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Blood pressure and α-vascular reactivity in hypertensive rats treated with amlodipine and dietary Ca

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Abstract

It has been suggested that the combination of dietary Ca and Ca^{2+} channel antagonists could have a synergic antihypertensive effect. In this study, 3-week-old male spontaneously hypertensive rats (SHR) were randomized into four groups of animals. Two of these groups were fed on a normal Ca diet (Ca 1%) and the other two groups were fed on a Ca-enriched diet (Ca 2.5%). One of the groups fed on each diet also received amlodipine (1 mg/kg/day) in their drinking water. Systolic and diastolic arterial blood pressure were measured weekly in the rats, from the 6th week of life until the 25th week of life, by the tail-cuff method, and we also calculated the corresponding pulse pressure values (systolic blood pressure−diastolic blood pressure). Determination of plasma Ca levels by colourimetric methods, and measurement in pithed rats of the pressor responses to the α-adrenoceptor agonists methoxamine and B-HT 920 (5-allyl-2-amino-5,6,7,8-tetrahydro-4*H*-thiazolo-(4,5-D)-acepin-dihydrochloride, talixepole) were also performed using 16- and 23-week-old animals from the different groups. The Ca-enriched diet decreased systolic and diastolic blood pressure in SHR. Almodipine also decreased systolic and diastolic blood pressure in SHR, and this drug intensified the antihypertensive effect of the Ca 2.5% diet in the SHR between weeks 13 and 18. Nevertheless, in the 19- to 25-week-old SHR amlodipine antagonized the effect of dietary Ca on arterial blood pressure. A decrease in the pulse pressure was seen only in the 15- to 20-week-old SHR which had been simultaneously treated with dietary Ca and amlodipine. All the treatments used increased calcaemia, and the highest plasma Ca levels were obtained in the animals which had received the combined treatment with Ca and amlodipine. The responses to methoxamine and to B-HT 920 in the pithed 16-week-old SHR were similar in the four groups of animals. The responses to these agonists in the pithed 23-week-old SHR fed on the Ca-enriched diet were smaller than the corresponding responses in 23-week-old SHR of the untreated group. By contrast, the responses to these agonists were slightly higher in the pithed 23-week-old SHR which were treated with amlodipine than in the pithed 23-week-old SHR in the untreated group. Moreover, amlodipine partially reversed the effect of dietary Ca on α-vascular reactivity. According to our results, it would seem inadvisable to use dietary Ca with a Ca^{2+} channel antagonist with the aim of controlling arterial blood pressure.

Keywords: Dietary Ca; Amlodipine; Arterial blood pressure; α-Vascular reactivity

1. Introduction

The administration of Ca is paradoxically associated with a decrease in arterial blood pressure and there are many rat studies which demonstrate that Ca-enriched diets can control hypertension in these animals (Aleixandre and Puerro, 1993; Hatton and McCarron, 1994; Buassi, 1998). Dietary Ca supplements also have beneficial effects in hypertensive patients (Aleixandre and Puerro, 1993; Pryer et al., 1995; Allender et al., 1996; Resnick, 1999). There is, nevertheless, a great variation in the data provided by different researchers with regard to the changes in systolic and diastolic arterial blood pressure caused by Ca-enriched diets. In addition, no existing study has looked at the change in pulse pressure following administration of dietary Ca supplements, but the pulse pressure is considered nowadays to be an excellent marker and predictor of cardiovascular risk in hypertensive patients (Khattar et al., 1999a,b; Blacher et al., 2000).

Treatment with Ca^{2+} channel antagonists is a very useful option for hypertensive patients, and it has been known for some time that, paradoxically, these agents are efficient in controlling arterial blood pressure in the same patients who respond favourably to an increase in dietary Ca (Resnick and Laragh, 1985). Of particular note in this

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context is also the study of Pang et al. in 1992. The normal content of Ca in rat food is 1%, but in that study adult male spontaneously hypertensive rats (SHR) were fed on one of three lower dietary levels of Ca (0.2%, 0.4%, or 0.8%), as well as on one of four doses of nifedipine or verapamil. These researchers showed that as dietary Ca increased within this range, the response to both drugs became more pronounced and dose-dependent. Bearing in mind these results, Pang et al. (1992) proposed that supplementary Ca and Ca^{2+} channel antagonists acted by different mechanisms in lowering blood pressure, and suggested that the combination of these different mechanisms of action might have potential therapeutic benefit. Nevertheless, there are no experimental or clinical studies to test this hypothesis.

Various mechanisms seem to be implicated in the blood pressure-lowering effect of Ca (Aleixandre et al., 1993), and it has been suggested that Ca administration may cause, among other changes, variations in α-vascular reactivity (Pernot et al., 1990; Hano et al., 1991; Hatton et al., 1993). Our research group has used the pithed rat preparation in many studies to evaluate the vascular reactivity of the animals. This is an experimental model in which the animal's central nervous system is destroyed and the drug-induced pressor responses only reflect peripheral effects (Shipley and Tilden, 1947). In 1999 we measured the α_1- and the α_2-adrenoceptor pressor responses in pithed Sprague–Dawley and SHR rats fed on diets with three different Ca contents. In that study, the values obtained when the animals were fed on a Ca 1% diet were considered as the control data, and we showed that the Ca-deficient diet (Ca 0.1%) did not modify the α-vascular reactivity of the rats. By contrast, the high-Ca diet (Ca 2.5%) caused a definite decrease in α_1- and α_2-adrenoceptor-mediated vasoconstrictor responses in both strains (Civantos et al., 1999). In an earlier study, we had observed that the acute administration of nifedipine also decreased the pressor responses to

