Br. J. Pharmac. (1984), **81**, 393–400

Comparative effects of verapamil and sodium nitroprusside on contraction and ^{45}Ca uptake in the smooth muscle of rabbit aorta, rat aorta and guinea-pig taenia coli

H. Karaki, H. Nakagawa & N. Urakawa

Department of Veterinary Pharmacology, Faculty of Agriculture, The University of Tokyo, Bunko-ku, Tokyo 113 Japan

1 The effects of verapamil and sodium nitroprusside on muscle tension and ^{45}Ca uptake activated in different ways were compared in rabbit aorta, rat aorta and guinea-pig taenia coli.

2 In rabbit aorta, K-induced contraction was specifically inhibited by verapamil and noradrenaline-induced contraction by sodium notroprusside. In rat aorta, both K-induced and noradrenaline-induced contractions were inhibited by verapamil or by sodium nitroprusside also. In taenia, both K- and histamine-induced sustained contractions were inhibited by verapamil but not by sodium nitroprusside. The effect of varapamil was competitively antagonized by external Ca, while that of sodium nitroprusside was not.

3 High K, noradrenaline and histamine increased the rate of ^{45}Ca uptake in aortae and taenia. In rabbit aorta the increment in response to high K was specifically inhibited by verapamil and the increment induced by noradrenaline was specifically inhibited by sodium nitroprusside. In rat aorta, increments induced by both high K and noradrenaline were inhibited by verapamil and by sodium nitroprusside. In taenia, the increments induced by high K and by histamine were inhibited by verapamil but not by sodium nitroprusside.

4 These results suggest different characteristics of Ca entry systems in these smooth muscles. In rabbit aorta, there seem to be two Ca channels, one of which is activated by high K and inhibited by verapamil, while the other is activated by noradrenaline and inhibited by sodium nitroprusside. In rat aorta, both K- and noradrenaline-activated Ca pathways are sensitive to both verapamil and sodium nitroprusside whereas, in taenia, both K- and histamine-activated Ca pathways are sensitive only to verapamil.

Introduction

It has become accepted that the calcium needed for smooth muscle contraction may come from either an extracellular source or cellular store. Extracellular Ca may enter the cell through voltage-dependent Ca channels or through receptor-linked Ca channels which are not dependent on membrane potential (Bolton, 1979; Van Breemen *et al.*, 1979; Triggle, 1981; Weiss, 1981). A group of compounds termed organic Ca antagonists are reported to inhibit voltage-dependent Ca channels (Fleckenstein, 1977; Bolton, 1979). Golenhofen (1976; 1981) also proposed that sodium nitroprusside is a selective inhibitor of one of these Ca activation systems in the membrane of the smooth muscle cell. In this paper, we have examined the effects of verapamil and sodium nitroprusside on tension development and ^{45}Ca uptake in smooth muscle of rabbit aorta, rat aorta and guinea-pig taenia in order to clarify the properties of Ca entry systems in these smooth muscle preparations.

Methods

Tissue preparation

Three muscle preparations were used. (1) Male New Zealand rabbits, weighing 2.0–2.5 kg, were killed by

a rapid injection of sodium pentobarbitone (25 mg kg^{-1}) and air into an ear vein. The thoracic aorta was rapidly removed and cut into a spiral strip, 3–4 mm wide. The adventitial layer was then separated from the media-intimal layer as described by Karaki & Urakawa (1977) and muscle strips, 4–8 mm long, were prepared. (2) Male Wistar rats, weighing about 200 g, were stunned and bled under light ether anaesthesia. The thoracic aorta was dissected out and spiral strips, 2–3 mm wide and 5–8 mm long, were prepared. (3) Albino male guinea-pigs, weighing 250–300 g, were killed by a blow on the neck and a section of taenia, 5–10 mm in length, was dissected from the caecum. Each muscle strip was weighed, attached to a holder under a resting tension of 1 g for aortae and 0.2 g for taenia and equilibrated in the bathing solution for 60–90 min before experiments were started. The rate of ^{45}Ca uptake by the muscle strips was expressed in terms of their initial weight which ranged from 5–10 mg.

Solutions

The normal bathing solution (pH 7.4, 37 °C) contained (mM): NaCl 136.9, KCl 5.4, $CaCl_2$ 1.5, $MgCl_2$ 1.0, $NaHCO_3$ 23.8 and glucose 5.5 (Karaki *et al.*, 1981). The concentration of $CaCl_2$ was changed to 0.3 mM or 7.5 mM in some experiments. High K solutions were made either by substituting 60 mM or all of the NaCl in the normal solution with equimolar K (isosmotic 65.4 mM K and 160.7 mM K solutions, respectively), or by adding 40 mM KCl to the normal solution (hyperosmotic 45.4 mM). The solutions were aerated with a 95% O_2 and 5% CO_2 mixture.

