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European Journal of Pharmacology 382 (1999) 91–101

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α-Adrenoceptor-mediated pressor responses in pithed rats fed diets with different calcium contents

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Received 18 February 1999; received in revised form 27 July 1999; accepted 30 July 1999

Abstract

Results of many clinical and experimental studies indicate an inverse relationship between dietary calcium and the prevalence of hypertension. Our study was designed to evaluate the alterations in arterial blood pressure and the changes in α-adrenoceptor-mediated vascular reactivity in normotensive Sprague–Dawley and spontaneously hypertensive rats (SHR) fed from weaning (3 weeks of life) three diets: normal calcium (Ca 1%), low calcium (Ca 0.1%), and high calcium (Ca 2.5%). The systolic and the diastolic arterial blood pressures were measured weekly by the tail cuff method. The plasma calcium levels in the animals were also measured regularly by colourimetric methods, and the α-adrenoceptor-mediated vascular reactivity was evaluated by measuring the pressor responses to α-adrenoceptor agonists in pithed rats. These determinations were carried out at the end of the feeding periods (9 weeks of life in Sprague–Dawley rats and 20 weeks of life in SHR) and also at the moments when maximal differences in arterial blood pressure were observed between the conscious animals fed the normal calcium diet and those fed the other two diets. Dietary calcium deficiency increased arterial blood pressure in both strains but calcium supplements were effective to lower this only in hypertensive animals. The plasma calcium levels were altered in both strains when calcium administration was not normal. The low-calcium diet did not modify the pressor responses to either the α_1-adrenoceptor agonist, methoxamine, or the α_2-adrenoceptor agonist, B-HT 920 (5-allyl-2-amino-5,6,7,8-tetrahydro-4*H*-thiazolo-(4,5-D)-acepin-dihydrochloride, talixepole), in the normotensive and the hypertensive rats. On the contrary, the high-calcium diet caused a definite decrease in α_1- and α_2-adrenoceptor-mediated vascular reactivity in both strains. The changes in the α-adrenoceptor-mediated vasoconstrictor responses were observed in pithed 9-week old Sprague–Dawley rats and in pithed 20-week old SHR, but none were observed in pithed 15-week old SHR, although at this age maximal differences in arterial blood pressure between the animals fed the high- and the normal calcium diet were observed. The results of this study suggest that the mechanisms implicated in the effects of dietary calcium supplements on arterial blood pressure are clearly different from the mechanisms, which bring about changes in arterial blood pressure when the diet is deficient in calcium. The results of this study also show that calcium administration causes variations in α-adrenoceptor-mediated vascular reactivity, but this is probably not the only mechanism implicated in the calcium effect on arterial blood pressure.

Keywords: Calcium, dietary; Blood pressure; α-Adrenoceptor

1. Introduction

It is known that the administration of calcium is paradoxically associated with a decrease in arterial blood pressure. For this reason, clinical trials have been carried out with the aim of controlling arterial blood pressure with calcium supplements, and it has been observed that calcium administration shows its effects in hypertensive patients, yet causes little variation in systolic and diastolic arterial blood pressure in normotensive subjects. Dietary calcium deficiency may, on the contrary, be associated with an increase in arterial blood pressure, and could potentiate the pressor effect of the sodium-enriched diets (Grobbee and Waal-Manning, 1990; Singh et al., 1990; Aleixandre and Puerro, 1993; Aleixandre et al., 1993; Pryer et al., 1995; Allender et al., 1996).

Many experimental studies in the last few years have also shown that calcium decreases arterial blood pressure

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0014-2999/99/$ - see front matter
PII: S0014-2999(99)00556-7

in rats and that this effect is particularly significant in hypertensive animals. Arterial blood pressure in young or pregnant rats was likewise increased by dietary calcium deficiency (Aleixandre and Puerro, 1993; Aleixandre et al., 1993; Hatton and McCarron, 1994).

Various mechanisms seem to be implicated in the blood pressure-lowering effect of calcium and it has been suggested that calcium administration may cause, among other changes, variations in vascular reactivity. However, studies with arteries obtained from rats fed diets with different calcium contents produced contradictory results (Arvola et al., 1990; Mangiarua et al., 1990; Porsti, 1992; Porsti et al., 1990a,b, 1992; Mäkynen et al., 1994, 1995, 1996). Of course, it is not always possible for in vitro studies to reflect the changes, which occur in vivo. Nevertheless, we also have to bear in mind that experimental studies have on occasion shown changes in pressor responses when an agent was administered to animals given calcium supplements, but these modifications were not found when a different agonist was used (Stern et al., 1987; Scrogin et al., 1991; Sallinen et al., 1996). Of particular note is the work of Hatton et al., which demonstrates a decrease in the pressor responses to noradrenaline in spontaneously hypertensive rats (SHR) fed a calcium-enriched diet, although the responses to angiotensin II remain unchanged in these animals (Hatton et al., 1989, 1993). In addition, the results of the study, which this group of researchers carried out in 1993 (Hatton et al., 1993), showed that calcium in the diet especially modifies the responses mediated by the stimulation of vascular α_1-adrenoceptors. Other researchers had also suggested that variations in vascular reactivity caused by changes in dietary calcium could be especially associated with alterations in the pressor responses mediated by the stimulation of vascular α-adrenoceptors (Pernot et al., 1990a,b; Hano et al., 1991).

We now studied the possible alterations in arterial blood pressure and in plasma calcium levels caused by changes in dietary calcium in hypertensive and normotensive rats when these animals are fed diets with different calcium contents for long periods. The main objective was to establish whether these possible changes are accompanied by alterations in the α_1- and α_2-adrenoceptor-mediated vasoconstrictor responses. The SHR is a well-established model for human hypertension. Therefore, we have chosen these animals as hypertensive strain. In previous studies (López-Miranda et al., 1996a, 1998), and also in the present one, we have chosen the Sprague–Dawley rats as a normotensive strain. This strain is clearly normotensive and has no genetic condition, which predisposes it to develop hypertension (Wright and Rankin, 1982). In order to evaluate the α_1- and α_2-adrenoceptor-mediated vasoconstrictor responses, we have chosen the pithed rat preparation. This is an experimental model in which the animal's central nervous system is destroyed thereby permitting an evaluation of in vivo vascular reactivity, since the drug-induced pressor responses only reflect peripheral effects.