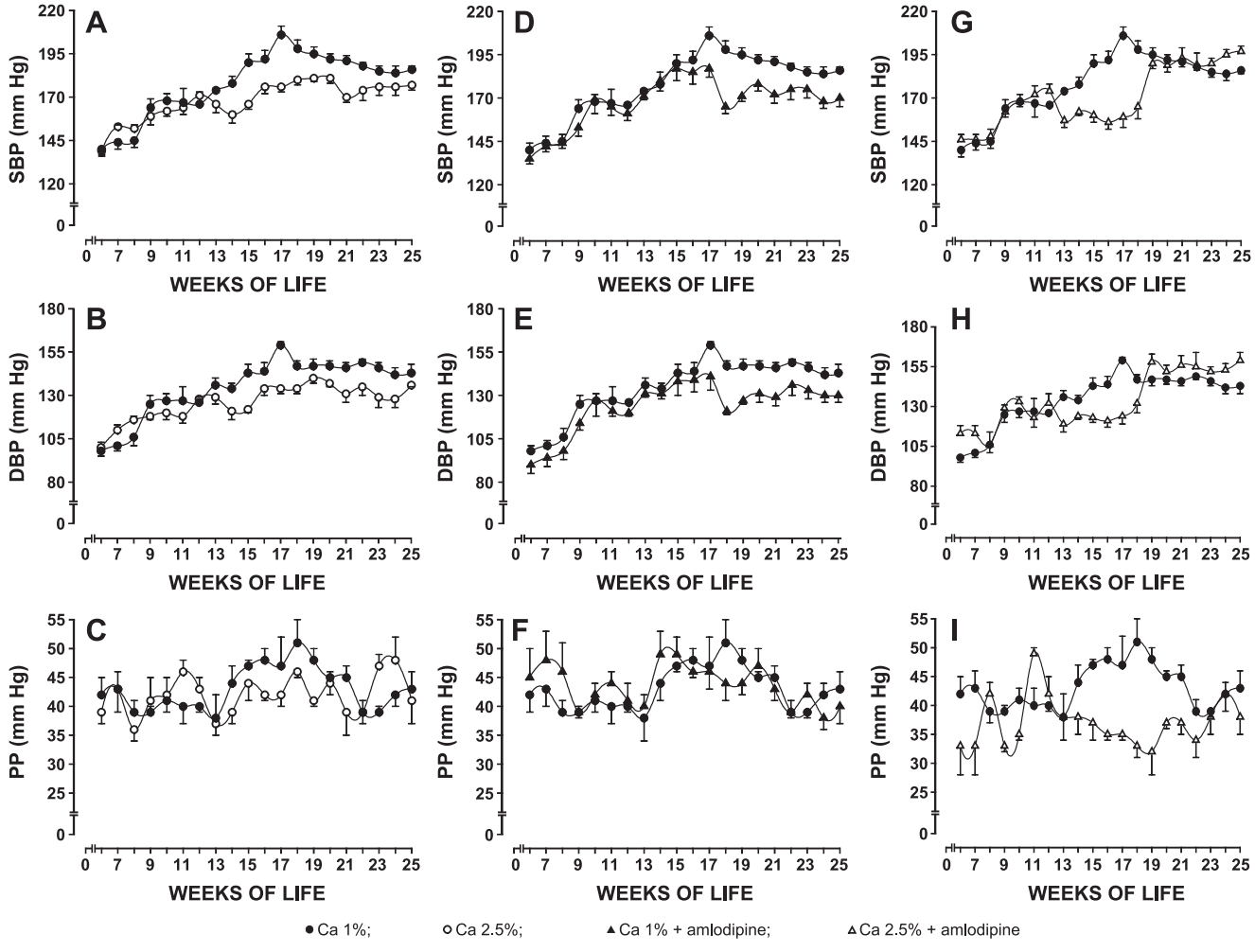

Fig. 1. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse pressure (PP) of four different groups of spontaneously hypertensive rats fed on a normal Ca diet (Ca 1%) (●), a Ca-enriched diet (Ca 2.5%) (○), a normal Ca diet and treated with amlodipine (▲), or a Ca-enriched diet and treated with amlodipine (△). Data represent mean values ± S.E.M. for a minimum of six animals.

selective α_1- and α_2-adrenoceptor agonists in pithed rats (Aleixandre et al., 1995). Nevertheless, the changes in α-vascular reactivity caused by chronic administration of Ca^{2+} channel antagonists have yet to be clarified, as do the changes in the responses of vascular α-adrenoceptors when Ca supplements and a Ca^{2+} channel antagonist are administered simultaneously.

Amlodipine is a long-acting second-generation dihydropyridine Ca^{2+} channel antagonist which is very selective for vascular tissue and has shown clear therapeutic advantages in the control of arterial blood pressure in hypertensive patients (Kaplan, 1991; Murdoch and Heel, 1991; Haria and Wagstaff, 1995). The aim of this study is to investigate the possible alterations in systolic blood pressure, diastolic blood pressure, pulse pressure, and α-vascular reactivity in SHR treated with dietary Ca supplements, amlodipine, or amlodipine in combination with the Ca-enriched diet for long periods. In order to facilitate interpretation of our results, we also determined the plasma Ca levels to evaluate the α-vascular reactivity of the rats.