Muscle tension

Muscle tension was recorded isometrically with a force-displacement transducer connected to a Nihon Kohden polygraph. Isosmotic 65.4 mM K-induced contractions in rabbit and rat aortae and hyperosmotic 45.4 mM K-induced contraction in taenia were used for standard K-induced contractions. Noradrenaline-induced contractions, 10^{-6}M and 10^{-7}M, in rabbit and rat aortae, respectively, and 10^{-6}M histamine-induced contraction in taenia were also used as standards because these contractions had magnitudes similar to those of the standard K-induced contractions in the respective smooth muscle preparations. Inhibitors were added 30 min before the application of agonists, or cumulatively applied when the contractile tension induced by an agonist reached a steady level. The concentration of inhibitors required to induce a 50% inhibition of contration (IC_{50}) was calculated from the cumulative concentration-inhibition curves.

Rates of ^{45}Ca uptake

The rate of ^{45}Ca uptake (exchange) of the lanthanum-inaccessible (cellular) Ca fraction was determined as described by Karaki & Weiss (1979). Muscle strips were incubated in solutions containing 1.5 mM Ca and a tracer amount of ^{45}Ca (0.4–1.6 μCi ml^{-1}) for 5 min (Karaki *et al.*,1983). Inhibitors were added 30 min prior to and during the ^{45}Ca incubation period. Agonists were added simultaneously with ^{45}Ca. After incubation with ^{45}Ca, the strips were washed for 60 min at 0.5 °C in the La-substituted solution containing 73.8 mM $LaCl_3$, 5.5 mM glucose and 24 mM tris(hydroxymethyl) aminomethane (Tris). This solution was adjusted to pH 6.8–6.9 at 0.5 °C with 1 N maleic acid. After the La-wash period, tissues were removed from the holders, blotted, placed in scintillation vials and the ^{45}Ca was extracted overnight in 1 ml of 20 mM EGTA solution. Scintillation mixture (ACS II, Amersham, 2 ml) was added to the extract and tissues; radioactivity was counted with a Packard Tri-Carb 3380 liquid scintillation spectrometer.

Statistics

Results of the experiments are expressed as mean ± s.e.mean. Student's t test was used for statistical analysis of the results and a P value less than 0.01 was taken as significant.

Drugs and chemicals

Verapamil (donated by Eisai), sodium nitroprusside (Wako Pure Chemical Industries), noradrenaline bitartrate (Wako), histamine dihydrochloride (Wako), Tris (Sigma), and $^{45}CaCl_2$ (New England Nuclear) were used.

Results

Muscle tension

Differential effects of verapamil and sodium nitroprusside on smooth muscle contractions are shown in Figure 1. Contraction of rabbit aorta induced by 65.4 mM K was inhibited by verapamil 10^{-6}M applied 30 min before the addition of high K. However, sodium nitroprusside 10^{-6}M had little effect on the K-induced contraction. In contrast, the 10^{-6}M noradrenaline-induced contraction of rabbit aorta was inhibited by sodium nitroprusside, but not by verapamil. In rat aorta, varapamil inhibited both 65.4 mM K- and 10^{-7}M noradrenaline-induced contraction as did sodium nitroprusside. In taenia, verapamil inhibited the sustained contractions, but not

