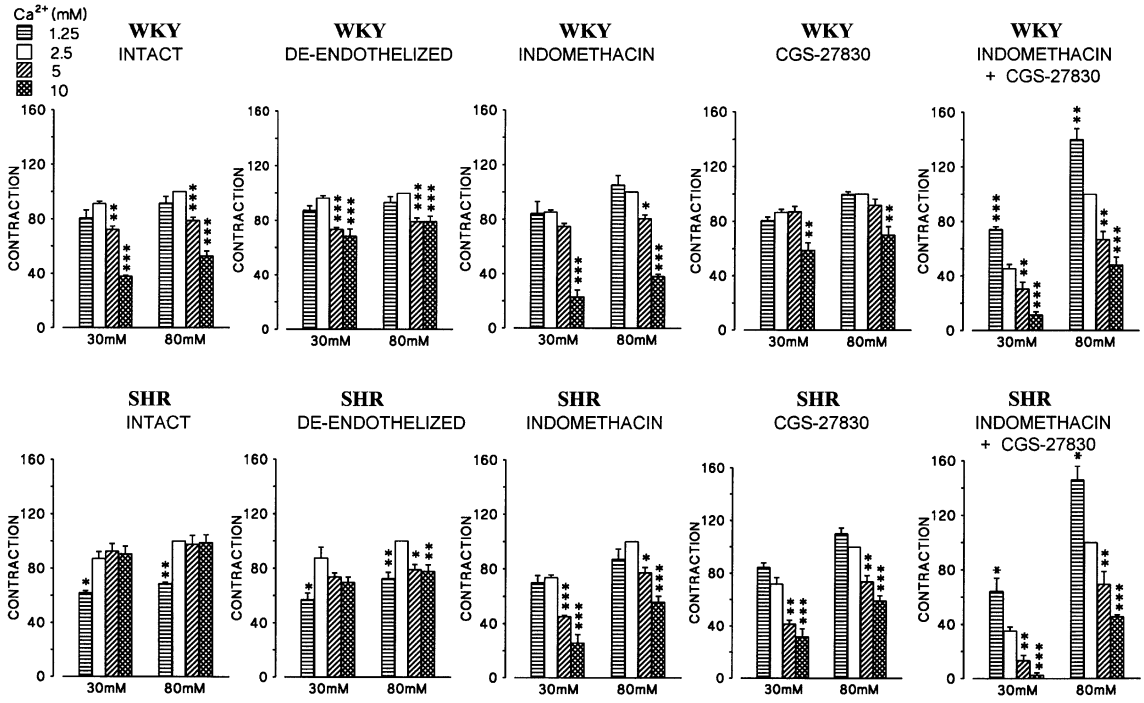

Fig. 2. Histograms of the contractions produced by 30 mM KCl and 80 mM KCl, in different aorta ring preparations (intact, de-endothelized, 10^{-5} M indomethacin-treated, 10^{-6} M CGS-27830-treated, and treated simultaneously with 10^{-5} M indomethacin and 10^{-6} M CGS-27830) from Wistar Kyoto (WKY) rats and spontaneously hypertensive rats (SHR), with different Ca^{2+} (mM) concentrations in the bath. The data represent the mean ± S.E.M. for five to seven experiments, taking the 80 mM KCl-induced contraction in 2.5 mM Ca^{2+} as 100. The asterisks indicate significant differences from the contractions obtained in the 2.5 mM Ca^{2+} solution ($*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$).

water. Indomethacin was dissolved in distilled water containing 50% ethanol, and CGS-27830 was dissolved in dimethyl sulfoxide (DMSO). The drug concentrations are expressed as final molar concentrations in the bath solutions and when we used a medium other than distilled water to dissolve the drug, we confirmed that the maximum quantity added of this medium had no effect on the preparations.

3. Results

In the intact aorta ring preparations from WKY rats, the responses to KCl were maximal when the Ca^{2+} concentration in the organ bath was 2.5 mM, and these responses were very similar to those obtained when the Ca^{2+} concentration was 1.25 mM. Nevertheless, KCl contractions decreased when the Ca^{2+} concentration in the organ bath was higher than 2.5 mM, and the decrease was more pronounced when the Ca^{2+} concentration was 10 mM than when the Ca^{2+} concentration was 5 mM. On the other hand, in intact aorta ring preparations from SHR, the responses to KCl were similar when the Ca^{2+} concentration in the organ bath was 2.5, 5 or 10 mM, and they were smaller when the Ca^{2+} concentration was 1.25 mM.