2. Methods

2.1. Experimental procedure

After being weaned at 3 weeks, male SHR were caged in groups of five at a temperature of 23 °C with 12-h light/dark cycles. They were randomized with ad libitum intake into four groups of animals. Two of these groups were fed on a control semi-synthetic casein diet with a normal Ca content (Ca 1%), and the other two groups were fed on a similar diet with a high-Ca content (Ca 2.5%) (UAR, Villemoison, France). One of the groups fed on each diet also received amlodipine (1 mg/kg/day) in the drinking water. The dose of amlodipine selected was based on a previous study in which it was observed that this dose caused a slight but sustained decrease in arterial blood pressure in SHR (López-Miranda et al., 1995). Therefore, in this study we established the following groups of SHR: fed on the normal Ca diet and without pharmacological treatment (considered to be the reference group), fed on the Ca-enriched diet and without pharmacological treatment, fed on the normal Ca diet and treated with amlodipine, and fed on the Ca-enriched diet and treated with amlodipine.

Systolic and diastolic blood pressure were measured weekly, from the 6th week of life until the 25th week of life, by the tail-cuff method (Buñag, 1973), and we also calculated the corresponding pulse pressure (systolic blood pressure−diastolic blood pressure) values. The original method for measuring arterial blood pressure using the tail-cuff provides only systolic blood pressure values, but the equipment used in this study, LE 5001 (Letica, Hospitalet, Barcelona, Spain), has a high sensitivity pulse transducer coupled with an accurate microprocessor program, and allows us to distinguish between systolic and diastolic blood pressure. The indirect measurement of blood pressure with this equipment is basically sphyngomanometric and the process is the same as

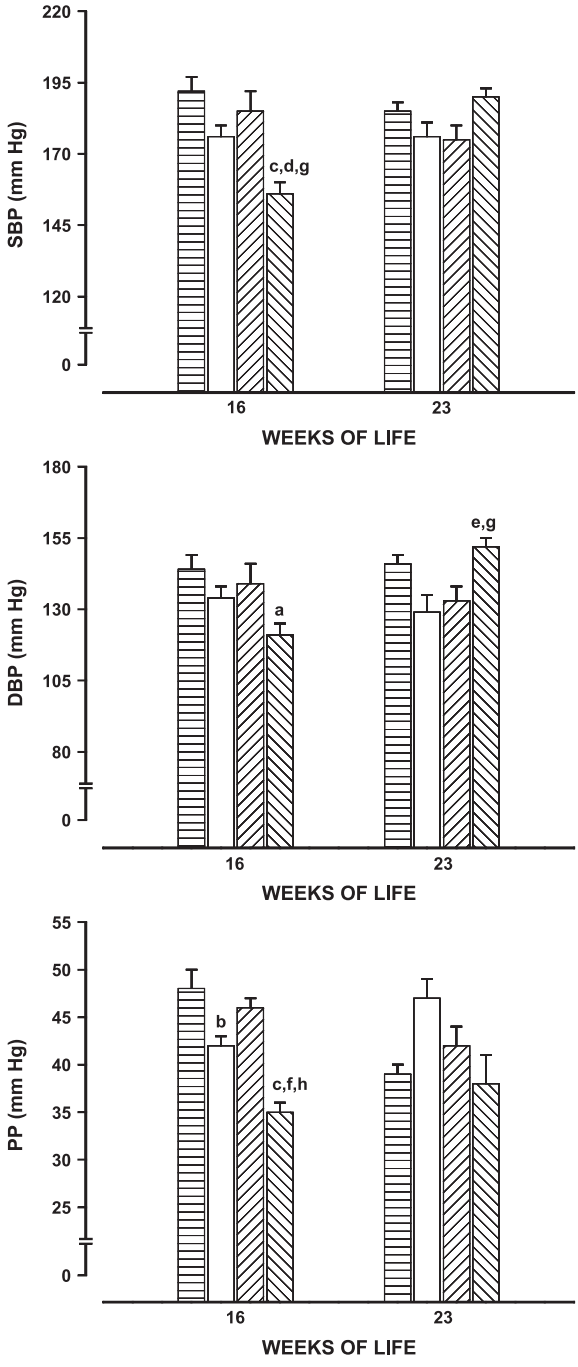

Fig. 2. Histograms of the systolic blood pressure (SBP), the diastolic blood pressure (DBP), and the pulse pressure (PP) of four different groups of 16- and 23-week-old spontaneously hypertensive rats fed on a normal Ca diet (Ca 1%) (≡), a Ca-enriched diet (Ca 2.5%) (□), a normal Ca diet and treated with amlodipine (▨), or a Ca-enriched diet and treated with amlodipine (▧). Data represent mean values ± S.E.M. for a minimum of six experiments. Letters show significant differences (one-way ANOVA and Bonferroni test: $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ vs. Ca 1%; $^{d}P < 0.05$, $^{e}P < 0.01$, $^{f}P < 0.001$ vs. Ca 2.5%; $^{g}P < 0.01$, $^{h}P < 0.001$ vs. Ca 1% + amlodipine).

that used in blood pressure measurements in humans. Once a good pulse signal is achieved, the attainment of reliable systolic or diastolic blood pressure values only depends on the accuracy of the microprocessor program to detect changes in the pulse wave level. Both values, systolic and diastolic blood pressure, are derived from analysis of the pulse wave amplitude. The systolic blood pressure is reached when the pulse wave is again detected after the collapse of the tail artery, and the diastolic blood pressure is reached when the pulse wave recovers its pre-measurement amplitude. Experiments were performed at the same time of day in order to avoid the influence of the circadian rhythm. In addition, to avoid the effect of stress, the animals were lightly anaesthetized with ether just before the actual start of the measurements, according to the procedure used in 1978 by Dietz et al. (1978). In this context, it should be remembered that SHR are very nervous animals, and that the pulse signal using the Letica's detector is better with a calm animal than with a vasodilated animal. This equipment nevertheless makes it possible to pick-up very good pulse signals in anaesthetized animals.