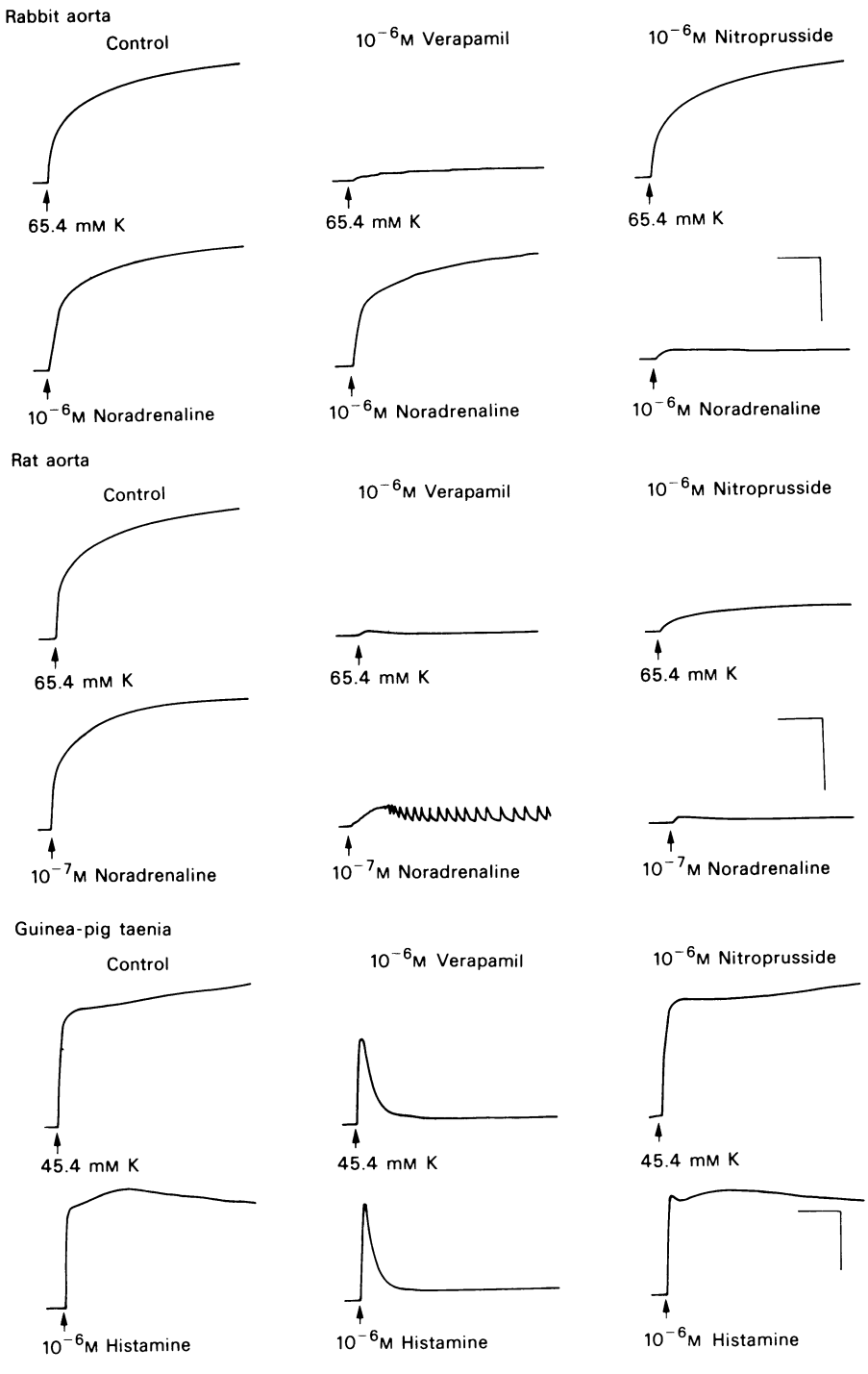

Figure 1 Effects of verapamil and sodium nitroprusside on the contractions induced in smooth muscles. Verapamil 10^{-6}M or sodium nitroprusside 10^{-6}M was added 30 min before the addition of stimulants. Horizontal bar indicates 2 min and vertical bar indicates 0.5 g tension for aortae and 5 g tension for taenia.

Table 1 Concentration of verapamil required to inhibit contractions by 50% (IC_{50}) in the presence of different concentrations of Ca

Smooth muscle	*Stimulant*	Ca 0.3 mM	Ca 1.5 mM	Ca 7.5 mM
			IC_{50}, $\times 10^{-7}$M	
Rabbit aorta	K 65.4 mM	0.15±0.02	0.68±0.06	1.1±0.2
	Noradrenaline 10^{-6}M	—	>100	—
Rat aorta	K 65.4 mM	0.47±0.04	1.0±0.1	2.5±0.1
	Noradrenaline 10^{-7}M	0.42±0.02	1.8±0.1	7.5±0.3
Guinea-pig taenia	K 45.4 mM	0.076±0.005	0.18±0.01	0.76±0.01
	Histamine 10^{-6}M	0.62±0.06	1.5±0.1	15.6±1.5

Values are mean ± s.e. mean of 4 to 6 experiments.

