Mechanisms of the Enhanced Contractile Response to Phenylephrine in Thoracic Aorta Isolated from Rats with Dietary Magnesium Deficiency

Hideaki Sakaguchi and Akira Nishio*

Department of Veterinary Pharmacology, Faculty of Agriculture, Kagoshima University, Kagoshima 890, Japan

Received September 24, 1993 Accepted January 18, 1994

ABSTRACT—The mechanisms underlying the enhanced contractile response to phenylephrine (PE) and increased susceptibility to nifedipine of de-endothelialized thoracic aorta isolated from rats with dietary magnesium deficiency (Mg-deficient rats) were examined by functional and radioligand binding studies. Enhanced PE-induced contractions and increased susceptibility to nifedipine in Mg-deficient rats were not observed in the presence of 10 μ M H7. PE significantly decreased the K_D value without changing B_{max} in the binding of [3 H]PN200-110 to de-endothelialized aortic strips. The K_D value obtained in the Mg-deficient rats was significantly smaller than that in the controls. Nifedipine displaced the binding of [3 H]PN200-110 concentration-dependently, and the pK_i value in Mg-deficient rats was significantly larger than that in the controls. A combination of PE and H7 abolished this difference. These results indicate that the modulation of L-type Ca^{2+} channels via the stimulation of α_1 -adrenoceptors may be involved in the enhancement of vasoconstriction and increased susceptibility to nifedipine in aortas isolated from Mg-deficient rats. The H7-sensitive mechanisms may play an important role in these phenomena.

Keywords: Mg-deficient rat, Phenylephrine, Nifedipine, [3 H]PN200-110 binding, H7

It is well known that magnesium ions (Mg^{2+}) influence the contractile response of isolated blood vessels to some endogenous vasoactive agents such as norepinephrine and serotonin (1). These experiments showed that low concentrations of Mg^{2+} in physiological salt solution enhance Ca^{2+} -entry into smooth muscle cells, resulting in enhanced muscular contraction. An *ex vivo* study using rats with dietary magnesium deficiency has shown that an enhanced contractile response to norepinephrine is observed in the isolated thoracic aorta after 30 days, but not after 15 days, while hypomagnesemia is observed after only 5 days (2). These results suggest that there are differences between the effects of *in vivo* dietary magnesium (Mg)-deficiency (at day 30) and *in vitro* Mg^{2+} depletion on the mechanisms of the enhanced contractile response of aortas to vasoactive agents.

Our previous studies showed that in thoracic aorta isolated from Mg-deficient rats, enhanced Ca^{2+} influx via L-type Ca^{2+} channels resulted in enhanced norepinephrine- and phenylephrine (PE)-induced contractions (3, 4), and that dietary Mg-deficiency increased the maximal density (B_{max}) of [3 H]PN200-110 specific binding, but not the B_{max}

of [3 H]prazosin, and did not alter the binding affinities of either ligand to aortic membranes (4). These results suggest that an increase in the density of L-type Ca^{2+} channels is involved in the enhancement of the contractile response to PE in thoracic aorta from Mg-deficient rats.

However, it is well known that L-type Ca^{2+} channels exist in different conformational states (i.e., closed, open and inactivated) and that each state has a different affinity for dihydropyridines (5). It therefore seems important to correlate binding data with functional data.

The present study was undertaken to clarify the mechanisms underlying the enhanced contractile response to PE and increased susceptibility to nifedipine in thoracic aorta isolated from rats with dietary Mg deficiency, using functional and radioligand binding studies.

MATERIALS AND METHODS

Animals

Adult male Wistar rats (7- to 9-week-old) were fed a Mg-deficient diet (Mg^{2+} : 0.001%) for 30 days. A control group received a normal diet (Mg^{2+} : 0.07%). The composition of the purified experimental diet has been described in detail in a previous paper (2). The rats were pair-fed

*To whom correspondence should be addressed.

and allowed free access to deionized water. Each rat was housed individually in a stainless steel cage at an ambient temperature of 22–24°C under a 12-hr light-dark cycle.