In the de-endothelized aorta ring preparations from WKY rats, the responses to KCl also decreased when the Ca^{2+} concentration in the organ bath was higher than 2.5 mM. Nevertheless, this was a slightly smaller decrease than that observed in intact preparations from the same strain. In the de-endothelized preparations from SHR we found a decrease in KCl responses when the Ca^{2+} concentration in the organ bath was higher than 2.5 mM, and it should be noted that this decrease was not observed in intact preparations from the animals.

The responses to KCl in the indomethacin-treated preparation from WKY rats and those in the indomethacin-treated preparations from SHR both decreased when the Ca^{2+} concentration in the organ bath was higher than 2.5 mM, and the decrease was more accentuated when the Ca^{2+} concentration was 10 mM than when it was 5 mM.

The responses to KCl in the CGS-27830-treated preparations from WKY rats were similar when the Ca^{2+} concentration in the organ bath was 1.25, 2.5 and 5 mM, but these contractions decreased when the Ca^{2+} concentration in the organ bath was 10 mM. In CGS-27830-treated preparations from SHR, the greatest KCl contractions were obtained using 1.25 mM Ca^{2+} in the organ bath, and the contractions for this agent were similar with 2.5 mM Ca^{2+}

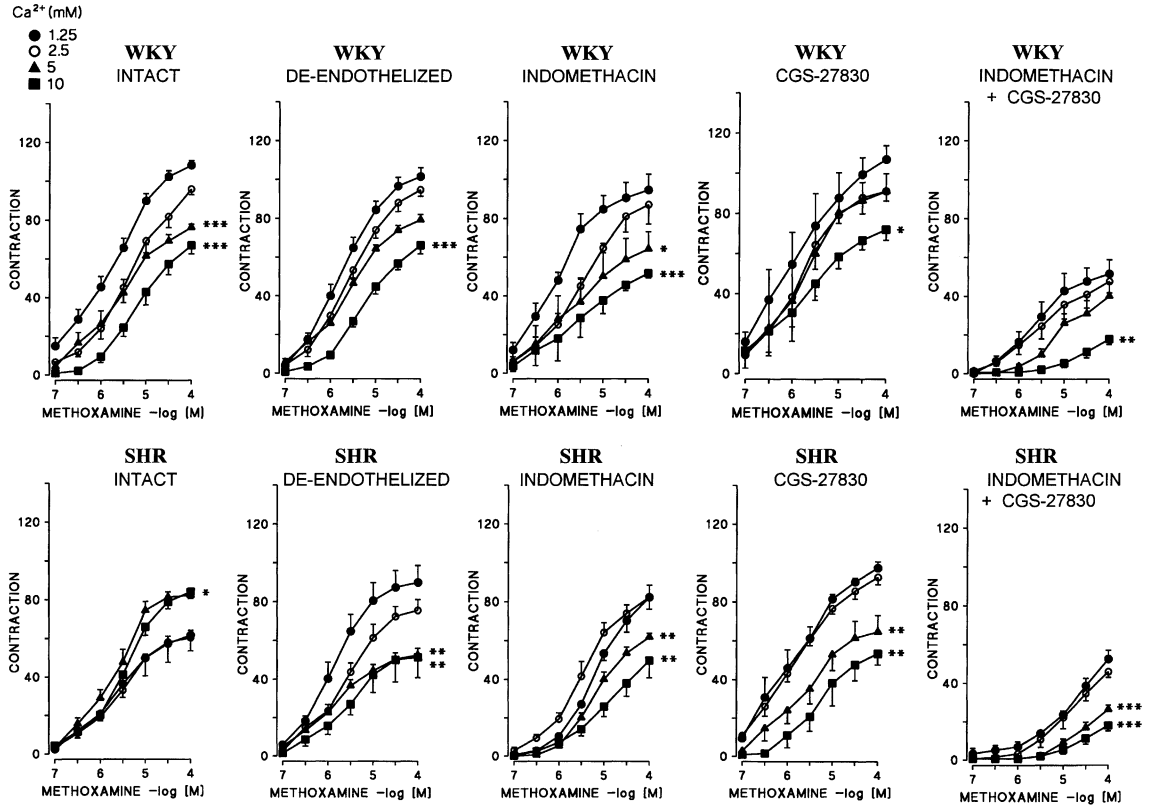

Fig. 3. Cumulative dose-response curve for methoxamine with different aorta ring preparations (intact, de-endothelized, 10^{-5} M indomethacin-treated, 10^{-6} M CGS-27830-treated, and treated simultaneously with 10^{-5} M indomethacin and 10^{-6} M CGS-27830) from Wistar Kyoto (WKY) rats and spontaneously hypertensive rats (SHR), with different Ca^{2+} (mM) concentrations in the bath. The data represent the mean ± S.E.M. for five to seven experiments, taking the 80 mM KCl induced contraction in 2.5 mM Ca^{2+} as 100. The asterisks indicate significant differences from the contractions obtained in the 2.5 mM Ca^{2+} solution ($*P \leq 0.5$; $**P \leq 0.01$; $***P \leq 0.001$).