Table 2 Concentration of sodium nitroprusside required to inhibit contractions by 50% (IC_{50}) in the presence of different concentrations of Ca

Smooth muscle	*Stimulant*	Ca 0.3 mM	Ca 1.5 mM	Ca 7.5 mM
			IC_{50}, $\times 10^{-7}$M	
Rabbit aorta	K 65.4 mM	—	>100	—
	Noradrenaline 10^{-6}M	0.79±0.07	1.2±0.1	1.6±0.1
Rat aorta	K 65.4 mM	0.046±0.001	0.047±0.003	0.16±0.01
	Noradrenaline 10^{-7}M	0.012±0.002	0.016±0.003	0.014±0.001
Guinea-pig taenia	K 45.4 mM	—	>100	—
	Histamine 10^{-6}M	—	>100	—

Values are mean ± s.e. mean of 4 to 6 experiments.

the initial transient contractions induced by K 45.4 mM and histamine 10^{-6}M. Sodium nitroprusside had little effect on the contractions induced by K and histamine in taenia.

In Table 1, IC_{50} values calculated from cumulative concentration-inhibition curves for the inhibitory effects of verapamil are shown. The IC_{50} for K-induced contractions in rabbit aorta, rat aorta and taenia was 0.68×10^{-7}M, 1.0×10^{-7}M, and 0.18×10^{-7}M, respectively. Decreasing the concentration of external Ca from control level of 1.5 mM to 0.3 mM shifted the concentration-inhibition curves to the left (IC_{50} decreased), whereas increasing the concentration of Ca to 7.5 mM shifted the curves to the right (IC_{50} increased). In rabbit aorta, verapamil 10^{-6}M which abolished the K-induced contraction, inhibited the noradrenaline-induced contraction by only 18%. The noradrenaline-induced contraction in rat aorta was inhibited by verapamil at a concentration 1.8 times higher than that needed to inhibit the K-induced contraction. Further, an approximately 8 times higher concentration of verapamil was necessary to inhibit the histamine-induced contraction than to inhibit the K-induced contraction in taenia. Changes in external Ca shifted the concentration-inhibition curves in a manner similar to that observed with K-induced contractions.

In Table 2, IC_{50} values for the inhibitory effects of sodium nitroprusside are shown. Noradrenaline-induced contractions in aortae were inhibited by sodium nitroprusside, but rat aorta was approximately 75 times more sensitive than rabbit aorta. The K-induced contraction in rabbit aorta was almost insensitive to sodium nitroprusside, whereas the K-induced contraction in rat aorta was inhibited by sodium nitroprusside. In rat aorta, the noradrenaline-induced contraction was approximately 3 times more sensitive to sodium nitroprusside than the K-induced contraction. The K- and the histamine-induced contractions in taenia were relatively insensitive to sodium nitroprusside; 10^{-5}M sodium nitroprusside inhibited K-induced contractions to 88.7±2.1% ($n=6$) and histamine-induced contractions to 75.6±1.7% ($n=4$) of the respective controls. Changing the concentration of external Ca modified only slightly the concentration-inhibition curves of the noradrenaline-induced contractions in aortae. In the K-induced contraction in rat aorta, a part of the contraction was insensitive to the inhibitory effect of sodium nitroprusside (10^{-4}M); an in-

crease in external Ca increased the insensitive portion of the contraction from $7.3 \pm 3.2\%$ ($n = 4$) in 1.5 mM Ca to $25.3 \pm 5.0\%$ ($n = 4$) in 7.5 mM Ca. This portion of the contraction was inhibited by 10^{-6}M verapamil.

Rate of ^{45}Ca uptake

As shown in Figure 2, 65.4 mM K and 10^{-6}M noradrenaline increased the rate of ^{45}Ca uptake in rabbit aorta. The increase induced by high K was inhibited by 10^{-6}M verapamil, while 10^{-6}M sodium nitroprusside had no effect. On the other hand, the increase in the rate of ^{45}Ca uptake induced by noradrenaline was inhibited by sodium nitroprusside, but not by verapamil. Neither verapamil nor sodium nitroprusside changed the rate of resting ^{45}Ca uptake in rabbit aorta. In rat aorta, neither of the inhibitors modified the rate of resting ^{45}Ca uptake. K 65.4 mM and noradrenaline 10^{-7}M increased the rate of ^{45}Ca uptake and the increment induced by noradrenaline was inhibited by either verapamil 10^{-6}M or sodium nitroprusside 10^{-6}M. The increment induced by high K was also inhibited by verapamil and sodium nitroprusside to the control level. However, compared with the rate of ^{45}Ca uptake in non-stimulated, sodium nitroprusside-treated muscle, the value in the K-stimulated, sodium nitroprusside-treated muscle was slightly higher ($0.01 < P < 0.05$). In guinea-pig taenia, K 45.4 mM and histamine 10^{-6}M increased the rate of ^{45}Ca uptake. The increment induced by high K was inhibited by verapamil 10^{-6}M but not by sodium nitroprusside 10^{-6}M. The increment induced by histamine was also inhibited by verapamil, but not by sodium nitroprusside. Neither verapamil nor sodium nitroprusside affected the rate of resting ^{45}Ca uptake in *taenia*.