Functional study

After an experimental period of 30 days, each rat was decapitated and the thoracic aorta was removed immediately. The aorta was cleaned of all fat and connective tissue and cut into rings about 2-mm-wide. Each ring was mounted in an organ bath containing 10 ml physiological salt solution (PSS) with the following composition: 119 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl_2 , 1.2 mM MgCl_2 , 1.2 mM KH_2PO_4 , 25 mM NaHCO_3 and 10 mM glucose. The organ bath solution was maintained at 37°C and gassed with a 95% O_2 and 5% CO_2 gas.

A 10-mN resting tension, which was optimal for inducing the maximal contraction in both aortas, was applied to each aortic strip, and an equilibration period of 2 hr was allowed before the aortic ring was exposed to drugs. The isometric muscle tension was recorded with a force-transducer (TB-611T; Nihon Kohden Kogyo Co., Tokyo) connected to a polygraph (AP-621G, Nihon Kohden Kogyo Co.). Cumulative concentration-response curves were obtained by a stepwise increase in the concentration of the agonists; additions were made as soon as a steady response was obtained with the preceding concentration. When antagonists were used, each was added to the organ bath 30 min (nifedipine) or 90 min (H7) before the concentration-response curves of the agonists were obtained.

In both functional and binding studies, we used preparations denuded of their endothelium to avoid the effects of this tissue (6). To remove the endothelium, the intimal surface was rubbed gently with a swab wetted with PSS. The absence of endothelium was verified by a lack of relaxation when acetylcholine was added.

Binding study

The binding of [^3H]PN200-110 to de-endothelialized aortic strips was determined by the method of Morel and Godfraind (7). Strips of aorta (0.8–1.0 mg) were incubated in PSS with [^3H]PN200-110 (0.02–0.75 nM) for 90 min at 37°C in the dark. When the effects of PE or KCl were examined, these were added to the PSS during the last 30 min of incubation. At the end of incubation, each strip was washed 3 times with ice-cold PSS and then dried on filter paper, weighed and dissolved in 0.1 ml of a mixture of perchloric acid : hydrogen peroxide (1 : 1). Radioactivity was counted by a liquid scintillation counter (LSC-3050; Aloka Co., Tokyo). To determine non-specific binding, 3 μM nifedipine was included in the incubation medium. The difference between total binding and non-specific binding was taken as specific binding.

Analysis of parameters

The dissociation constant (K_D), the maximal density of specific binding sites (B_{max}) and the Hill coefficient were determined by Scatchard analysis using an equilibrium binding data analysis computer program (8). In displacement studies, the IC_{50} value (the concentration of a Ca^{2+} channel antagonist inducing a 50% inhibition of the binding of [^3H]PN200-110) was determined by regression analysis, using a Hofstee plot, and the inhibitor constant (K_i) was calculated by the computer program (8).

Statistical analyses

The data were expressed as the mean $\pm$ S.E. and were analyzed statistically by Student's *t*-test and Sheffé's test for comparisons involving three or more values. Unless stated otherwise, *n* refers to the number of animals. A probability of less than 0.05 was considered to indicate a significant difference.

Drugs

Drugs used were: (–)-phenylephrine hydrochloride (Wako Pure Chemical Industries, Ltd., Osaka), nifedipine (Sigma Chemical Co., St. Louis, MO, USA), (+)-[^3H]PN 200-110 (83 Ci/mmol; Amersham, Tokyo) and 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride (H7) (Research Biochemicals Inc., Natic, MA, USA).

RESULTS

General observations

Even though the control and Mg-deficient rats were pair-fed, the body weight gain in Mg-deficient rats (57.56 ± 7.52 g, *n* = 15) was significantly smaller than that in the controls (113.9 ± 6.31 g, *n* = 15). However, the wet weight of thoracic aortas in Mg-deficient rats (51 ± 4 mg, *n* = 23) was not significantly different from that in the controls (51 ± 3 mg, *n* = 23). As previously reported (2), there were no morphological changes in aortas from Mg-deficient rats. Plasma Mg, Ca, potassium (K) and sodium (Na) were determined by an atomic absorption spectrophotometer (170-50A; Hitachi, Ltd., Tokyo) (9). Plasma magnesium levels in the control (*n* = 8) and Mg-deficient (*n* = 8) rats, after 30 days, were 1.75 ± 0.07 mEq/l and 0.52 ± 0.04 mEq/l, respectively. Plasma levels of Ca, K and Na were 4.96 ± 0.08 mEq/l, 5.54 ± 0.50 mEq/l and 148.3 ± 0.9 mEq/l in the Mg-deficient rats (*n* = 8) after 30 days. Except for magnesium, these values were not significantly different from those of the controls (*n* = 8).