2. Methods

2.1. Experimental procedure

Two rat strains were used for this study: Sprague–Dawley and SHR. After being weaned at 3 weeks, males of both strains were caged in groups of five at a temperature of 23°C with 12 h light/dark cycles, and were randomized with ad libitum intake of one of three possible diets: control semi-synthetic casein with a normal calcium content (Ca 1%), low calcium (Ca 0.1%) and high calcium (Ca 2.5%) (UAR, Villemoison, France). The composition of the three diets was the same in all respects (protein, carbohydrate, fat, vitamins and minerals) except for the calcium content. Most studies with rats use diets with a calcium content between 0.5% and 1% as a normal value. The lower figure corresponds to the level of calcium recommended for rats in the American Institute for Nutrition report (American Institute for Nutrition Ad Hoc Committee on Animal Nutrition, 1977), but in fact most normal feeds for rats have a 1% calcium content. Therefore, the

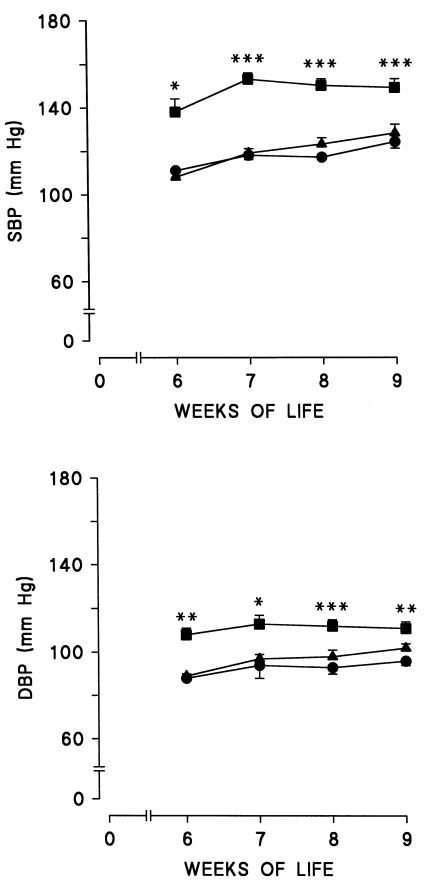

Fig. 1. Systolic (SBP) and diastolic (DBP) arterial blood pressure of Sprague–Dawley rats fed a normal calcium diet (Ca 1%) (circle), a low-calcium diet (Ca 0.1%) (square), or a high-calcium diet (Ca 2.5%) (triangle) from weaning. Data are shown as mean values ± S.E.M. for a minimum of six animals. The asterisks show significant differences from animals fed the Ca 1% diet (* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$).

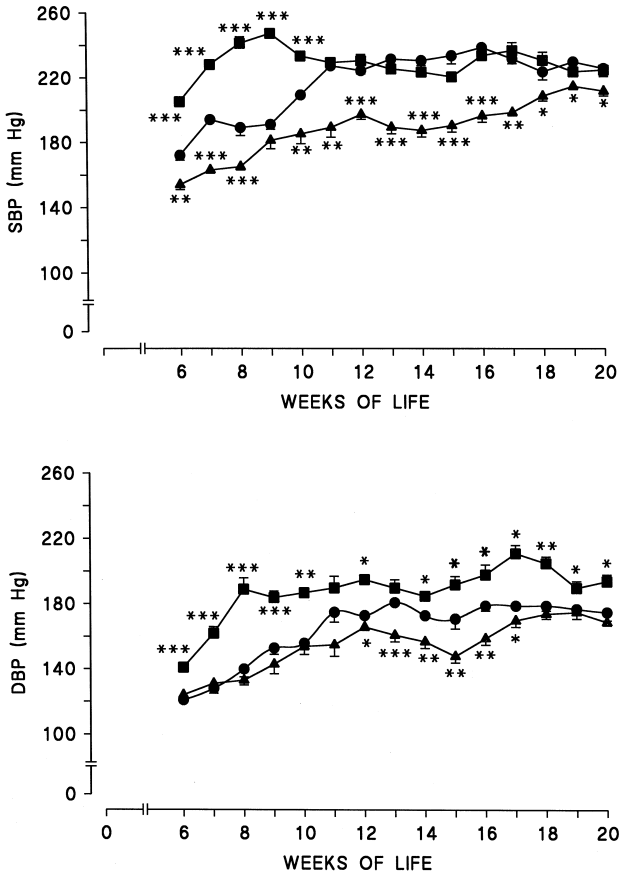

Fig. 2. Systolic (SBP) and diastolic (DBP) arterial blood pressure of SHR fed a normal calcium diet (Ca 1%) (circle), a low-calcium diet (Ca 0.1%) (square), or a high-calcium diet (Ca 2.5%) (triangle) from weaning. Data are shown as mean values ± S.E.M. for a minimum of six animals. The asterisks show significant differences from animals fed the Ca 1% diet (* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$).

values obtained when the animals were fed the Ca 1% diet were considered as the control data in this study.

Both systolic and diastolic arterial blood pressures were measured weekly, from the 6th week of life, in the conscious rats by the tail cuff method (Buñag, 1973). Even though the original method for measuring arterial blood pressure using the tail cuff provides only systolic arterial blood pressure values, the equipment used in this study, LE 5001 (Letica, Hospitalet, Barcelona, Spain), allows us to distinguish between systolic and diastolic arterial blood pressure as both measurements are given automatically. The rats were accustomed to the procedure during the 5th week of life, the age at which the animals are big enough to permit the use of our equipment for measuring arterial blood pressure. Experiments were performed at the same time of day in order to avoid the influence of the circadian rhythm. In addition, to minimize the effect of stress, the rats were also daily accustomed to the procedure before the actual start of the experiment. In Sprague–Dawley rats, a strain which does not usually develop hypertension, measurements were taken until the 9th week of life. At this age, the males of this strain are adult as they reach sexual maturity between 40–51 days of life (Poole, 1987). However, in the SHR, measurements were taken until the 20th week of life because this strain has a reduced growth rate as compared with the normotensive strain (López-Miranda et al., 1996a) and usually develops hypertension progressively when fed normal diets, reaching maximum hypertension levels only at the 18th–20th week of life (Okamoto and Aoki, 1963).