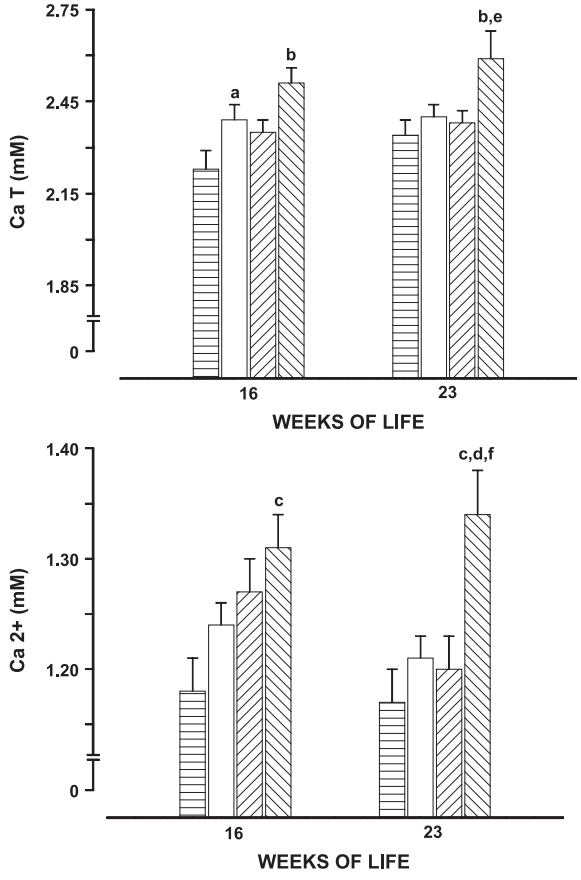

Fig. 3. Histograms of the total plasma Ca (Ca_T) and the plasma ionic Ca^{2+} (Ca^{2+}) concentrations of four different groups of 16- and 23-week-old spontaneously hypertensive rats fed on a normal Ca diet (Ca 1%) (≡), a Ca-enriched diet (Ca 2.5%) (□), a normal Ca diet and treated with amlodipine (▨), or a Ca-enriched diet and treated with amlodipine (▧). Data represent mean values±S.E.M. for a minimum of 12 experiments. Letters show significant differences (one-way ANOVA and Bonferroni test: [a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$ vs. Ca 1%; [d]$P<0.05$ vs. Ca 2.5%; [e]$P<0.01$, [f]$P<0.001$ vs. Ca 1%+amlodipine).

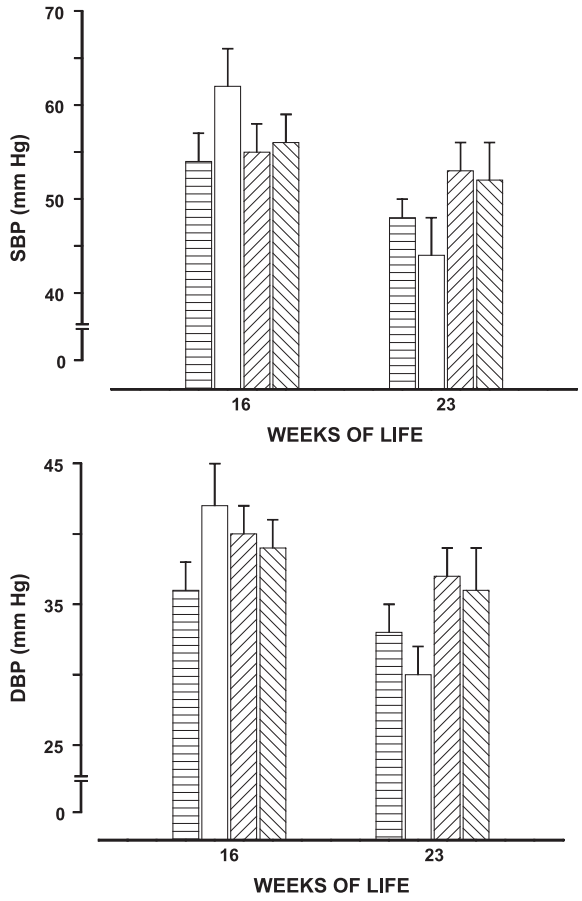

Fig. 4. Histograms of the systolic blood pressure (SBP) and the diastolic blood pressure (DBP) of four different groups of pithed 16- and 23-week-old spontaneously hypertensive rats fed on a normal Ca diet (Ca 1%) (≡), a Ca-enriched diet (Ca 2.5%) (□), a normal Ca diet and treated with amlodipine (▨), or a Ca-enriched diet and treated with amlodipine (▧). Data represent mean values±S.E.M. for a minimum of six experiments. No significant differences between animals from the different groups (one-way ANOVA and Bonferroni test).

Determination of plasma Ca levels and experiments to evaluate α-vascular reactivity were carried out in the 16- and 23-week-old animals.

In order to measure plasma Ca levels, the rats 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 Ca and proteins were measured using the *O*-Cresolphthalein complex and the Biuret colourimetric methods, respectively, and the ionic Ca^{2+} concentration was indirectly estimated using these values according to Zeisler's formula (Weissman and Pileggi, 1980). The results are expressed as mmol/l concentrations of total Ca and ionic Ca^{2+}.

In order to evaluate the α-vascular reactivity of the animals, dose–response curves were obtained for the selective α_1-adrenoceptor agonist methoxamine (10–3000 μg/kg) and the selective 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) (3–1000 μg/kg) in pithed SHR from the four groups of animals. For these experiments, the rats were anaesthetized with ether and pithed by introducing a blunt needle into the spinal canal via the orbit, as described by Shipley and Tilden (1947); the animals were ventilated artificialy. 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 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 and were analysed by one-way analysis of variance (ANOVA). Differences between the groups were assessed by the Bonferroni test, and differences between the means were considered significant when $P \leq 0.05$.