Contractile responses to PE and KCl

Figure 1 shows the concentration-response curves for PE, a selective α_1 -agonist, in thoracic aortas isolated from the control and Mg-deficient rats. Contractile responses

Fig. 1. Concentration-response curves for phenylephrine in de-endothelialized thoracic aortas isolated from the control (○) and Mg-deficient (●) rats. Each point represents the mean value from 6–7 rats $\pm$ S.E. Contractions induced by 30 mM KCl were taken as 100%; mean absolute values were 7.0 ± 0.7 mN in Mg-deficient rats and 7.2 ± 0.6 mN in control rats. There was no significant difference between the two values. * Significantly different from the corresponding controls ($P < 0.05$).

to PE (3×10^{-8} – 10^{-5} M) were significantly greater in Mg-deficient rats than in the controls. However, there was no significant difference in the pD_2 value between the control (7.64 ± 0.11) and Mg-deficient rats (7.63 ± 0.55). There was no significant difference in contractile response to KCl (15–60 mM) between the control and Mg-deficient rats. The pD_2 values for KCl were 1.65 ± 0.01 in Mg-deficient rats and 1.69 ± 0.03 in the controls.

Effects of nifedipine on the contractile responses to PE and KCl

The inhibitory effects of nifedipine on the contractile response to PE in thoracic aortas isolated from the control and Mg-deficient rats are shown in Fig. 2. Nifedipine (0.1–10 nM) inhibited the contractile response to PE in

Table 1. pIC_{50} values for nifedipine in the contractile response to phenylephrine (PE) with or without H7 in isolated thoracic aortas

	PE 10 μ M	PE 10 μ M + H7 10 μ M
Control	8.12 ± 0.31 (n=8)	7.90 ± 0.14 (n=6)
Mg-deficient	9.21 ± 0.23^a (n=8)	7.92 ± 0.33^b (n=6)

^a Significantly different from the control ($P < 0.05$). ^b Significantly different from H7-non-treated strips ($P < 0.05$). n, Number of rats used.

both groups. The degree of inhibition with nifedipine was significantly greater in Mg-deficient rats than in the controls. As shown in Table 1, the pIC_{50} value of nifedipine on the contractile response to 10 μ M PE was significantly greater in Mg-deficient rats than in the controls. However, the inhibitory effect of nifedipine on contractions induced by 60 mM KCl in Mg-deficient rats was not significantly different from that in the controls; the pIC_{50} value of nifedipine in the control and Mg-deficient rats was 9.05 ± 0.08 and 9.12 ± 0.08 , respectively.

Effects of H7 on the contractile response to PE

Figure 3 shows the concentration-response curves for PE in thoracic aortas isolated from the control and Mg-deficient rats in the presence or absence of 10 μ M H7 (a protein kinase C inhibitor). In the absence of H7, PE-induced contractions in Mg-deficient rats were significantly greater than those in the controls, as in Fig. 1. In the presence of H7, however, the contractile response to PE was not significantly different in the control and Mg-deficient rats.

Fig. 2. Effects of nifedipine on the contractile response to phenylephrine (PE) (●) in de-endothelialized thoracic aortas isolated from the control (a) and Mg-deficient (b) rats. Nifedipine (○, 0.1 nM; □, 1.0 nM; △, 10 nM) was added to the organ bath 30 min before the concentration-response curves for PE were obtained. Contractions induced by 10 μ M PE were taken as 100%. Each point represents the mean of 8 rats $\pm$ S.E.

Fig. 3. Concentration-response curves for phenylephrine (PE) in de-endothelialized thoracic aortas isolated from the control (○, □) and Mg-deficient (●, ■) rats in the presence (□, ■) or absence (○, ●) of $10 \mu\text{M}$ H7. Contractions induced by 30 mM KCl were taken 100%; mean absolute values were $7.4 \pm 0.5 \text{ mN}$ in Mg-deficient rats and $7.6 \pm 0.5 \text{ mN}$ in control rats. There was no significant difference between the two values. H7 was added to the organ bath 90 min before the concentration-response curves for PE were obtained. Each point represents the mean of 6 rats $\pm$ S.E. * Significantly different from the corresponding controls ($P < 0.05$).