in the bath. In these preparations, KCl contractions decreased when the Ca^{2+} concentration in the bath was even higher, and the decrease was more accentuated with 10 mM Ca^{2+} in the bath than with 5 mM Ca^{2+}.

The responses to KCl in the preparations from WKY and SHR treated with both indomethacin and CGS-27830 decreased when the Ca^{2+} concentration in the organ bath increased, and the greatest KCl contractions in all these preparations were obtained using 1.25 mM in the organ bath.

Fig. 2 shows the contractile effect of KCl in the above-mentioned rat aorta ring preparations from WKY and SHR with different Ca^{2+} concentrations in the organ bath.

In aorta ring preparations from WKY rats, maximal responses to methoxamine and phenylephrine were obtained with 1.25 mM Ca^{2+} in the organ bath, and we observed a decrease of these contractions with higher Ca^{2+} concentrations. Moreover, the greater the increase in the Ca^{2+} concentration the sharper the decrease in these contractions. On the contrary, we did not observe a decrease in α_1-adrenoceptor contractions in the aorta ring preparations from SHR when we increased the Ca^{2+} bath concentration over 1.25 mM. The contractions in response to methoxamine in these preparations were greater with 5 and 10 mM Ca^{2+} than with 1.25 and 2.5 mM Ca^{2+}. The contractions in response to phenylephrine were similar with all the Ca^{2+} concentrations.

When the endothelium was disrupted in both the preparations from WKY rats and SHR, the greatest contractions to methoxamine were obtained using 1.25 mM Ca^{2+} in the organ bath, and these contractions decreased when Ca^{2+} concentration increased over this value. Moreover, as was the case with the intact WKY rat preparations, the greater the increase in the Ca^{2+} concentration the sharper the decrease in these contractions. When the agonist was phenylephrine, the decrease in contractions caused by high Ca^{2+} concentrations was also clear in de-endothelized preparations from WKY. In the de-endothelized preparations from SHR the contractions were similar with all the Ca^{2+} concentrations used, although in this case they were also slightly smaller with the higher Ca^{2+} concentrations.

The responses to methoxamine and phenylephrine in the indomethacin-treated preparations from WKY rats always decreased when the Ca^{2+} concentration in the organ bath increased. The responses to phenylephrine in the indomethacin-treated preparations from SHR also decreased when the Ca^{2+} concentration in the organ bath increased. The responses to methoxamine in these preparations were similar