Discussion

In rabbit aorta, Ito *et al.* (1977) have found that

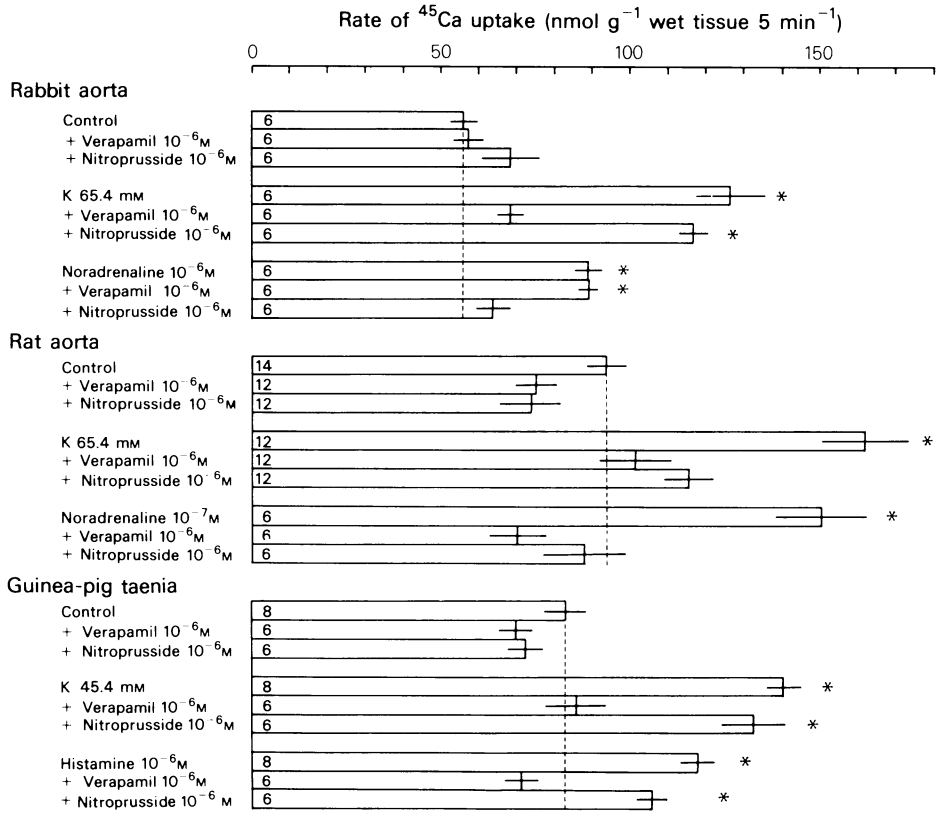

Figure 2 Changes in the rate of ^{45}Ca uptake in rabbit and rat aortae and taenia of guinea-pig. Experiments were done in similar conditions to those in Figure 1. Number in each column indicates number of experiments. *Different from respective control ($P < 0.01$).

verapamil inhibited the K-induced contraction but had only a slight effect on the noradrenaline-induced contraction, whereas sodium nitroprusside inhibited the noradrenaline-induced but not the K-induced contraction. It has also been shown (Karaki *et al.*, 1983) that both K and noradrenaline increased the rate of ^{45}Ca uptake (exchange). In the present experiments, we found in rabbit aorta that sodium nitroprusside inhibits the increase in the rate of ^{45}Ca uptake induced by noradrenaline, but not the increase induced by high K. It is known that the response of rabbit aorta to noradrenaline persists in Ca-deficient solution after the response to high K is abolished (Hudgins & Weiss, 1968). However, the response to noradrenaline is not entirely attributable to the release of cellular Ca stores because the addition of EGTA to the Ca-depleted solution rapidly inhibits the remaining sustained contraction (Karaki *et al.*, 1979). In aorta pretreated with EGTA, only an initial transient contraction is induced by noradrenaline (Deth & van Breemen, 1977; Karaki *et al.*, 1979). These results support the interpretation that the K-induced contraction in rabbit aorta is attributable to an influx of extracellular Ca through a voltage-sensitive Ca channel which is specifically inhibited by verapamil; that only the initial transient contraction induced by noradrenaline is due to a release of cellular Ca; that the sustained contraction induced by noradrenaline is the result of an inward translocation of Ca which is bound to the membrane surface in such a manner that the bound Ca is not easily removed by a Ca-deficient solution, but is removed by EGTA; and the latter Ca influx, through a receptor-linked Ca channel, is specifically inhibited by sodium nitroprusside.