Effects of nifedipine on PE-induced contraction in the presence of H7

Figure 4 shows the inhibitory effects of nifedipine on PE-induced contractions in thoracic aortas isolated from control and Mg-deficient rats in the presence of $10 \mu\text{M}$ H7. In the presence of H7, there was no significant difference in the inhibitory effect of nifedipine between the control and Mg-deficient rats. As shown in Table 1, the pIC_{50} value of nifedipine in Mg-deficient rats was not significantly different from that in the controls, and it was significantly smaller than that observed in the absence of H7.

Fig. 4. Effects of nifedipine on the contractile response to phenylephrine (PE) (●) in de-endothelialized thoracic aortas isolated from the control (a) and Mg-deficient (b) rats in the presence of $10 \mu\text{M}$ H7. H7 was added to the organ bath 90 min before the concentration-response curves for PE were obtained. Nifedipine (○, 0.1 nM; □, 1.0 nM; △, 10 nM) was added to the organ bath 30 min before the concentration-response curves for PE were obtained. Contractions induced by $10 \mu\text{M}$ PE in the presence of H7 were taken as 100%. Each point represents the mean of 6 rats $\pm$ S.E.

Binding of [^3H]PN200-110 to aortic strip

The specific binding of [^3H]PN200-110 to an aortic strip was determined in the control and Mg-deficient rats. In physiological salt solution (resting state), [^3H]PN200-110 bound with high affinity and in a saturable manner (Fig. 5a). Scatchard plots of the specific binding were linear in both groups (Fig. 5b), indicating a single class of binding sites. The K_D value obtained from Mg-deficient rats was not significantly different to that from the controls; however, B_{max} was significantly larger in Mg-deficient rats than in the controls (Table 2).

In the presence of $10 \mu\text{M}$ PE or 60 mM KCl, the specific binding of [^3H]PN200-110 increased without any change in the non-specific binding in both groups, and the Scatchard plots were linear in both cases (data not shown), indicating a single class of binding sites. Calculated K_D and B_{max} values are shown in Table 2. PE and KCl significantly decreased K_D , but did not significantly affect B_{max} in both groups. In the presence of PE, the K_D value was significantly smaller in Mg-deficient rats than in the controls. This difference was not observed with 60 mM KCl.

Effects of nifedipine on [^3H]PN200-110 binding to aortic strip

The inhibitory effects of nifedipine on [^3H]PN200-110 binding to aortic strips isolated from the control and Mg-deficient rats were investigated. In the resting state, nifedipine displaced the specific binding of [^3H]PN200-110 in a concentration-dependent manner (Fig. 6), and the Hofstee plots were linear (data not shown). There was no significant difference in the pK_i of nifedipine between the control and Mg-deficient rats (Table 3).

In the presence of $10 \mu\text{M}$ PE or 60 mM KCl, the displacement curves for nifedipine were shifted to the left

Fig. 5. Saturation curves (a) and Scatchard plots (b) of the specific binding of [³H]PN200-110 to de-endothelialized thoracic aortas isolated from the control (○) and Mg-deficient (●) rats in physiological salt solution. Each point represents the mean of 8 rats ± S.E.

Table 2. Characteristics of [³H]PN200-110 binding to isolated thoracic aortas

	K _D (nM)		B _{max} (fmol/mg wet tissue)	
	Control	Mg-deficient	Control	Mg-deficient
Vehicle	0.234 ± 0.016	0.258 ± 0.011	11.4 ± 1.4	15.8 ± 1.2 ^a
PE 10 μM	0.151 ± 0.009 ^b	0.106 ± 0.007 ^{a,b}	15.2 ± 1.3	16.1 ± 1.3
KCl 60 mM	0.136 ± 0.023 ^b	0.140 ± 0.014 ^b	17.9 ± 3.5	18.7 ± 3.0

Each value represents the mean of 5–8 rats ± S.E. PE, phenylephrine. ^aSignificantly different from the corresponding control ($P < 0.05$). ^bSignificantly different from the corresponding vehicle ($P < 0.05$).