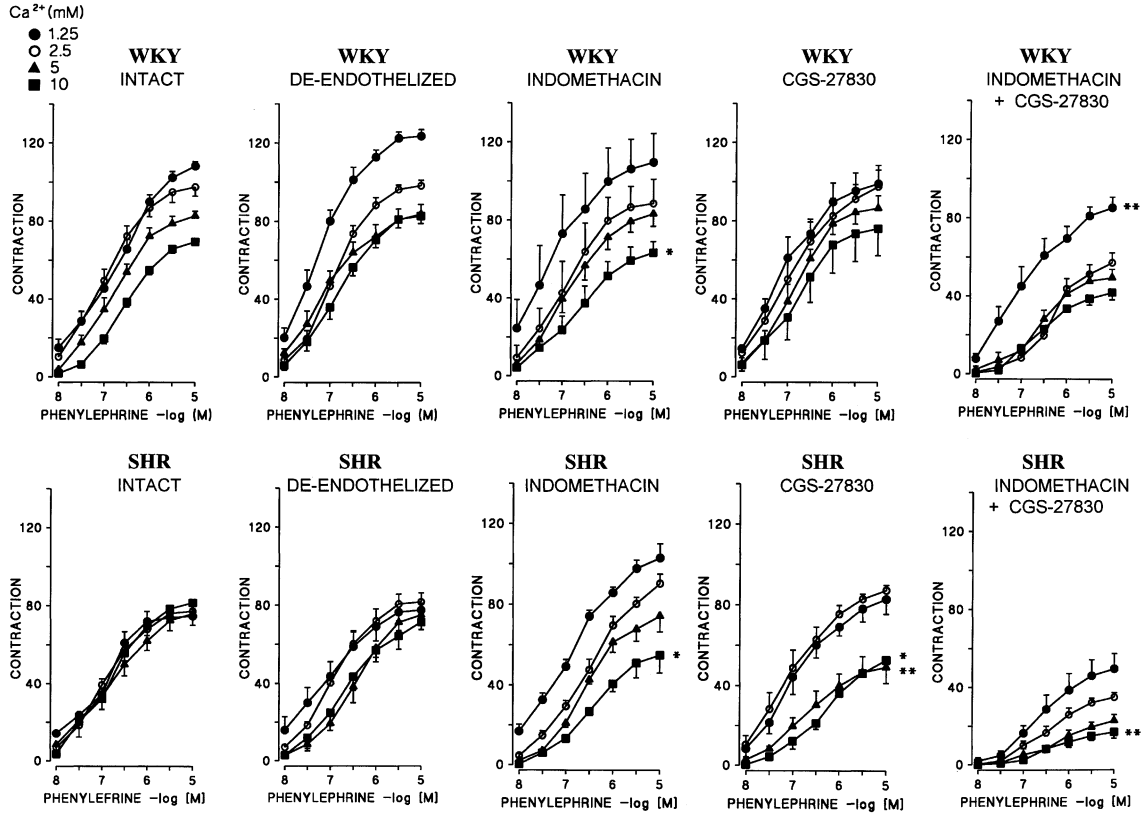

Fig. 4. Cumulative dose-response curve for phenylephrine with different aorta ring preparations (intact, de-endothelized, 10^{-5} M indomethacin-treated, 10^{-6} M CGS-27830-treated, and treated simultaneously with 10^{-5} M indomethacin and 10^{-6} M CGS-27830) from Wistar Kyoto (WKY) rats and spontaneously hypertensive rats (SHR), with different Ca^{2+} (mM) concentrations in the bath. The data represent the mean ± S.E.M. for five to seven experiments, taking the 80 mM KCl induced contraction in 2.5 mM Ca^{2+} as 100. The asterisks indicate significant differences from the contractions obtained in the 2.5 mM Ca^{2+} solution (* $P \leq 0.5$; * * $P \leq 0.01$; * * * $P \leq 0.001$).

with 1.25 and 2.5 mM Ca^{2+} and decreased with higher Ca^{2+} concentrations (5 and 10 mM).

Likewise, the responses to methoxamine and phenylephrine in the CGS-27830-treated preparations from WKY rats always decreased when the Ca^{2+} concentration in the organ bath increased. The responses to both methoxamine and phenylephrine in CGS-27830-treated preparations from SHR were very similar with 1.25 and 2.5 mM Ca^{2+} and also decreased with higher Ca^{2+} concentrations (5 and 10 mM).

The contractile responses to the α_1-adrenoceptor agonists in the preparations from WKY and SHR treated with indomethacin and CGS-27830 were smaller than those observed when these agonists had been added in the other groups of preparations. The decrease was more accentuated in the preparations from the SHR, nevertheless, the contractions elicited by the α_1-adrenoceptor agonists in all the preparations of both strains treated with indomethacin and CGS-27830 decreased even more when the Ca^{2+} concentration in the organ bath increased, and the greatest contractions elicited by methoxamine and phenylephrine in the preparations treated simultaneously with both drugs were observed using 1.25 mM Ca^{2+} in the organ bath.

Figs. 3 and 4 show the contractile effect of methoxamine and phenylephrine, respectively, in the above mentioned rat aorta ring preparations from WKY and SHR with different Ca^{2+} concentrations in the organ bath.

4. Discussion

We now studied the modification of vascular smooth muscle contraction in hypertensive situations when extracellular Ca^{2+} increased. We used aorta preparations from SHR and from their normotensive control, WKY rats.