Determinations of plasma calcium levels, and experiments to evaluate vascular reactivity were carried out at the moments when maximal differences in the arterial blood pressure were observed between the conscious animals fed the normal calcium diet and those fed the other two diets, and also at the end of the respective feeding periods. In the Sprague–Dawley strain, maximal differences in arterial blood pressure were observed at the end of the feeding period when the 8- to 9-week old animals fed the low- or on the high-calcium diet were compared with the 8- to 9-week old animals fed the normal calcium diet. Therefore, these determinations and experiments were carried out on Sprague–Dawley rats only when the animals were 9 weeks old. However, bearing in mind the above-mentioned criteria, measurements in the SHR were performed at different times of the animals' life. Measurements were performed in the SHR fed the low-calcium diet when the animals were 8 weeks old (maximal differences in arterial blood pressure) and 20 weeks old (end of the feeding period), and in the SHR fed the high-calcium diet when the animals were 15 weeks old (age, which could represent the period with maximal differences in the arterial blood pressure) and 20 weeks old (end of the feeding period). In the SHR fed the normal calcium diet, plasma

Table 1
Total plasma calcium (Ca_T) (mM) and ionic plasma calcium (Ca_i^{2+}) (mM) in Sprague–Dawley rats (SDR) and spontaneously hypertensive rats (SHR) fed three diets with different calcium contents. Data are shown as mean values ± S.E.M. for a minimum of six experiments

	Dietary calcium		
	1%	0.1%	2.5%
SDR 9-week old			
Ca_T	2.40 ± 0.04	1.94 ± 0.06[a]	2.65 ± 0.04[a]
Ca_i^{2+}	1.20 ± 0.05	0.97 ± 0.02[a]	1.31 ± 0.02[a]
SHR 8-week old			
Ca_T	2.30 ± 0.01	1.58 ± 0.04[a]	
Ca_i^{2+}	1.15 ± 0.01	0.81 ± 0.02[a]	
SHR 15-week old			
Ca_T	2.33 ± 0.04		2.51 ± 0.03[b]
Ca_i^{2+}	1.19 ± 0.03		1.26 ± 0.02[c]
SHR 20-week old			
Ca_T	2.30 ± 0.03	2.30 ± 0.02	2.28 ± 0.04
Ca_i^{2+}	1.12 ± 0.01	1.08 ± 0.01[a]	1.17 ± 0.03

[a] Significant differences from the control diet, Ca 1%: $P \leq 0.001$.
[b] Significant differences from the control diet, Ca 1%: $P \leq 0.01$.
[c] Significant differences from the control diet, Ca 1%: $P \leq 0.05$.

Table 2
Systolic arterial blood pressure (SBP) (mm Hg) and diastolic arterial blood pressure (DBP) (mm Hg) in pithed SDR and pithed SHR fed three diets with different calcium contents. Data are shown as mean values ± S.E.M. for a minimum of six experiments. Significant differences from the control diet, Ca 1%: $P \leq 0.05$

	Dietary calcium		
	1%	0.1%	2.5%
SDR 9-week old			
SBP	58 ± 3	52 ± 3	37 ± 3[a]
DBP	47 ± 3	44 ± 3	28 ± 2[a]
SHR 8-week old			
SBP	31 ± 2	29 ± 2	
DBP	24 ± 1	22 ± 1	
SDR 15-week old			
SBP	55 ± 4		61 ± 6
DBP	38 ± 3		40 ± 4
SDR 20-week old			
SBP	54 ± 4	50 ± 2	35 ± 3[b]
DBP	39 ± 3	37 ± 2	26 ± 3[b]

[a] Significant differences from the control diet, Ca 1%: $P \leq 0.001$.
[b] Significant differences from the control diet, Ca 1%: $P \leq 0.01$.

calcium level determinations and experiments to evaluate vascular reactivity were performed when the animals were 8, 15, and 20 weeks old, and these could be used as a control for the rats fed the above diets. Moreover, in order to interpret our results, we also compared the vascular reactivity of the SHR fed the normal calcium diet at different times of the SHR's life.

In order to measure plasma calcium levels, Sprague–Dawley rats and SHR were killed by decapitation. The blood for these determinations was collected in heparinized tubes, which were centrifuged at 5000 × *g* at 2°C for 15 min. Following this, plasma was separated off. The total plasma calcium and proteins were measured using the O-Cresolphthalein complexone and the biuret colourimetric methods, respectively, and the ionic calcium concentration was indirectly estimated using these values according to Zeisler's formula (Weissman and Pileggi, 1980). The results are expressed as millimolar concentrations of total and ionic calcium.

In order to evaluate the vascular reactivity of the animals, dose–response curves were obtained for methoxamine (10 μg/kg–3000 μg/kg) and B-HT 920 (5-allyl-2-amino-5,6,7,8-tetrahydro-4*H*-thiazolo-(4,5-D)-acepin-dihydrochloride, talixepole) (3 μg/kg–1000 μg/kg) in pithed Sprague–Dawley rats and SHR fed the three diets. For