(Fig. 6), and the Hofstee plots were linear in both groups (data not shown). As shown in Table 3, PE and KCl significantly increased the pK_i for nifedipine in both groups. In the presence of PE, the pK_i value in Mg-deficient rats was significantly larger than that in the controls. This

difference was not observed with 60 mM KCl.

Effects of H7 on [³H]PN200-110 binding to aortic strip

The effects of H7 on [³H]PN200-110 specific binding were investigated in PE-stimulated aortas. Scatchard

Fig. 6. Effects of nifedipine on the specific binding of [³H]PN200-110 to de-endothelialized thoracic aortas isolated from the control (a) and Mg-deficient (b) rats. Binding studies were carried out with 0.2 nM [³H]PN200-110. Aortic strips were incubated for 60 min in physiological salt solution and further incubated for 30 min in solution containing vehicle (○), 10 μM PE (●) or 60 mM KCl (□). Each point represents the mean of 4–5 rats ± S.E.

Table 3. pK_i values for nifedipine in [3 H]PN200-110 binding to isolated thoracic aortas in the presence of vehicle, PE or KCl

	Vehicle	PE 10 μ M	KCl 60 mM
Control	8.28 $\pm$ 0.05 (n=4)	8.69 $\pm$ 0.07 ^b (n=5)	9.00 $\pm$ 0.04 ^b (n=4)
Mg-deficient	8.36 $\pm$ 0.11 (n=4)	9.54 $\pm$ 0.04 ^{a,b} (n=5)	9.03 $\pm$ 0.07 ^b (n=4)

^a Significantly different from the control ($P < 0.05$). ^b Significantly different from the vehicle ($P < 0.05$). PE, phenylephrine; n, number of rats used.

Table 4. Effects of H7 on [3 H]PN200-110 binding to isolated thoracic aortas

		K_D (nM)	B_{max} (fmol/mg wet tissue)
PE 10 μ M + H7 10 μ M			
Control	(n=4)	0.194 $\pm$ 0.014	15.7 $\pm$ 1.8
Mg-deficient	(n=4)	0.188 $\pm$ 0.012	19.2 $\pm$ 1.2

PE, phenylephrine; n, number of rats used.

plots of specific binding were linear in both the control and Mg-deficient rat aortas (data not shown), indicating a single class of binding sites. Calculated K_D and B_{max} values are shown in Table 4. There were no significant differences in K_D and B_{max} values between the control and Mg-deficient rats. K_D values in the presence of both PE and H7 were significantly larger than those in the presence of PE only as shown in Table 2.

The inhibitory effect of nifedipine on the specific binding of [3 H]PN200-110 in the presence of both 10 μ M PE and 10 μ M H7 was examined in the control and Mg-deficient rats. In Mg-deficient rats, the pK_i value for nifedipine decreased significantly from 9.54 $\pm$ 0.04 (Table 3) to 8.92 $\pm$ 0.03 (n=4) in the presence of both PE and H7. However, in control rats (n=4), it was not significantly different from that obtained in the presence of PE only (8.79 $\pm$ 0.08 vs. 8.69 $\pm$ 0.07 respectively, see Table 3).

DISCUSSION

The potentiated vascular contractile response to an α_1 -agonist, PE, in the thoracic aorta isolated from rats with dietary Mg deficiency confirms our previous data (4). It has been shown that PE causes the contractile response in rat aorta via extracellular Ca^{2+} -entry, intracellular Ca^{2+} release and the increase to Ca^{2+} -sensitization of contractile elements in smooth muscle cells (10–12). The contractile response to PE of thoracic aorta in Ca^{2+} -free PSS showed no significant difference between the control and

Mg-deficient rats (A. Nishio and H. Sakaguchi, unpublished data). These results suggest that the enhanced contractile response to PE in thoracic aorta isolated from Mg-deficient rats depends mainly on Ca^{2+} -entry via the stimulation of α_1 -adrenoceptors. The possibility that changes in the L-type Ca^{2+} channel were involved in the enhanced contractile response to PE during Mg-deficiency was examined using nifedipine (an L-type Ca^{2+} channel antagonist) (13). Nifedipine depressed PE-induced contraction of aortas in both Mg-deficient rats and controls, and the degree of depression was significantly greater in the Mg-deficient rats. These results suggest that functional changes in the L-type Ca^{2+} channel may occur during Mg-deficiency and participate in the enhancement of PE-induced contraction. However, contractions induced by 60 mM KCl and the inhibitory effect of nifedipine on these contractile responses were unchanged during Mg-deficiency. These results clearly show that enhanced vasoconstriction and increased susceptibility to nifedipine are observed in Mg-deficient rat aorta during α_1 -adrenoceptor stimulation, but not during membrane depolarization.