We observed that high Ca^{2+} concentrations (5 and 10 mM) caused a clear decrease in KCl and α_1-adrenoceptor-mediated contractions in the preparations from normotensive rats, but in contrast, high extracellular Ca^{2+} concentrations were not able to diminish KCl and phenylephrine contractions in SHR preparations, and even caused an increase in methoxamine contractions in these preparations. In 1992, Chai and Webb (Chai and Webb, 1992) also reported that the decrease in noradrenaline contraction caused by high extracellular Ca^{2+} concentrations was greater in the tail artery of normotensive rats than in the same artery of hypertensive rats. These differences between arterial preparations from normotensive and hypertensive rats are undoubtedly a consequence of the changes produced in vascular tissue with the onset of hypertension. As we mentioned in the Introduction, the effect of high extracellular Ca^{2+} levels may be linked to a change in the release of the endothelial factors, which control vascular tone, and indeed many experimental (Konishi and Su, 1983; Gray and De Mey, 1985; Hongo et al., 1988; Dohi et al., 1990; Lüscher et al., 1992) and clinical studies (Calver et al., 1992; Panza et al., 1990, 1993, 1994, 1995; Taddei et al., 1993; Lyons et al., 1997) associate hypertension with alterations in the synthesis and/or the effect of these factors. Bearing all this in mind, we also carried out experiments with de-endothelized aorta preparations from WKY and SHR. In these experiments, we observed that endothelial disruption in normotensive rat preparations did not clearly prevent a decrease in KCl and α_1-adrenoceptor-mediated responses when Ca^{2+} was increased in the organ bath. The study carried out by our research group in 1997 also demonstrated that high extracellular Ca^{2+} concentrations continued to cause a decrease in contractility when de-endothelized rabbit aorta ring preparations were used (Ortega et al., 1997). It would therefore be logical to think that, under normotensive conditions, the main mechanism involved in the high extracellular Ca^{2+} relaxing effect is not the change in endothelial function. The results obtained with de-endothelized preparations from SHR were however different from those obtained with the intact SHR preparations. In the latter, we observed no decrease in contractions when Ca^{2+} was increased in the organ bath, but when the endothelium was destroyed in the aorta from these animals, we observed a decrease in the effect of all the vasoconstrictors used after the increase in extracellular Ca^{2+}. It would seem paradoxical that this decrease was more accentuated when methoxamine was used and this agonist had caused greater contractions with high Ca^{2+} levels in the intact SHR preparations. The differences between the results obtained with methoxamine and those obtained with phenylephrine should be investigated further but these differences could be a consequence of the α-adrenoceptor subtypes implicated in the responses. The responses to methoxamine could be more influenced by the extracellular Ca^{2+} levels because this agonist selectively stimulates the α_{1A}-adrenoceptor subtype that couples with Ca^{2+} influx (Tsujimoto et al., 1989). In contrast, phenylephrine stimulates α_{1B}-adrenoceptors, and the contractile responses are mainly caused by the release of Ca^{2+} from the internal pools (Han et al., 1987; Minneman, 1988; Ruffolo and Hieble, 1994; Buscher et al., 1999). In any case, all these data suggest that the endothelium of hypertensive rats synthesizes contracting factors, which make it impossible to see the relaxing effect of high extracellular Ca^{2+} levels.

The results obtained with de-endothelized preparations from SHR led us to include experiments in which the preparations from such rats were treated with drugs able to inhibit the synthesis of vasoconstrictor cyclooxygenase-dependent endoperoxides (indomethacin) or the effect of endothelin (CGS-27830), both of which are endothelial factors specifically associated with hypertension in these animals. Similar experiments were carried out with WKY.

The inhibition of cyclooxygenase in the SHR preparations also made it possible to observe the decrease in KCl and α_1-adrenoceptor-mediated responses when Ca^{2+} was increased in the bathing solution. Moreover, the decrease in KCl and phenylephrine contractions was greater than that in

the de-endothelized preparations from these animals. The synthesis of vasoconstrictor cyclooxygenase-derived compounds in hypertensive rats could therefore play an important role in countering the relaxing effect of high extracellular Ca^{2+} levels. In addition, according to some workers these vasoconstrictors could be synthesized in the vascular smooth muscle from the hypertensive rat itself (Vanhoutte, 1996). For this reason, the treatment with indomethacin could have been more effective for observing the Ca^{2+} effect in these animals than the disruption of the endothelium. It should also be mentioned that, surprisingly, when Ca^{2+} increased the decrease in contraction was greater in the indomethacin-treated preparations from WKY rats, than that in their intact or de-endothelized preparations. It had been held that WKY rats are prehypertensive because the SHR strain derives from them (Wright and Rankin, 1982). This prehypertensive condition could perhaps explain the results we obtained in indomethacin-treated preparations from WKY rats in this study. An imbalance between the synthesis of the various factors that control arterial tone might exist in the endothelium of these animals. The relationship between the contractile factors, which counteract the extracellular Ca^{2+} effect, and the relaxing factors, may be stronger in these rats than the same relationship in the endothelium of purely normotensive animals.