Since it was difficult to obtain the maximum effect and the pD_2 value (−log of the dose producing 50% of the maximum effect) for the drugs in the in vivo experiments, the effect of dietary Ca content and/or the pharmacological treatment on α-adrenoceptor-mediated pressor responses is expressed as the area under each dose–response curve, taking as 100 the area under the curve for the reference mean values (obtained when the animals had been fed on the normal Ca diet and had not been treated with amlodipine).

2.3. Drugs

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

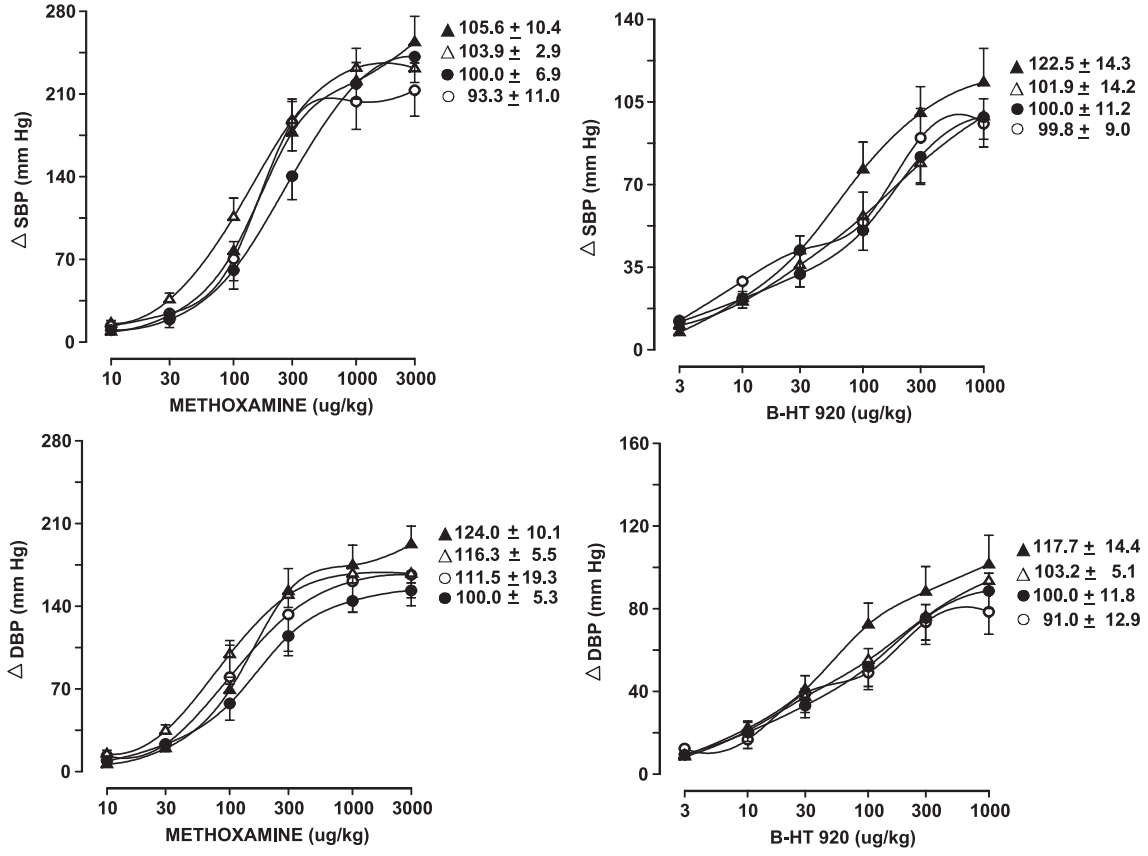

Fig. 5. Log dose–response curves for the increase in systolic blood pressure (SBP) and diastolic blood pressure (DBP) caused by intravenously administered methoxamine, or B-HT 920, in four different groups of pithed 16-week-old spontaneously hypertensive rats fed on a normal Ca diet (Ca 1%) (●), a Ca-enriched diet (Ca 2.5%) (○), a normal Ca diet and treated with amlodipine (▲), or a Ca-enriched diet and treated with amlodipine (△). Data represent 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 between animals from the different groups (one-way ANOVA and Bonferroni test).

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 daily in normal saline solution. The doses mentioned in the text and figures refer to the salts for these drugs.

3. Results

3.1. Arterial blood pressure

The SHR fed on the normal Ca diet and without pharmacological treatment (reference group) showed a gradual increase in systolic and diastolic blood pressure, which reached maximum values at 17 weeks of life. From this age, the arterial blood pressure of these rats remained constantly high and their systolic and diastolic blood pressure values were similar between weeks 17 and 25 (see Fig. 1).

The systolic and diastolic blood pressure values obtained between weeks 6 and 13 were very similar in the four groups of animals. A definite decrease in systolic and diastolic blood pressure could be observed in the rats fed on the Ca-enriched diet without pharmacological treatment from the 13th week of life. The decrease in systolic blood pressure was a little more accentuated than the decrease in diastolic blood pressure, but in the animals treated with dietary Ca we did not see a clear decrease in pulse pressure (see Fig. 1A–C).