In vascular smooth muscle stimulated by an α_1 -agonist, it has been reported that the functions of the L-type Ca^{2+} channels are controlled by membrane depolarization (14–16), by direct coupling of pertussis toxin-sensitive G protein (17, 18) or by channel phosphorylation by protein kinase C (19, 20). It is well known that stimulation of α_1 -adrenoceptors activates phospholipase C and produces diacylglycerol which is an activator of protein kinase C (11). Recently, it has been shown that a pertussis toxin-insensitive G protein is involved in the activation of phospholipase C by norepinephrine stimulation (19). Pertussis toxin did not alter PE-induced contraction in rat thoracic aorta in this study (A. Nishio and H. Sakaguchi, unpublished data). Therefore, the effects of H7 (a protein kinase C inhibitor) (21) on the contractile response to PE and on the inhibitory effects of nifedipine were investigated. PE-induced vasoconstriction and the inhibitory effects of nifedipine were not significantly different in Mg-deficient rats and controls in the presence of H7. Hidaka and Kobayashi (21) have shown that H7 inhibits also cyclic AMP- and cyclic GMP-dependent protein kinases. In vascular smooth muscles, however, it has been shown that the activation of cyclic AMP- and cyclic GMP-dependent protein kinases depresses the voltage dependent Ca^{2+} channel current and that of protein kinase C increases it (22–24). Therefore, PE-induced contraction in Mg-deficient rats may be enhanced through the activation of protein kinase C.

Our previous study (4), using [3 H]PN200-110 specific binding to rat aorta membranes, showed that the enhanced contractile response to PE during Mg-deficiency

might depend on an increase in the number of L-type Ca²⁺ channels. However, it is well known that L-type Ca²⁺ channels exist in different conformational states and that dihydropyridines have a high affinity for the inactivated state (25, 26). Therefore, we tried to characterize the L-type Ca²⁺ channels using [³H]PN200-110 binding to aortic strip in the presence of PE. It has been reported that both membrane depolarization and the stimulation of α_1 -adrenoceptors increases the binding affinity of dihydropyridines without changing the number of dihydropyridine receptors in vascular smooth muscles (7, 16, 20, 27). Similar results were obtained in the present study (Table 2), although the K_D value obtained from Mg-deficient rats was significantly smaller than that from controls in the presence of PE, but not KCl. Also, potentiation of the inhibitory effects of nifedipine on [³H]PN200-110 binding in Mg-deficient rats was observed only during the stimulation of α_1 -adrenoceptors. The pK_i values for nifedipine (9.54 ± 0.04) obtained from the binding studies were close to the pIC₅₀ (9.21 ± 0.23) values obtained from the functional studies. These results indicate that the increased affinity of [³H]PN200-110 binding to the aortic strip may play an important role in the enhancement of PE-induced contraction during Mg-deficiency.

It has been shown that activation of protein kinase C by the stimulation of α_1 -adrenoceptors modulates [³H]PN200-110 binding to voltage-dependent Ca²⁺ channels independently of a separate modulation by membrane depolarization (20). Therefore, the effects of H7 on the increased affinity of [³H]PN200-110 binding and on the inhibitory effects of nifedipine on [³H]PN200-110 binding were examined. In the presence of both PE and H7, the K_D and pK_i values for nifedipine obtained from Mg-deficient rats were not significantly different from those in the controls (Table 4). These results strongly suggest that protein kinase C is involved in the increased affinity of [³H]PN200-110 binding to PE-stimulated aorta isolated from Mg-deficient rats. However, the K_D values obtained in the presence of both PE and H7 were significantly smaller than those obtained in the resting state in both groups. These results clearly indicate that another transduction mechanism is involved in the increased affinity of [³H]PN200-110 binding via the stimulation of α_1 -adrenoceptors. Morel and Godfraind (16) have shown that the increase in affinity of [³H]PN200-110 by the stimulation of α_1 -adrenoceptors by 10 μ M norepinephrine may depend on membrane depolarization, which is comparable to 25 mM KCl in rat aorta. One could speculate that the K_D values obtained in the presence of both PE and H7 (Table 4) are modified by membrane depolarization in both groups. There is a possibility that in rat aorta, the increased affinity of [³H]PN200-110 binding following the stimulation of α_1 -adrenoceptors may be due to: 1) mem-