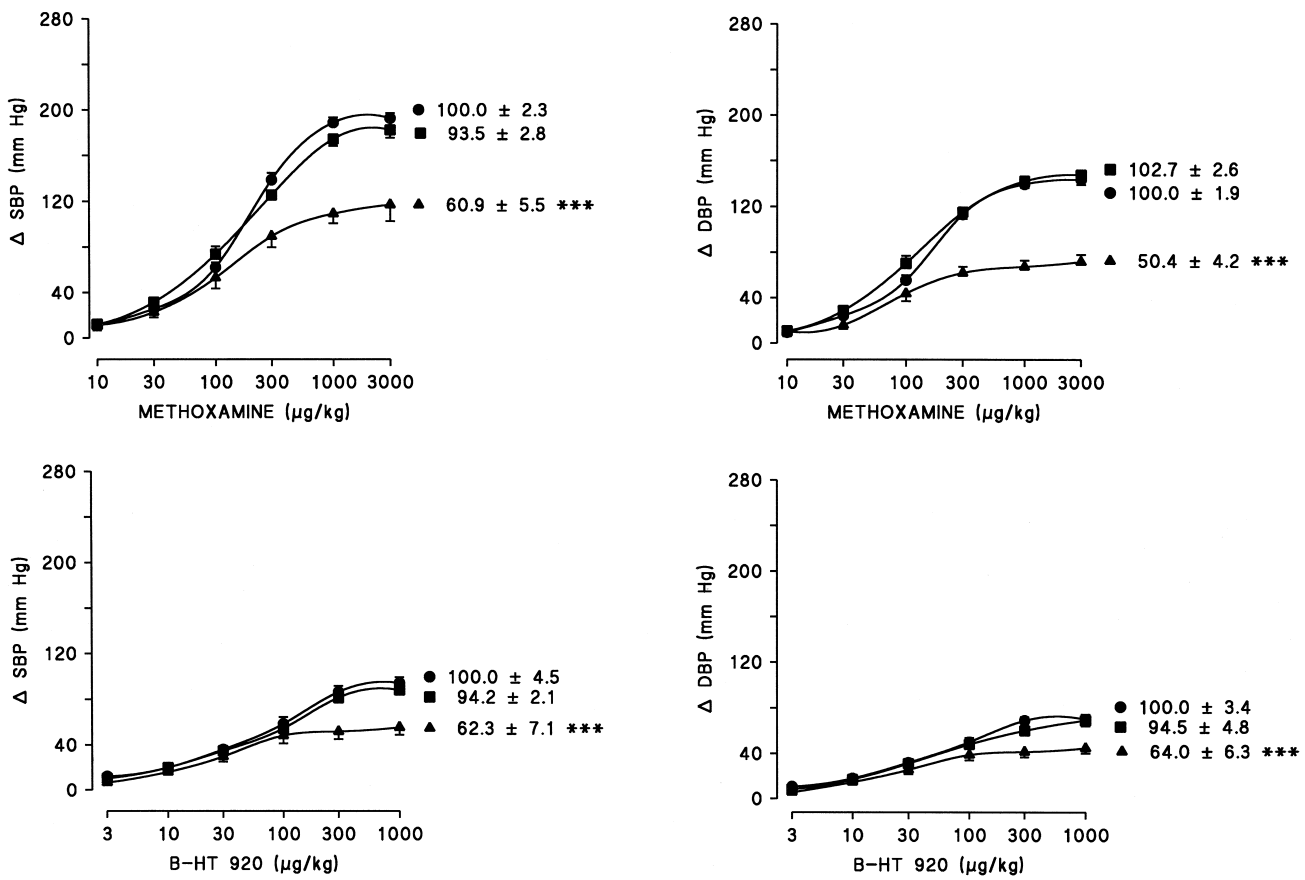

Fig. 3. Log dose–response curves for the increase in systolic blood pressure (SBP) and diastolic blood pressure (DBP) caused by intravenously administered methoxamine, or B-HT 920, in pithed 9-week old Sprague–Dawley rats fed a normal calcium diet (Ca 1%) (circle), a low-calcium diet (Ca 0.1%) (square), or a high-calcium diet (Ca 2.5%) (triangle) from weaning. Data are shown as mean values ± S.E.M. for a minimum of six experiments. The number at the top right of each dose–response curve represents the normalized area under the curve. The asterisks show significant differences from animals fed the Ca 1% diet (* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$).

these experiments, the rats were anaesthetized with ether and pithed by introducing a blunt needle into the spinal canal via the orbit by the Shipley and Tilden method (Shipley and Tilden, 1947), then immediately artificially respired. In these rats, the left jugular vein and the right carotid artery were cannulated for administration of drugs and recording of arterial blood pressure, respectively. Increasing doses of the agonist were administered, and only one dose–response curve was obtained for each animal. The maximum increases (mm Hg) in systolic and diastolic arterial blood pressure were measured for each dose with a Panlab 8C Datasystem (Panlab, Barcelona, Spain).

All the above-mentioned experiments were performed as authorized for scientific research (European Directive 86/609/CEE and Royal Decree 223/1988 of the Spanish Ministry of Agriculture, Fisheries and Food).

2.2. Statistical analysis

The results are always expressed as mean values ± S.E.M. for a minimum of six experiments. In both strains, statistical comparisons were made between the data obtained when the normal calcium diet was administered, and when the other diets were used. Since it was difficult to obtain the maximal effect and the pD_2 value ($-\log$ of the dose producing 50% of the maximal effect) for the drugs in the in vivo experiments, the effect of dietary calcium content on α-adrenoceptor-mediated pressor responses was expressed as the area under each dose–response curve, taking the area under the curve for the control mean values (obtained when the animals were fed with the normal calcium diet) as 100. In order to compare the vascular reactivity of the SHR fed the normal calcium diet at different times of the SHR's life, we used the absolute values of the area under the corresponding dose–response curves, although these figures are not shown. Student's *t*-test with a P value ≤ 0.05 indicating statistical significance was used to compare the mean of the arterial blood pressure and the plasma calcium values. It was also used to compare the areas under the α-adrenoceptor agonist dose–response curves.

2.3. Drugs

The following drugs were used in this study: methoxamine HCl (Sigma, USA), and B-HT 920 2HCl (5-allyl-2-