The results obtained in the CGS-27830-treated preparations from SHR indicate that the vasoconstrictor effects of endothelin in these animals could be an additional factor preventing the Ca^{2+} relaxing effect. In fact, as occurred with the indomethacin-treated preparations from SHR, in these preparations treated with this endothelin receptor antagonist, we observed that the contractions induced by KCl, methoxamine and phenylephrine clearly decreased when high extracellular Ca^{2+} concentrations were used. In the case of KCl and phenylephrine, the decrease was greater than that in the de-endothelized preparations from SHR and this could be explained by the fact that endothelin-1 synthesis has also been detected in smooth muscle cells from SHR aorta (Hahn et al., 1990). The results obtained when the extracellular Ca^{2+} concentration increased in the CGS-27830-treated preparations from WKY rats are not very different from those obtained when we increased Ca^{2+} in the organ bath with intact preparations from these animals. We observed that the contraction decreased slightly less in the former but it should not be forgotten that endothelin also has vasodilator effects and that these effects are particularly evident under physiological conditions (Miyauchi et al., 1989; Warner et al., 1989; Masaki, 1995). The vasodilator endothelin action could perhaps favour the weakening of the contraction produced by high extracellular concentrations in these animals.

It was not surprising to have found a clear decrease in the responses elicited by KCl and the α_1-adrenoceptor agonists in the preparations from SHR treated simultaneously with indomethacin and CGS-27830, when the Ca^{2+} concentration in the organ bath was increased, because in these preparations neither the vasoconstrictor cyclooxygenase-derived compounds nor endothelin can counteract the Ca^{2+} relaxing effect. It is therefore evident that the vasoconstrictor cyclooxygenase-derived compounds and endothelin play an important role in the excess of arterial tone that characterizes hypertension in SHR. As mentioned in Results, when the α_1-adrenoceptor agonists were added to the preparations from SHR and WKY treated with indomethacin and CGS-27830, the contractile responses were clearly smaller than the corresponding responses of the remaining preparations from these rats. Moreover, the decrease in contractions elicited by the α_1-adrenoceptor agonists was greater in the preparations from the hypertensive animals. This fact could also be explained by the role that endothelium-derived vasoconstrictors play in modulating vascular smooth muscle contractile activity in hypertensive and prehypertensive situations.

The present results may serve to emphasize the differences between the endothelium of hypertensive animals and that of normotensive animals, and also to an awareness that these differences may condition the changes in the responses of the vascular smooth muscle when extracellular Ca^{2+} increases. It should, however, be pointed out that our results obtained in vitro using arteries from normotensive and hypertensive animals and increasing Ca^{2+} in the organ bath are to some degree paradoxical, if we bear in mind the results obtained by different researchers (see reviews Aleixandre and Puerro, 1993; Aleixandre et al., 1993; Hatton and McCarron, 1994) and also by our research group (Civantos et al., 1998) when the arterial blood pressure of normotensive and hypertensive rats was measured following the administration of Ca^{2+} supplements in the diet. In these studies it was observed that the increase in dietary Ca^{2+} and the subsequent increase in the calcemia of the animals led to a decrease in arterial blood pressure, which was greater in the hypertensive animals. Ca^{2+} supplements are also more effective for modifying blood pressure in hypertensive patients than in normotensive subjects (Grobbee and Waal-Manning, 1990; Singh et al., 1990; Pryer et al., 1995; Allender et al., 1996). It should however be remembered that some of the physiological mechanisms, which control arterial blood pressure and which may be modified when dietary Ca^{2+} supplements are administered, cannot condition the results obtained from experiments with isolated arteries. Moreover, a further difference between in vitro experiments with high extracellular Ca^{2+} levels and experiments in animals fed a Ca^{2+}-enriched diet is that, in the organ bath, Ca^{2+} may increase much more than in the plasma. It should also be borne in mind that the effect of high extracellular Ca^{2+} may vary in different vascular beds. It would perhaps be a good idea to carry out experiments with high Ca^{2+} bath concentrations, using isolated resistance arteries, as well as to study the relaxing effect of high extracellular Ca^{2+} in other species. In any case, our study is an important

contribution to this line of research as our results show that the vascular smooth muscle from normotensive and hypertensive animals responds very differently when extracellular Ca^{2+} increases, and also highlight the importance of endothelial alterations in maintaining the arterial tone in hypertensive situations.

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