brane depolarization (which is unchanged in Mg-deficiency) and 2) activation of protein kinase C (which is independent of membrane potential). Only the change of channel function modulated by protein kinase C via the stimulation of α_1 -adrenoceptors may participate in the enhanced contractile response to PE in thoracic aortas during Mg-deficiency.

The observation that changes in [³H]dihydropyridine binding sites occur in some pathological states (hypertension, cardiomyopathy) has led some workers to suggest that alterations in L-type Ca²⁺ channels may play a crucial role in the etiology of these diseases (28, 29). However, few reports have described the relationship between the characteristics of Ca²⁺ channels stimulated by agonists and agonist-induced functional changes in these pathological states. The present study does not clarify how protein kinase C participates in the enhancement of the contractile response to PE in thoracic aortas isolated from Mg-deficient rats. Therefore, further studies using a direct protein kinase C activator, such as phorbol 12-myristate 13-acetate, are needed.

In conclusion, the present results indicate that modulation of L-type Ca²⁺ channels via the stimulation of α_1 -adrenoceptors participates in enhanced vasoconstriction and increased susceptibility to nifedipine in aortas isolated from rats with dietary Mg deficiency. The H7-sensitive mechanisms may play an important role in these phenomena.

REFERENCES

- 1 Altura BM, Altura BT, Carella A, Gebrewold A, Murakawa T and Nishio A: Magnesium-calcium interaction in contractility of vascular smooth muscle: Magnesium versus organic calcium channel blockers on myogenic tone and agonist-induced responsiveness of blood vessels. *Can J Physiol Pharmacol* **65**, 729–745 (1987)
- 2 Nishio A, Ishiguro S and Matsumoto M: Ex vivo study of the vascular reactivity to some vaso-active agents in isolated thoracic aorta from Mg-deficient rats. *Magnesium Res* **1**, 169–175 (1988)
- 3 Nishio A, Ishiguro S, Shimonishi I and Hirota A: Enhanced contractile response to noradrenaline and calcium influx in thoracic aorta isolated from dietary magnesium deficient rats. *Magnesium Res* **2**, 173–178 (1989)
- 4 Sakaguchi H, Ishiguro S and Nishio A: Enhanced contractile response to phenylephrine and increased density of [³H]PN200-110 binding sites in thoracic aorta isolated from Mg-deficient rats. *Jpn J Pharmacol* **61**, Supp I, 118P (1993)
- 5 Spedding M and Paoletti R: Classification of calcium channels and the sites of action of drugs modifying channel function. *Pharmacol Rev* **44**, 363–376 (1992)
- 6 Furchgott RF and Zawadzki JV: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* **288**, 373–376 (1980)
- 7 Morel N and Godfraind T: Prolonged depolarization increases