Organic Ca antagonists are thought to bind directly to Ca channels in the cell membrane and competitively inhibit Ca entry (Karaki & Weiss, 1980; Meisheri *et al.*, 1981; Triggle, 1981). The mechanism of action of sodium nitroprusside on Ca influx seems to be different from that of verapamil because changes in external Ca concentration did not seem to modify the effect of sodium nitroprusside. There are conflicting reports on the effect of sodium nitroprusside on ^{45}Ca movements (Zsoster *et al.*, 1977; Hester *et al.*, 1979; Karaki *et al.*, 1980; Karaki & Weiss, 1980; Ozaki *et al.*, 1981), which may be due to the difference in the smooth muscle preparations used and also to the different experimental procedure. Further, sodium nitroprusside seems to have multiple sites of action: in addition to the inhibitory effect on Ca influx, this agent seems to inhibit Ca release and/or to increase Ca sequestration because it inhibits the noradrenaline-induced initial transient contraction which is not dependent on Ca influx. The latter possibility has been suggested in various smooth muscle preparations (Kreye *et al.*, 1975; Verhaeghe & Shepherd, 1976; Hester *et al.*, 1979; Karaki *et al.*, 1980; Ozaki *et al.*, 1981). Recently, Rapoport & Murad (1983) suggested that the inhibitory effect of sodium nitroprusside may be attributable to a membrane hyperpolarization following stimulation of an electrogenic Na pump. However, this might not be the only mechanism of action because sodium nitroprusside is effective in high K-depolarized rat aorta in the presence of an inhibitor of the Na pump, ouabain (Karaki *et al.*, 1984).

In rat aorta, it has been suggested that the initial transient contraction induced by noradrenaline is the result of release of cellular Ca, while sustained contraction induced either by high K or by noradrenaline is due to Ca influx (Godfraind & Kaba, 1972). This possibility is supported by the previous (Godfraind, 1976; 1983) and the present findings that both high K and noradrenaline increase the rate of ^{45}Ca uptake in rat aorta. Thus, Ca movements in rabbit and rat aortae seem to be similar. However, the experiments with inhibitors showed that there is no specific correlation between inhibitors and Ca pathways; the K- and the noradrenaline-induced increases in tension and ^{45}Ca uptake were nonspecifically inhibited by either verapamil or sodium nitroprusside.

Although verapamil strongly inhibited the aortic contractions induced by either high K^+ or noradrenaline in male Wistar rats (present results) and also in male Sprague-Dawley rats (Lincoln, 1983), Peiper *et al.* (1971) and Bilek *et al.* (1974) found that noradrenaline-induced contraction was not, or only slightly, inhibited by verapamil in their rat aorta (strain not specified). Furthermore, it was found that, in the aorta of the rabbits of a closed colony, noradrenaline-induced contraction was much more sensitive to verapamil than that in New Zealand rabbits used in the present experiments (unpublished observations). In other vascular smooth muscles including mesenteric, coronary and cerebral arteries and portal vein, not only K^+-induced contractions but also the contractions induced by noradrenaline and other receptor-agonists are inhibited in varying degrees by verapamil (see Triggle, 1981; Flaim, 1982). These results suggest that the differences between rabbit and rat aorta in terms of their sensitivity to verapamil are qualitative rather than quantitative; sensitivity of most vascular smooth muscles to verapamil may be more or less nonspecific.

In guinea-pig taenia, both K- and histamine-induced sustained contractions are dependent on external Ca (Nasu *et al.*, 1971). It was found that verapamil inhibits only the sustained contraction, but not the initial transient contraction induced by high K or histamine. In contrast, sodium nitroprusside had little effect on taenia. Kreye *et al.* (1975) have also observed that the smooth muscle preparations from the splanchnic region with varying degree of phasic

contractility are less sensitive to sodium nitroprusside. Since the increase in the rate of ^{45}Ca uptake induced by high K or histamine was inhibited by verapamil, but not by sodium nitroprusside, Ca pathways in taenia seem to be insensitive to sodium nitroprusside.