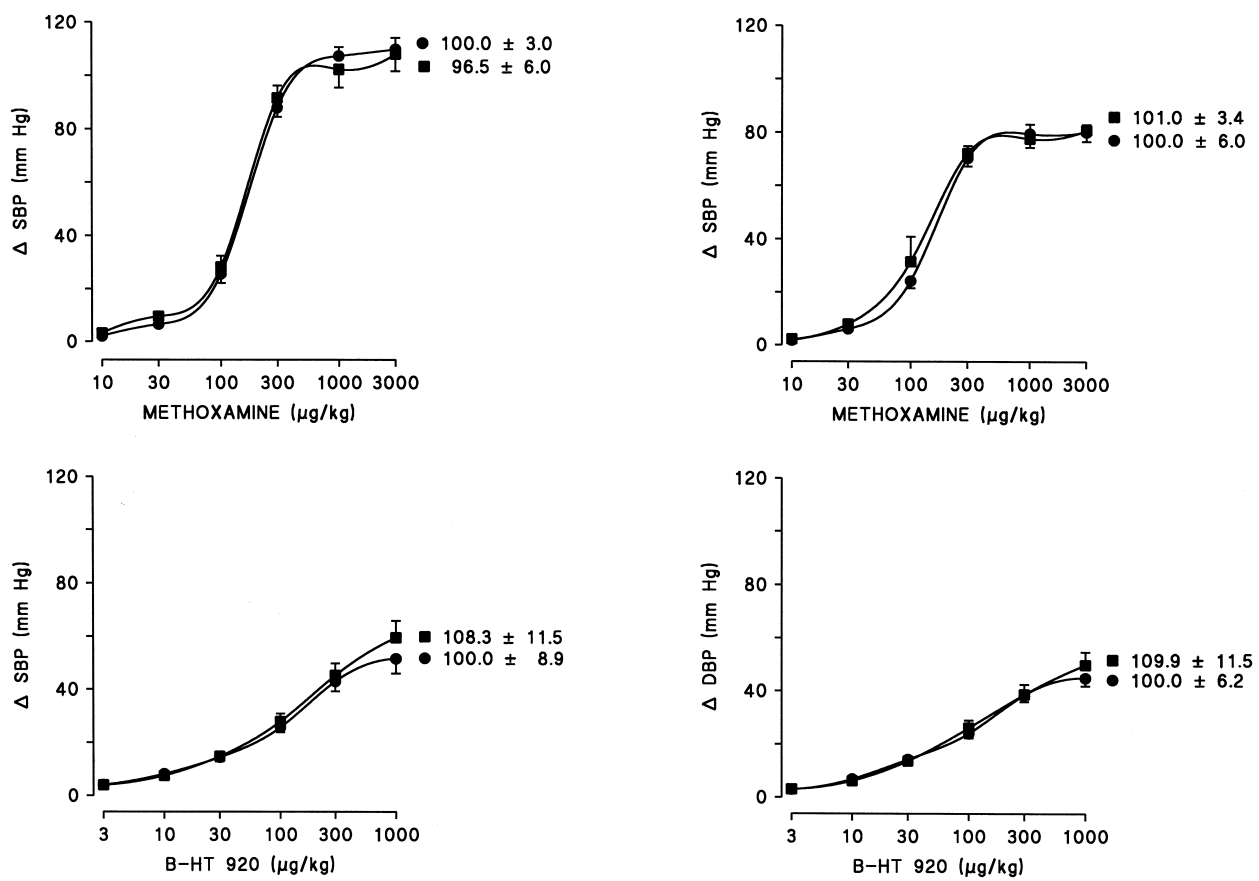

Fig. 4. Log dose–response curves for the increase in SBP and DBP caused by intravenously administered methoxamine, or B-HT 920, in pithed 8-week old SHR fed a normal calcium diet (Ca 1%) (circle), or a low-calcium diet (Ca 0.1%) (square). Data are shown as mean values ± S.E.M. for a minimum of six experiments. The number at the top right of each dose–response curve represents the normalized area under the curve. (No significant differences from animals fed the Ca 1% diet).

amino-5,6,7,8-tetrahydro-4*H*-thiazolo-(4,5-D)-acepin-dihydrochloride, talixepole) (Supplied by Boehringer Ingelheim, Germany). The α-adrenoceptor agonists were dissolved freshly daily in normal saline solution. The doses mentioned in the text and figures refer to the salts for these drugs.

3. Results

The 9-week old Sprague–Dawley rats showed arterial blood pressure values slightly higher than the younger animals. Nevertheless, this strain did not develop hypertension, and the measurements always corresponded to normotensive values (Fig. 1). The low-calcium diet caused an increase in the systolic and diastolic arterial blood pressure of the conscious Sprague–Dawley rats. The conscious 8- to 9-week old rats showed the maximum statistical differences in arterial blood pressure when the animals of this strain fed the low-calcium diet were compared with those fed the normal calcium diet. On the contrary, the high-calcium diet did not change the systolic and the diastolic arterial blood pressure of the conscious Sprague–Dawley rats (Fig. 1).

The SHR fed the normal calcium diet showed a gradual increase in systolic and diastolic arterial blood pressure which reached maximum values between weeks 16–20 (Fig. 2). Hypertension developed most rapidly in SHR fed the low-calcium diet. This diet modified the systolic and the diastolic arterial blood pressure of the animals. Diastolic arterial blood pressure values remained consistently high, while systolic arterial blood pressure values were similar to those of the animals of this strain fed a normal calcium diet from the 11th week of life. Maximum differences in arterial blood pressure between the animals fed the low-calcium diet and those fed the normal calcium diet were obtained in 8-week old SHR. The high-calcium diet caused a decrease in the SHR arterial blood pressure and clearly slowed the development of hypertension in this rat strain. With this diet, a definite decrease in systolic arterial blood pressure could be observed in the SHR from the time when measurements started and a lessening of diastolic arterial blood pressure was observed from the 12th week of life. Maximum differences in arterial blood pressure between the animals fed the high-calcium diet and those fed the normal calcium diet were obtained in SHR at 13–16 weeks of life, and therefore, the values at 15 weeks