- the pharmacological effect of dihydropyridines and their binding affinity for calcium channels of vascular smooth muscle. *J Pharmacol Exp Ther* **243**, 711–715 (1987)
- 8 McPherson GA: Analysis of radioligand binding experiments. A collection of computer programs for IBM PC. *J Pharmacol Methods* **14**, 213–228 (1985)
- 9 Sakaguchi H, Sakaguchi R, Ishiguro S and Nishio A: Beneficial effects of calcium antagonist, nifedipine, on death and cardiovascular calcinosis induced by dietary magnesium deficiency in adult mice. *Magnesium Res* **5**, 121–125 (1992)
- 10 Godfraind T, Miller RC and Lima JS: Selective α_1 - and α_2 -adrenoceptor agonist-induced contraction and ^{45}Ca fluxes in the rat isolated aorta. *Br J Pharmacol* **77**, 597–604 (1982)
- 11 Minneman KP: α_1 -Adrenergic receptor subtypes, inositol phosphates, and sources of cell Ca^{2+} . *Pharmacol Rev* **40**, 87–119 (1988)
- 12 Karaki H, Sato K and Ozaki H: Different effects of verapamil on cytosolic Ca^{2+} and contraction in norepinephrine-stimulated vascular smooth muscle. *Jpn J Pharmacol* **55**, 35–42 (1991)
- 13 Hosey MM and Lazdunski M: Calcium channels: molecular pharmacology, structure and regulation. *J Membr Biol* **104**, 81–105 (1988)
- 14 Nelson MT, Standen NB, Brayden JE and Worley JF III: Noradrenaline contracts arteries by activating voltage-dependent calcium channels. *Nature* **336**, 382–385 (1988)
- 15 Haeusler G and De Peyer J-E: Rabbit aorta: electrical properties and agonist-induced depolarization. *Eur J Pharmacol* **166**, 175–182 (1989)
- 16 Morel N and Godfraind T: Characterization in rat aorta of the binding sites responsible for blockade of noradrenaline-evoked calcium entry by nisoldipine. *Br J Pharmacol* **102**, 467–477 (1991)
- 17 Nichols AJ, Motley ED and Ruffolo RR Jr: Effect of pertussis toxin treatment on postjunctional α_1 - and α_2 -adrenoceptor function in the cardiovascular system of the pithed rat. *J Pharmacol Exp Ther* **249**, 203–209 (1989)
- 18 Rakotoarisoa L, Mironneau C, Sayet I and Mironneau J: Guanine nucleotide-binding proteins modulate desmethoxyverapamil binding to calcium channels in vascular smooth muscle. *J Pharmacol Exp Ther* **259**, 164–168 (1991)
- 19 Loirand G, Pacaud P, Mironneau C and Mironneau J: GTP-binding proteins mediate noradrenaline effects on calcium and chloride currents in rat portal vein myocytes. *J Physiol (Lond)* **428**, 517–529 (1990)
- 20 Mironneau C, Rakotoarisoa L, Sayet I and Mironneau J: Modulation of [^3H]dihydropyridine binding by activation of protein kinase C in vascular smooth muscle. *Eur J Pharmacol (Mol Pharmacol Sec)* **208**, 223–230 (1991)
- 21 Hidaka H and Kobayashi R: Pharmacology of protein kinase inhibitors. *Annu Rev Pharmacol Toxicol* **32**, 377–397 (1992)
- 22 Sperelakis N and Ohya Y: Regulation of calcium slow channels in vascular smooth muscle cells. In *Ion Channels of Vascular Smooth Muscle Cells and Endothelial Cells*, Edited by Sperelakis N and Kuriyama H, pp 27–38, Elsevier Sci Publ Co, New York (1991)
- 23 Ousterhout JM and Sperelakis N: Cyclic nucleotides depress action potentials in cultured aortic smooth muscle cells. *Eur J Pharmacol* **144**, 7–14 (1987)
- 24 Fish RD, Sperti G, Colucci WS and Clapham DE: Phorbol ester increases the dihydropyridine-sensitive calcium conductance in a vascular smooth muscle cell line. *Circ Res* **62**, 1049–1054 (1988)
- 25 Bean BP: Nitrendipine block of cardiac calcium channels: High-affinity binding to the inactivated state. *Proc Natl Acad Sci USA* **81**, 6388–6392 (1984)
- 26 Cohen CJ and McCarthy RT: Nimodipine block of calcium channels in rat anterior pituitary cells. *J Physiol (Lond)* **387**, 195–225 (1987)
- 27 Dacquet C, Loiland G, Rakotoarisoa L, Mironneau C and Mironneau J: (+)-[^3H]-PN 200-110 binding to cell membranes and intact strips of portal vein smooth muscle: characterization and modulation by membrane potential and divalent cations. *Br J Pharmacol* **97**, 256–262 (1989)
- 28 Chatelain P, Demol D and Roba J: Comparison of [^3H]nitrendipine binding to heart membranes of normotensive and spontaneously hypertensive rats. *J Cardiovasc Pharmacol* **6**, 220–223 (1984)
- 29 Wagner JA, Reynolds IJ, Weisman HF, Dudeck P, Weisfeldt ML and Snyder SH: Calcium antagonist receptors in cardiomyopathic hamster: selective increased in heart, muscle, brain. *Science* **232**, 515–518 (1986)