These results raise the question as to whether or not the Ca channels opened by K-depolarization and by receptor agonists are different in rat aorta and taenia. In rat aorta, increase in external Ca increased a portion of K-induced contraction which is insensitive to sodium nitroprusside and is inhibited by verapamil. Further, noradrenaline-induced contraction in rat aorta was more sensitive to sodium nitroprusside and less sensitive to verapamil than K-induced contraction. Also in taenia, K-induced contraction was more sensitive to verapamil than histamine-induced contraction. These differences suggest the existence of two different Ca channels activated by K-depolarization and receptor-agonists, respectively.

It is concluded that verapamil is a specific inhibitor of the voltage-dependent Ca channel in rabbit aorta, but is a relatively nonspecific inhibitor of Ca channels in rat aorta and taenia; and that sodium nitroprusside is able to inhibit specifically the receptor-linked Ca channel in rabbit aorta, to inhibit relatively nonspecifically the Ca channels in rat aorta, and has little effect on Ca influx in taenia.

We gratefully acknowledge the helpful suggestions of Dr Phyllis Kutsky, Department of Physiology, Texas College of Osteopathic Medicine.

References

BILEK, I., LAWEN, R., PEIPER, U. & REGNAT, K. (1974). The effect of verapamil on the response to noradrenaline or to potassium-depolarization in isolated vascular strips. *Microvasc. Res.*, **7**, 181–189.

BOLTON, T.B. (1979). Mechanisms of action of transmitters and other substances on smooth muscle. *Physiol. Rev.*, **59**, 606–718.

BREEMEN, C. VAN, AARONSON, P. & LOUTZENHISER, R. (1979). Sodium-calcium interactions in mammalian smooth muscle. *Pharmac. Rev.*, **30**, 167–208.

DETH, R. & VAN BREEMEN, C. (1977). Agonists induced release of intracellular Ca^{2+} in the rabbit aorta. *J. Membrane Biol.*, **30**, 363–380.

FLAIM, S. F. (1982). Comparative pharmacology of calcium blockers based on studies on vascular smooth muscle. In *Calcium Blockers, Mechanisms of Action and Clinical Applications*. ed. Flaim, S.F. & Zelis, R., pp. 155–178. Baltimore: Urban & Schwarzenberg.

FLECKENSTEIN, A. (1977). Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. *A. Rev. Pharmac.*, **17**, 149–166.

GODFRAIND, T. (1976). Calcium exchange in vascular smooth muscle, action of noradrenaline and lanthanum, *J. Physiol.*, **260**, 21–35.

GODFRAIND, T. (1983). Actions of nifedipine on calcium fluxes and contraction in isolated rat arteries. *J. Pharmac. exp. Ther.*, **224**, 443–450.

GODFRAIND, T. & KABA, A. (1972). The role of calcium in the action of drugs on vascular smooth muscle. *Archs. int. Pharmacodyn. Thér.*, **196**, (suppl.), 35–49.

GOLENHOFEN, K. (1976). Theory of P and T systems for calcium activation in smooth muscle. In *Physiology of Smooth Muscle*. ed. Bülbring, E. & Shuba, M. F. pp. 197–202. New York: Raven.

GOLENHOFEN, K. (1981). Differentiation of calcium activation process in smooth muscle using selective antagonists. In *Smooth Muscle, and Assesment of Current Knowledge*, ed. Bülbring, E., Jones, A. W. & Tomita, T. pp. 157–170. London: Arnold.

HESTER, R. K., WEISS, G. B. & FRY, W. J. (1979). Differing actions of sodium nitroprusside and D-600 on tension and ^{45}Ca fluxes in canine renal arteries. *J. Pharmac. exp. Ther.* **208**, 155–160.

HUDGINS, P. & WEISS, G.B. (1968). Differential effects of calcium removal upon vascular smooth muscle contraction induced by norepinephrine, histamine and potassium. *J. Pharmac. exp. Ther.*, **159**, 91–97.

ITO, K., KARAKI, H. & URAKAWA, N. (1977). The mode of contractile action of palytoxin on vascular smooth muscle. *Eur. J. Pharmac.*, **46**, 9–14.

KARAKI, H., HATANO, K. & WEISS, G.B. (1983). Effects of magnesium on ^{45}Ca uptake and release at different sites in rabbit aortic smooth muscle. *Pflügers Arch. ges. Physiol.*, **398**, 27–32.