Amlodipine also decreased systolic and diastolic blood pressure in the rats, but the antihypertensive effect of the pharmacological treatment appeared more slowly than the effect of dietary Ca. In fact, from the time when measurements started until week 17, systolic and diastolic blood pressure values were similar in the group of rats fed on the normal Ca diet and treated with amlodipine and in the reference group. A drop in systolic and diastolic blood pressure was observed in the rats fed on the normal Ca diet and treated with amlodipine from the 17th to 18th week of life. The treatment with amlodipine did not change pulse pressure in the rats (see Fig. 1D–F).

The combined treatment with the Ca-enriched diet and amlodipine also caused a decrease in the arterial blood pressure in SHR between weeks 13 and 18. During this period, systolic and diastolic blood pressure values in the animals treated with dietary Ca and amlodipine were in fact lower than those of the remaining groups (see Fig. 1G and H). Moreover, the combined treatment caused a clear decrease in the pulse pressure in the SHR between weeks 15 and 20 (see Fig. 1I). Systolic blood pressure in the 19- to 25-week-old SHR that received the combined treatment was very similar to that of the rats of the same age in the reference groups. During this period, diastolic blood pressure was even slightly higher in the SHR that received the combined treatment than in the SHR of the reference group (see also Fig. 1G and H).

Considering the results presented above, we established two periods (between 13 and 18 weeks, and between 19 and 25 weeks) in which we observed relevant, but opposite, changes in the arterial blood pressure of SHR treated with dietary Ca and amlodipine. Bearing in mind these periods, we believe that systolic, diastolic and pulse pressure values in the 16- and 23-week-old animals (see Fig. 2) can be used to compare the changes in arterial blood pressure observed in the four groups of SHR established in this study.

3.2. Calcaemia

Dietary Ca supplements increased plasma Ca in SHR. Significant differences were obtained between the total plasma Ca levels in the 16-week-old SHR fed on the Ca-enriched diet without pharmacological treatment and the total plasma Ca levels in the 16-week-old SHR of the reference group (see Fig. 3).

Treatment with amlodipine slightly increased plasma Ca in SHR, but no statistical differences were obtained when the total plasma Ca and ionic Ca^{2+} concentrations in the animals fed on the normal Ca diet and treated with amlodipine were compared with the corresponding values in the animals of the same age in the reference group (see Fig. 3).

The highest total plasma Ca and ionic Ca^{2+} concentrations were measured in the animals treated with the Ca-enriched diet and amlodipine, and significant differences were obtained when these values were compared with those of the animals of the same age in the reference group (see Fig. 3).

3.3. Pithed rats

The pithed SHR had very low systolic and diastolic blood pressure values, and no statistical differences were obtained when these values were compared over the four groups of animals. Fig. 4 shows the systolic and diastolic blood pressure values in the four groups of animals after pithing.

Methoxamine and B-HT 920 increased systolic and diastolic blood pressure in the pithed SHR in a dose-dependent manner. The pressor responses to both agonists were similar in the pithed 16-week-old SHR from the four groups (see Fig. 5). Nevertheless, we obtained different responses to methoxamine and to B-HT 920 in the pithed 23-week-old SHR from all four groups. At this age, the highest responses to the agonists were measured in the pithed animals treated with amlodipine, and the lowest responses were seen in the pithed animals treated with dietary Ca. The responses to methoxamine and B-HT 920 in the pithed 23-week-old SHR treated with dietary Ca and amlodipine were smaller than the corresponding responses in the reference group but they were not as small as the responses to these agonists in