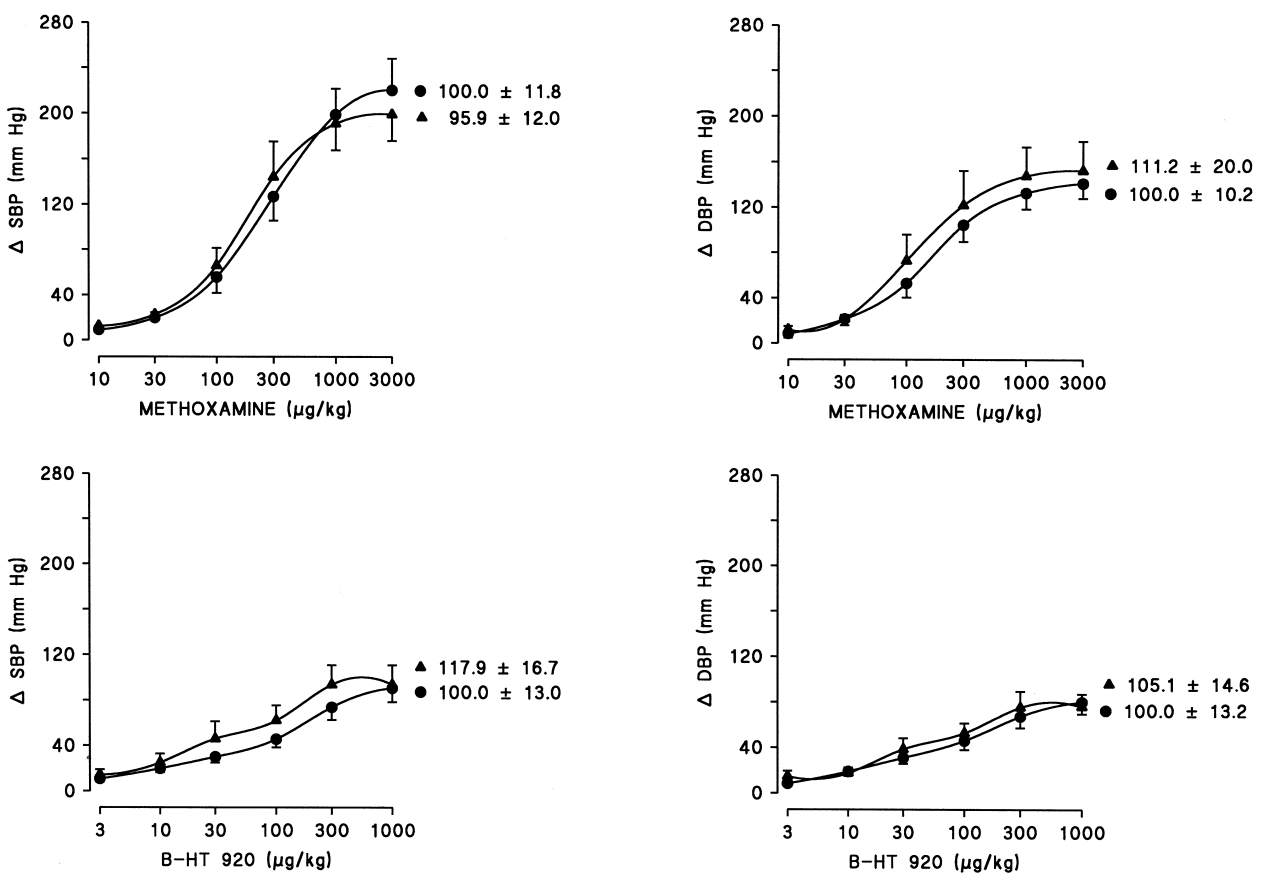

Fig. 5. Log dose–response curves for the increase in SBP and DBP caused by intravenously administered methoxamine, or B-HT 920, in pithed 15-week old SHR fed a normal calcium diet (Ca 1%) (circle), or a high-calcium diet (Ca 2.5%) (triangle). Data are shown as mean values ± S.E.M. for a minimum of six experiments. The number at the top right of each dose–response curve represents the normalized area under the curve. (No significant differences from animals fed the Ca 1% diet).

of life could represent this period of time. It should however be emphasized that, in the final weeks of treatment, the diastolic arterial blood pressure of the rats was similar whether the animals had been fed the high-calcium diet or the normal calcium diet, and there was a smaller difference between the systolic arterial blood pressure of these two groups of animals (Fig. 2).

The low-calcium diet decreased the plasma calcium levels in both strains, but in the SHR, significant differences in the total plasma calcium were only obtained when compared with 8-week old animals fed the control diet but not with 20-week old animals. Furthermore, the decrease in the ionic plasma calcium concentration was more marked in the 8-week old animals than in the 20-week old animals. The high-calcium diet increased the plasma calcium levels in Sprague–Dawley rats and in 15-week old SHR. Nevertheless, the total and the ionic plasma calcium values were similar in the 20-week old SHR fed the high-calcium diet and in the 20-week old SHR fed the control diet (Table 1). Table 1 shows that the plasma calcium levels of the SHR at all ages fed the normal calcium diet were somewhat lower than those of the adult Sprague–Dawley rats fed this diet. Although statistical comparisons were not usually made between the strains used for this study, there was a significant difference when the total plasma calcium values ($P \leq 0.01$) and the ionic plasma calcium values ($P \leq 0.001$) of the adult 9-week old Sprague–Dawley rats were compared with those of the adult 20-week old SHR.

The pithed Sprague–Dawley rats and the pithed SHR always showed very low arterial blood pressure values. The low-calcium diet did not alter the values, but the high-calcium diet caused a significant decrease in systolic and diastolic arterial blood pressure values in pithed 9-week old Sprague–Dawley rats and 20-week old SHR (Table 2). Responses to methoxamine and to B-HT 920 were similar in the pithed 9-week old Sprague–Dawley rats fed the low-calcium diet and in the pithed 9-week old Sprague–Dawley rats fed the normal calcium diet. Nevertheless, the responses to methoxamine and to B-HT 920 were smaller in the pithed 9-week old Sprague–Dawley rats fed the high-calcium diet than in the 9-week old Sprague–Dawley rats fed the normal calcium diet (Fig. 3). Responses to methoxamine and to B-HT 920 in the pithed 8- and 20-week old SHR fed the low-calcium diet were similar to the corresponding responses in the pithed 8- and 20-week old SHR fed the normal calcium diet. These responses