KARAKI, H., HESTER, R.K. & WEISS, G.B. (1980). Cellular basis of nitroprusside-induced relaxation of graded response to norepinephrine and potassium in canine renal arteries. *Archs. int. Pharmacodyn. Thér.*, **245**, 198–210.

KARAKI, H., KUBOTA, H. & URAKAWA, N. (1979). Mobilization of stored calcium for phasic contraction by norepinephrine in rabbit aorta. *Eur. J. Pharmac.*, **56**, 237–245.

KARAKI, H., MURAKAMI, K., NAKAGAWA, H. & URAKAWA, N. (1984). Nitroglycerine-induced biphasic relaxation in vascular smooth muscle of rat aorta. *Br. J. Pharmac.*, **81**, 387–392.

KARAKI, H., SUZUKI, T. & URAKAWA, N. (1981). Tris does not inhibit isolated vascular or intestinal smooth muscle contraction. *Am. J. Physiol.*, **241**, H337-H341.

KARAKI, H. & URAKAWA, N. (1977). Possible role of endogenous catecholamines in the contraction induced in rabbit aorta by ouabain, sodium depletion and potassium depletion. *Eur. J. Pharmac.*, **43**, 65–72.

KARAKI, H. & WEISS, G. B. (1979). Alterations in high and low affinity binding of ^{45}Ca in rabbit aortic smooth muscle by norepinephrine and potassium after exposure to lanthanum and low temperature. *J. Pharmac. exp. Ther.*, **211**, 86–92.

KARAKI, H. & WEISS, G.B. (1980). Effects of stimulatory agents on mobilization of high and low affinity site ^{45}Ca in rabbit aortic smooth muscle. *J. Pharmac. exp. Ther.*, **213,** 450–455.

KREYE, V.A.W., BARON, G. D., LÜTH, J.B. & SCHMIDT-GAYK, H. (1975). Mode of action of sodium nitroprusside on vascular smooth muscle. *Naunyn-Schmiedebergs Arch. Pharmac.*, **288,** 381–402.

LINCOLN, T.M. (1983). Effects of sodium nitroprusside and 8-bromo-cyclic GMP on the contractile activity of the rat aorta. *J. Pharmac. exp. Ther.*, **224,** 100–107.

MEISHERI, K.D., HWANG, O. & VAN BREEMEN, C. (1981). Evidence for two separate Ca^{2+} pathways in smooth muscle plasmalemma. *J. Membrane Biol.*, **59,** 19–25.

NASU, T., KARAKI, H., IKEDA, M. & URAKAWA, N. (1971). Effects of histamine on calcium movement in guinea pig taenia coli. *Jap. J. Pharmac.*, **21,** 597–603.

OZAKI, H., SHIBATA, S., KITANO, H., MATSUMOTO, P. & ISHIDA, Y. (1981). Comparative study of the relaxing effect of nitroprusside and verapamil on human umbilical vessels. *Blood Vessels*, **18,** 321–329.

PEIPER, U., GRIEBEL, L. & WENDE, W. (1971). Activation of vascular smooth muscle of rat aorta by noradrenaline and depolarization: two different mechanisms. *Pflüger's Arch.*, **330,** 74–89.

RAPOPORT, R.M. & MURAD, F. (1983). Effects of ouabain and alterations in potassium concentration on relaxation induced by sodium nitroprusside. *Blood Vessels*, **20,** 255–264.

TRIGGLE, T.J. (1981). Calcium antagonists. Basic chemical and pharmacological aspects. In *New Perspectives on Calcium Antagonists.* ed. Weiss, G.B. pp. 1–18. Baltimore: Williams & Wilkins.

VERHAEGHE, R.H. & SHEPHERD, J.T. (1976). Effects of nitroprusside on smooth muscle and adrenergic nerve terminals in isolated blood vessels. *J. Pharmac. exp. Ther.*, **199,** 269–277.

WEISS, G.B. (1981). Sites of action of calcium antagonists in vascular smooth muscle. In *New Perspectives on Calcium Antagonists.* ed. Weiss, G.B. pp. 83–94. Baltimore: Williams & Wilkins.

ZSOSTER, T.T., HENEIN, N.F. & WOLCHINSKY, C. (1977). The effect of sodium nitroprusside on the uptake and efflux of ^{45}Ca from rabbit and rat vessels. *Eur. J. Pharmac.*, **45,** 7–12.

(*Received August 31, 1983.*
Revised October 10, 1983.)