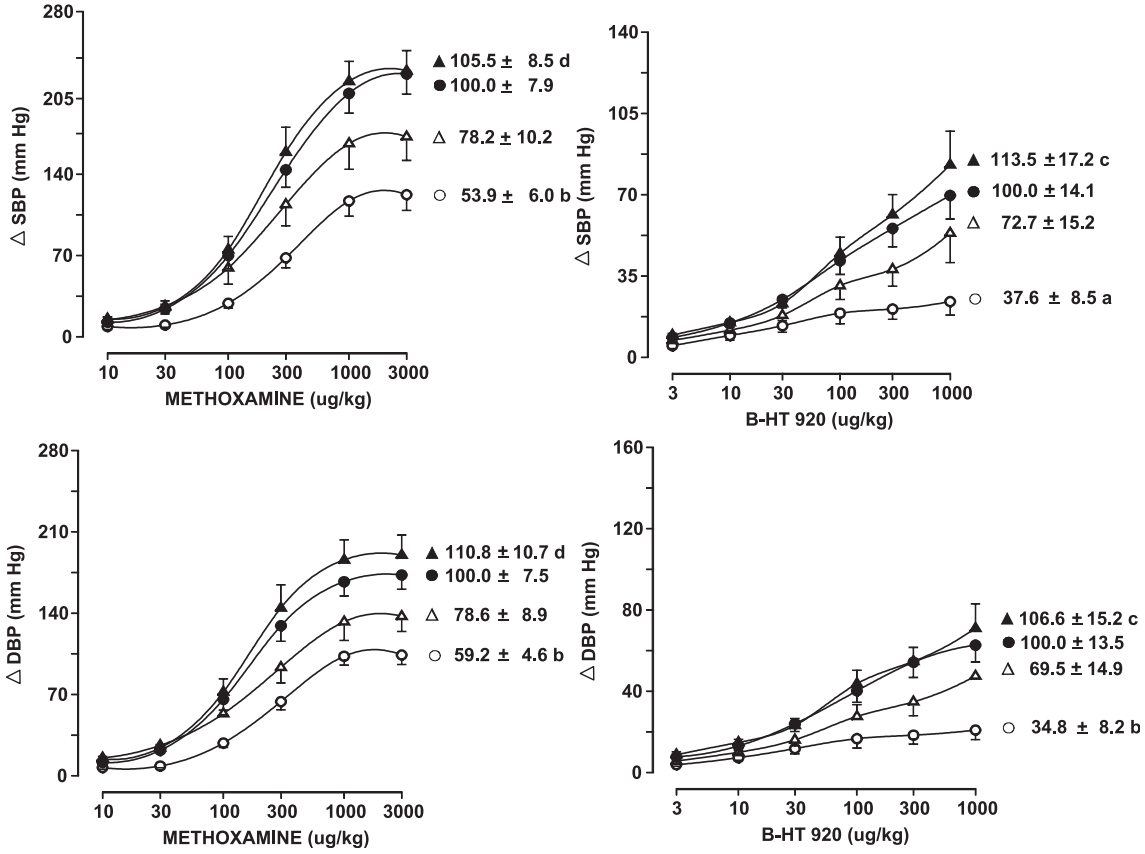

Fig. 6. Log dose–response curves for the increase in systolic blood pressure (SBP) and diastolic blood pressure (DBP) caused by intravenously administered methoxamine, or B-HT 920, in four different groups of pithed 23-week-old spontaneously hypertensive rats fed on a normal Ca diet (Ca 1%) (●), a Ca-enriched diet (Ca 2.5%) (○), a normal Ca diet and treated with amlodipine (▲), or a Ca-enriched diet and treated with amlodipine (△). Data represent 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. Letters show significant differences (one-way ANOVA and Bonferroni test: [a]$P<0.05$, [b]$P<0.01$ vs. Ca 1%; [c]$P<0.01$, [d]$P<0.001$ vs. Ca 2.5%).

the pithed 23-week-old SHR which had been treated with dietary Ca only (Fig. 6).

4. Discussion

Many studies have been carried out to evaluate the antihypertensive effect of Ca, but this is the first study to evaluate changes in arterial blood pressure following the joint administration of dietary Ca supplements and Ca^{2+} channel antagonists. When different antihypertensive measures are used simultaneously, interactions may occur which make long-term studies advisable. Moreover, hypertension is a chronic pathology which requires chronic treatment. For these reasons, the above treatments were administered to SHR over a long period of time. We also tried to establish whether calcaemia and α_1- and α_2-vasoconstrictor responses are modified by the administration of a Ca-enriched diet or a Ca^{2+} channel antagonist individually or in combination.

Various researchers, including our group, have established that before arterial blood pressure stabilizes in SHR, there is an initial period (weeks 11–12) when systolic and diastolic blood pressure increase strongly (López-Miranda et al., 1998; Civantos et al., 1999). To ensure that the measurements were reliable, in this study arterial blood pressure was measured after the animals were lightly anaesthetized. Perhaps for this reason, the arterial blood pressure values in the SHR fed on a normal Ca diet stabilized later (approximately at 17 weeks). It seems evident that SHR show a clear tendency to develop hypertension, and we have corroborated that dietary Ca supplements are able to decrease arterial blood pressure and specifically delay the onset of hypertension in these animals. Nevertheless, the Ca-enriched did not cause a clear decrease in the pulse pressure in the SHR, and its antihypertensive effect was smaller when treatment was prolonged. This was particularly noticeable for the systolic blood pressure. When antihypertensive compounds are administered, they always trigger adaptive processes which may sometimes cancel out their initial benefit. The contraregulatory mechanisms which exist to maintain arterial tone might in fact lessen the effect of the Ca supplements and partially decrease their antihypertensive effect when administered continuously. This should probably also be borne in mind when dietary Ca is administered clinically.

At the dose used (1 mg/kg/day), the antihypertensive effect of amlodipine could also be seen in SHR from the 17th week of life, but we noticed no changes in the pulse pressure of the SHR treated with amlodipine. Several studies have demonstrated the effectiveness of the dihidropyridines (Christensen, 1991; Tsoucaris et al., 1995; Zanchetti et al., 1998) and of some other Ca^{2+} channel antagonists (Hannes et al., 1993) in lowering the pulse pressure in hypertensive patients. In this study, the pulse pressure values were obtained by measuring systolic and diastolic blood pressure with the tail-cuff method, and since the caudal artery is a distal muscular conducting artery, our pulse pressure values may be somewhat different from the central pulse pressure. We demonstrated that the changes in arterial blood pressure measured when dietary Ca and amlodipine were simultaneously administered were not the same as those observed when these treatments were given long-term. We will try to link these changes with the corresponding modifications in calcaemia and vascular reactivity in the animals.

When hypertension exists, an increase in the plasma level of vasoconstrictor calciotropic hormones, such as parathormone, parathyroid hypertensive factor or calcitriol, may occur (Resnick et al., 1986; Pang et al., 1990a,b; Young et al., 1990, 1995; Lin et al., 1994). This pathology has also been linked to a decrease in cellular Ca^{2+} extrusion (Coca and De La Sierra, 1990; Timmermans et al., 1994). It is clear that when Ca intake is increased, its absortion increases and an increase in calcaemia occurs. Moreover, dietary Ca supplements would probably correct the above-mentioned changes in hypertensive rats, provoking in this way an even greater increase in extracellular Ca^{2+} and, paradoxically, a decrease in intracellular Ca^{2+} and arterial relaxation. In fact, total plasma Ca and ionic Ca^{2+} concentrations were higher in SHR when these animals had been fed from weaning on the Ca-enriched diet, and the arterial blood pressure of these animals was lower than that of rats of the same age fed on the normal Ca diet.