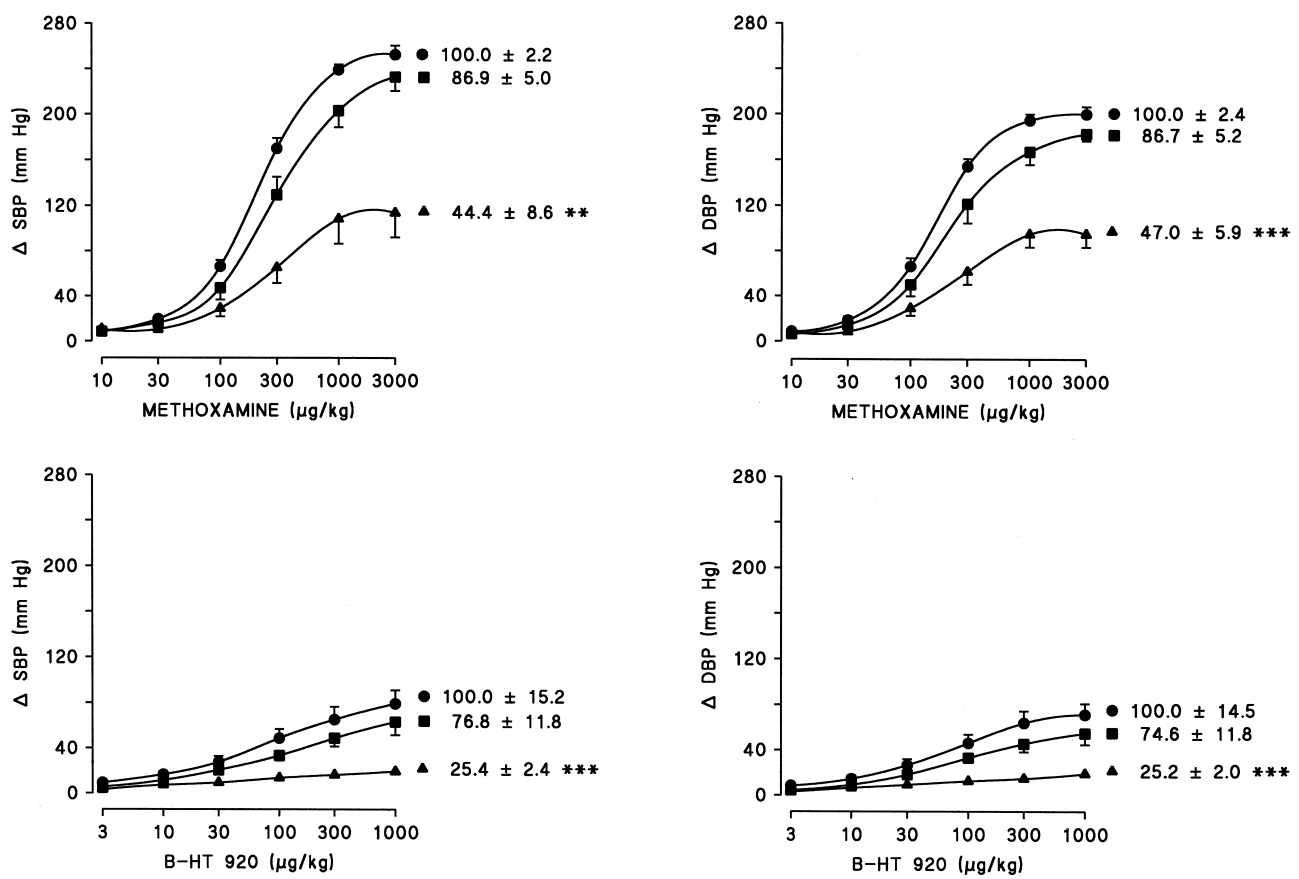

Fig. 6. Log dose–response curves for the increase in SBP and DBP caused by intravenously administered methoxamine, or B-HT 920, in pithed 20-week old SHR fed a normal calcium diet (Ca 1%) (circle), a low-calcium diet (Ca 0.1%) (square), or a high-calcium diet (Ca 2.5%) (triangle). Data are shown as mean values ± S.E.M. for a minimum of six experiments. The number at the top right of each dose–response curve represents the normalized area under the curve. The asterisks show significant differences from animals fed the Ca 1% diet (* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$).

were smaller in pithed 20-week old SHR fed the high-calcium diet than the corresponding ones in the pithed 20-week old SHR fed the normal calcium diet. Similar responses to both agonists were observed in pithed 15-week old SHR fed the high-calcium diet and in 15-week old SHR fed the normal calcium diet (Figs. 4–6).

It should also be emphasized that the responses to the α-adrenoceptor agonists in the pithed SHR fed the normal calcium diet increased with age. There was a marked difference in the responses to both agonists between the 8-week old animals and the older ones. The responses to methoxamine in the 15-week old pithed SHR fed the normal calcium diet were also smaller than the responses to this agonist in the 20-week old pithed SHR fed on this diet. These differences could be confirmed when the absolute values for the areas under the dose–response curves for the corresponding agonist in animals of different ages fed the normal calcium diet were compared (data not shown).

4. Discussion

A basic premise in this research was that the increases and decreases in extracellular calcium paradoxically cause a lessening and an increase, respectively, in the contraction of vascular smooth muscle cells. In the present study, rats of the Sprague–Dawley and the SHR strains were used, and were fed three diets with different calcium contents. It could be shown that modifications in dietary calcium are accompanied by significant alterations in plasma calcium levels and in arterial blood pressure.

The Sprague–Dawley rats were always normotensive when they were fed a normal calcium diet. It is however worth commenting on the arterial blood pressure values of the SHR when they were fed a normal calcium diet. Some researchers define the first weeks of life of the SHR as a prehypertensive phase (Smith and Hutchings, 1979; Karr-Dullien et al., 1981; Sánchez et al., 1986). In our study, the arterial blood pressure values of the 6-week old SHR fed the normal calcium diet suggested that the animals were probably already hypertensive before we began measuring. Bearing in mind the above-mentioned research, it seems obvious that the SHR display a prehypertensive phase which, being very short, could not be differentiated in our study. When we began measurements (6 weeks of life), we observed that the arterial blood pressure values of the SHR fed a normal calcium diet increased rapidly and did not begin to stabilize until the 11th week of life. Taking into account these arterial blood pressure modifications, we can see that there are two phases in the development of hypertension in these animals: an early phase with a rapid increase in arterial blood pressure values, and a later phase of stabilization of arterial blood pressure. Some of the groups mentioned above (Smith and Hutchings, 1979; Sánchez et al., 1986) have also described these two phases. These phases, however, may be subject to temporary modifications given that, in addition to arterial blood pressure measurements, other criteria could be used, such as measurements of flow, peripheral vascular resistance, sympathetic activity and the number of α-adrenoceptors.

There were several differences between the responses of normotensive and hypertensive animals when dietary calcium was modified. The low-calcium diet increased systolic and diastolic arterial blood pressure in both strains. The high-calcium diet did not modify the arterial blood pressure of the normotensive Sprague–Dawley rats, but did cause a decrease in the arterial blood pressure of SHR. We have already mentioned in Section 1 that dietary calcium supplements cause a decrease in arterial blood pressure, particularly in hypertensive patients and in animals with experimental hypertension. It should be borne in mind that hypertension in many patients and animals is associated with lower levels of plasma calcium (Resnick et al., 1983; Stern et al., 1984; Folsom et al., 1986; Bindels et al., 1987; Barbagallo et al., 1992; Young et al., 1992). Calcium supplements should correct these levels, and should therefore control arterial blood pressure. One might expect that normotensive persons and animals with normal plasma calcium levels would not respond to calcium supplements modifying arterial blood pressure but that when a dietary calcium deficiency exists, their arterial blood pressure probably would rise as a result of the drop in plasma calcium levels. This reasoning would explain the results obtained in our study and the differences found between Sprague–Dawley rats and SHR. The determination of plasma calcium levels in hypertensive patients with suspected deficient calcium ingestion might possibly be appropriate. This would permit correction in calcium ingestion, thereby controlling arterial blood pressure or facilitating its control, using additional therapies. The most useful determination would be that of ionic plasma calcium, which is the active fraction. Indeed, there are reports describing that the alteration in ionic plasma calcium levels is not necessarily associated with an alteration in total plasma calcium levels. This could be related to changes in calcium binding to plasma and membrane proteins (Folsom et al., 1986; Strazzullo et al., 1986; Young et al., 1992; Papagalanis et al., 1993). In the present study, a change in the plasma calcium levels of the animals appeared when calcium administration was not normal. Nevertheless, we should point out that when dietary calcium of the SHR was either low or high for a long period of time, both plasma calcium and arterial blood pressure levels approached the normal values for these animals. Adaptative mechanisms may exist which could perhaps be responsible for the disappearance of the alterations when an abnormal calcium diet is maintained. This may also apply for total plasma calcium when ionic plasma calcium is altered. Only the 20-week old SHR fed the low-calcium diet had similar total plasma calcium levels but significantly lower ionic plasma calcium levels than

did the rats of the same age and strain fed the normal calcium diet. In fact, some time ago, an increase in the binding of calcium to cellular membranes, adipocytes and erythrocytes was described in the rats of this strain, which could explain the discrepancy (Navarro-López et al., 1980; Channick et al., 1981).