The SHR treated with amlodipine also showed an increase in calcaemia. This increase in extracellular Ca^{2+} could reflect blockade of the entry of this ion into the cell, which by definition is caused by a Ca^{2+} channel antagonist. It is possible that the drop in arterial blood pressure was only noticeable in these animals from the 17th week of life because the intracellular Ca^{2+} reserves initially compensated for the effect of this dose of amlodipine on vascular smooth muscle.

The SHR treated with both Ca supplements and amlodipine had a higher level of calcaemia and lower intracellular Ca^{2+} levels than the animals that received only one of these treaments. We would expect that vascular smooth muscle contraction following the combined treatment would also be more difficult. This argument may explain why a synergy for controlling hypertension takes place when dietary Ca supplements and a Ca^{2+} channel antagonist are jointly administered, and more specifically explains the arterial blood pressure values observed in the 13- to 18-week-old SHR which had received the combined treatment. This synergism could be observed when both systolic and diastolic blood pressure were measured. Moreover, the animals which received the combined treatment showed a clear decrease in pulse pressure over a similar period.

Arterial contraction produced by stimulation of vascular α-adrenoceptors may be more difficult if tissue Ca^{2+} concentrations decrease. However, the pressor responses to the α-adrenoceptor agonists in the pithed 16-week-old SHR from the four groups were very similar. At this age, α-adrenoceptors have not yet matured and their quantity or sensitivity to vasoconstrictor hormones may still be increasing. The density of α-adrenoceptors in arterial tissue of SHR of different ages has not been measured, but the number of both α_1- and α_2-adrenoceptors of SHR may gradually increase during their lifetime (Otkay et al., 1986; Sánchez et al., 1986). Our research group demonstrated in a previous study that the pressor response to α-adrenoceptor agonists in pithed SHR fed on a normal Ca diet also increased gradually during the animals' lifetime. The pressor response was in fact lower when the rats were 15–16 weeks old than when they were 20 weeks old (Civantos et al., 1999). Thus dietary Ca supplements can lower arterial blood pressure without causing changes in α-vasoconstrictor responses. We are aware that other mechanisms exist which could justify the antihypertensive effect of Ca.

When treatment with Ca supplements was prolonged, a readjustment in calcaemia was observed. Cellular Ca^{2+} extrusion would be reduced and the cells would have more Ca^{2+} for contraction. Metabolic and hormonal changes may explain this. In fact, the antihypertensive effect of dietary Ca lessens with time, and we can therefore assume that modifications in calcaemia caused by dietary Ca are always accompanied by parallel modifications in arterial blood pressure. The α-adrenoceptors of the 23-week-old rats were already mature, and the animals of this age treated with dietary Ca supplements showed a decrease in α-adrenoceptor-mediated vasoconstrictor responses. It is clear that the Ca-enriched diet diminishes vascular smooth muscle tone and also decreases the vasoconstrictor response mediated by α-adrenoceptors. However, the effects on vascular tone and the specific effects on α-adrenoceptor-mediated vasoconstrictor responses did not necessarily occur simultaneously.

After administration of amlodipine for a long period, the concentration of free intracellular Ca^{2+} may decrease because Ca^{2+} stores are unable to compensate for the effect of Ca^{2+} channel blockage. This would explain the decrease in arterial blood pressure observed in rats treated with amlodipine for a long time. The rats treated with amlodipine nevertheless showed a slight increase in α-adrenoceptor vasoconstrictor responses. The increase in α-vascular reactivity may seem surprising, because we know that acute treatment with a Ca^{2+} channel antagonist always decreases α-adrenoceptor vasoconstrictor responses. It could be that this increase may in fact be a mechanism for maintaining

vascular tone and compensating for the decrease in intracellular Ca^{2+} produced by prolonged treatment with a Ca^{2+} channel antagonist.

Finally, by definition, Ca administration should antagonize the effect of Ca^{2+} channel antagonists on Ca^{2+} channels. In the rats treated for a short time with amlodipine, the concentration of free intracellular Ca^{2+} was probably still high, and dietary Ca would lead to Ca^{2+} extrusion from the cell. We assume that when treatment with amlodipine is prolonged, the intracellular concentration of this ion will decrease significantly. Under these conditions, it would be difficult to extrude more Ca^{2+} from the cell, and high levels of extracellular Ca^{2+} would displace the Ca^{2+} channel antagonist from its receptor, thus relieving the ion channel blockade. This would allow us to explain the effect which prolonged treatment with dietary Ca supplements and amlodipine had on the arterial blood pressure of SHR. The animals that received long-term combined treatment had high levels of calcaemia and their intracellular Ca^{2+} levels were probably slightly lower than those of the untreated animals of the same age. This might explain why the responses to the α-adrenoceptor agonists were, in spite of everything, slightly lower in the 23-week-old pithed SHR treated chronically with Ca supplements and amlodipine than in the pithed rats of the same age in the reference group. The decrease in the α-adrenoceptor vasoconstrictor responses was not as noticeable as that observed in the animals of the same age treated with Ca supplements only. This is understandable because chronic treatment with the Ca^{2+} channel antagonist increased these responses. It should also be pointed out that the arterial blood pressure levels which we measured in the pithed 23-week-old animals before administering the α-adrenoceptor agonists also reflected the differences in α-vascular reactivity in the different groups.

Studies in animals have many limitations, but our research results suggest that it would be inadvisable to attempt to improve the control of arterial blood pressure with Ca supplements in patients undergoing treatment with Ca^{2+} channel antagonists. The increase in dietary Ca could actually eradicate the antihypertensive effect of these drugs. Moreover, in many elderly hypertensive patients arterial blood pressure can only be controlled by means of a Ca^{2+} channel antagonist, but for other reasons they need to take Ca supplements. Our results also suggest that antihypertensive treatment should be closely monitored in these patients.

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