In any case, it seems clear that hypertension is usually associated with an increase in intracellular calcium and also appears to be linked to lower plasma ionic calcium. Some researchers have established that there is indeed a drop in plasma ionic calcium in SHR when compared with the corresponding level in their genetic control, the Wistar–Kyoto rats (McCarron et al., 1981; Stern et al., 1984; Bindels et al., 1987; Barbagallo et al., 1992). The present results and those of other researchers (Wright and Rankin, 1982) have also shown lower extracellular calcium in SHR than in normotensive Sprague–Dawley rats, although, as we know, this latter strain is not the normotensive control for SHR.

We have analyzed in detail the arterial blood pressure changes in conscious animals and the plasma calcium levels when dietary calcium is altered, but it is clear that the main objective of our work was to find whether these arterial blood pressure changes are accompanied by alterations in the α-adrenoceptor-mediated vascular reactivity of the animals. For this reason, it was important to determine the moments when the changes in dietary calcium caused the maximum alterations in arterial blood pressure. It was also important to use a good experimental model, such as the pithed rat preparation, to evaluate the in vivo α-adrenoceptor-mediated vascular responses. As we stated in Section 1, different studies have produced contradictory data about the alteration of α-adrenoceptor-mediated vascular reactivity caused by dietary calcium modifications (Hatton et al., 1989, 1993; Pernot et al., 1990a,b; Hano et al., 1991). We had already obtained more conclusive preliminary data when the pithed rat preparation was used for these evaluations (López-Miranda et al., 1996b; Civantos et al., 1998).

We showed that changes in dietary calcium content caused similar alterations in the basal arterial blood pressure of the pithed rats and in the pressor responses mediated by vascular α-adrenoceptors when methoxamine and B-HT 920 were administered to these animals. We have to bear in mind that arterial blood pressure in pithed rats is maintained mainly by the effect of circulating catecholamines on vascular α-adrenoceptors. Therefore, the changes in basal arterial blood pressure of the pithed rats as induced by variations in dietary calcium content should reflect the alterations in the responses mediated by these receptors.

The low-calcium diet did not modify the pressor responses to either the α_1-adrenoceptor agonist or the α_2-adrenoceptor agonist in the pithed Sprague–Dawley rats and SHR, although it did increase arterial blood pressure in both strains. The high-calcium diet, on the contrary, caused a definite decrease in α_1- and α_2-adrenoceptor-mediated vascular reactivity but, nevertheless, the drop in vascular reactivity in the normotensive rats fed this diet did not go hand in hand with a decrease in arterial blood pressure. In the case of the hypertensive strain, it was also surprising that it was the 20-week old animals fed the high-calcium diet that showed a clear decrease in the vascular α-adrenoceptor-mediated pressor responses, even though at that age rats of this strain display arterial blood pressure values close to those of the SHR fed a control diet. Besides, when a high-calcium diet was fed to the SHR, maximum arterial blood pressure differences were observed in 15-week old animals, but at that time there were no obvious differences in α_1- and α_2-adrenoceptor-mediated vasoconstrictor responses. The alterations in vascular reactivity without changes in the arterial blood pressure may, perhaps, be explained by considering that compensating mechanisms could exist which would tend to maintain arterial tone even if the vascular receptor responses were altered. To explain the changes in arterial blood pressure, which were not accompanied by alterations in vascular reactivity, we should of course remember that other mechanisms exist besides the alteration in α-adrenoceptor-mediated vasoconstrictor responses which could be implicated in the effect of calcium on arterial blood pressure. In fact, the arterial blood pressure modifications caused by dietary calcium changes have been linked to variations in parathormone, parathyroid hypertensive factor or calcitriol levels in both hypertensive patients (Resnick et al., 1986; Levey et al., 1995; Sánchez et al., 1997) and animals (Pang et al., 1992; Lin et al., 1994; López-Miranda et al., 1998). It has also been stated that, when the dietary calcium content is modified, the synthesis of endothelial nitric oxide also undergoes a change (López-Jaramillo et al., 1989; López-Jaramillo and De Félix, 1991a,b; Mäkynen et al., 1996). Moreover, it is also likely that, at least as far as the α_1-adrenoceptors are concerned, full maturity would not yet have been reached when the SHR were 15 weeks old. The progressive maturing of the vascular α-adrenoceptors and, in particular, the vascular α_1-adrenoceptors, which are particularly important in the control of arterial blood pressure, is illustrated by the differences obtained when we compared the absolute values of the areas under the dose–response curves of the α_1-adrenoceptor agonists in SHR of different ages fed the normal calcium diet. Specifically, when the absolute values for the areas under the dose–response curves for methoxamine in 15- and 20-week old SHR fed the normal calcium diet were compared, the areas were smaller for the younger animals, with a significant difference for diastolic arterial blood pressure ($P \leq 0.01$). This premise is supported by other researchers who carried out binding studies and also demonstrated that the SHR α-adrenoceptors mature throughout the life of the animals. Sánchez et al. (1986) have shown that the number of α_1-adrenoceptors in the kidney of SHR increases until the 8th week of life and that the number of α_2-adrenoceptors

in this tissue continues to grow even until the 18th week of life. Otkay et al. (1986), also using binding studies in SHR, showed that the levels of α_1-adrenoceptors in these animals could not be considered stable until the 8th week of life.

In any case, the present results suggest that the mechanisms implicated in the alterations of the arterial blood pressure caused by high-calcium diets are clearly different from those mechanisms, which bring about changes in arterial blood pressure when the diet is deficient in calcium. We can conclude that dietary calcium supplements cause a definite decrease in the vasoconstrictor responses mediated by vascular α_1- and α_2-adrenoceptors and that this could at least partly explain the calcium-induced drop in arterial blood pressure observed when hypertension is present.

Acknowledgements

This work was supported by the Spanish Ministry of Education and Science (DGICYT PB93-0065) and the University Complutense (PR 156/97-7099). We also thank the Danone Institute for the postdoctoral grant to Visitación López-Miranda González in order to carry out this study.

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