Br. J. Pharmac. (1986), **89**, 423–429

Effects of the calcium channel facilitator, CGP 28,392, on different modes of contraction in smooth muscle of rabbit and rat aortae and guinea-pig taenia caeci

H. Karaki[1], H. Nagase, H. Ozaki, N. Urakawa & G.B. Weiss*

Department of Veterinary Pharmacology, Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan and *Department of Physiology, University of Medicine and Dentistry of New Jersey, Newark, New Jersey 07103, U.S.A.

1 Effects of a Ca^{2+} channel facilitator, CGP 28,392, on smooth muscle contractions were examined in order to delineate characteristics of Ca^{2+} channels in rabbit and rat aortae and guinea-pig taenia caeci.

2 Application of increasing concentrations of KCl induced contractile responses in these smooth muscles and CGP 28,392 shifted the concentration-response curve for KCl to the left. The maximum response was also increased in rat aorta and guinea-pig taenia. CGP 28,392 also shifted the concentration-response curves for noradrenaline in rat aorta and for histamine in taenia to the left and increased the maximum response in rat aorta. However, the corresponding curve for noradrenaline in rabbit aorta was not affected by CGP 28,392.

3 The sustained contractions induced by KCl were inhibited by cumulative application of verapamil in these smooth muscles. Pretreatment of the muscle with CGP 28,392 decreased the inhibitory effect of verapamil. The noradrenaline-induced contraction in rat aorta and the histamine-induced contraction in taenia were also inhibited by verapamil, and CGP 28,392 antagonized the effect of verapamil. The noradrenaline-induced contraction in rabbit aorta was only slightly inhibited by verapamil, and CGP 28,392 did not modify the effect of verapamil.

4 In these smooth muscles, cumulative application of Ca^{2+} to the Ca^{2+}-depleted, KCl-treated muscle induced contraction, and the concentration-response curve for Ca^{2+} was shifted to the left by CGP 28,392 and to the right by verapamil. The concentration-response curves for Ca^{2+} in Ca^{2+}-depleted, noradrenaline-treated rabbit and rat aortae and in Ca^{2+}-depleted, histamine-treated taenia were also shifted to the left by CGP 28,392 and to the right by verapamil. In some contractions, CGP 28,392 increased and verapamil decreased the maximum responses. CGP 28,392 antagonized the inhibitory effect of verapamil.

5 These results suggest that the Ca^{2+} channel facilitator, CGP 28,392, has a relatively selective activating effect on voltage-dependent Ca^{2+} channels in rabbit aorta. However, it also activates receptor-linked Ca^{2+} channels in rabbit aorta when Ca^{2+} concentrations are low. In rat aorta and guinea-pig taenia this facilitator activates both types of Ca^{2+} channels.

Introduction

CGP 28,392 is a 2,4-dihydropyridine derivative which is structurally related to the dihydropyridine Ca^{2+} channel inhibitor, nifedipine. Although the Ca^{2+} channel inhibitors decrease Ca^{2+} influx through specific Ca^{2+} channels located in the membrane of myocardial and smooth muscle cells (Fleckenstein, 1977), CGP 28,392 is reported to have pharmacological effects opposite to those of the Ca^{2+} channel inhibitors (Truog *et al.*, 1984). YC-170 (Takenaka & Maeno, 1982) and Bay K 8644 (Schramm *et al.*, 1983) are also dihydropyridine derivatives with pharmacological properties similar to CGP 28,392, and these compounds have been termed Ca^{2+} channel facilitators.

In many vascular smooth muscle preparations, these Ca^{2+} channel facilitators have little or no effect on the resting tone unless the concentration of external KCl is raised to 10–15 mM (Schramm *et al.*, 1983; Su *et al.*, 1984; Loutzenhiser *et al.*, 1984; Cheung, 1985). However, the Ca^{2+} channel facilitators potentiate the

[1]Author for correspondence.

contraction induced by elevated concentrations of K^+ (Schramm *et al.*, 1983; Su *et al.*, 1984; Loutzenhiser *et al.*, 1984; Truog *et al.*, 1984; Kanmura *et al.*, 1984; Yamamoto *et al.*, 1984; Cheung, 1985; Dube *et al.*, 1985). Similar potentiation of the K^+-induced contraction has been reported for intestinal smooth muscle (Spedding & Berg, 1984). Furthermore, potentiation of K^+-induced contractions by Ca^{2+} channel facilitators is followed by an additive increase in $^{45}Ca^{2+}$ influx (Loutzenhiser *et al.*, 1984; Yamamoto *et al.*, 1984; Schramm *et al.*, 1985). These results suggest that Ca^{2+} channel facilitators increase Ca^{2+} entry through voltage-dendent Ca^{2+} channels in the smooth muscle.

In contrast to the above observations, effects of the Ca^{2+} channel facilitators on contractions induced by receptor agonists are inconsistent. The Ca^{2+} channel facilitators have little effect on the noradrenaline-induced contraction in rabbit aorta (Schramm *et al.*, 1983; Loutzenhiser *et al.*, 1984), a slight potentiating effect on the phenylephrine-induced contraction in rat tail artery (Su *et al.*, 1984), the noradrenaline-induced contraction in rabbit mesenteric artery (Kanmura *et al.*, 1984) and the histamine-induced contraction in porcine coronary artery (Dube *et al.*, 1985), and a pronounced potentiating effect on the BHT-920-induced contraction in rat tail artery (Su *et al.*, 1984) and the noradrenaline- and methoxamine-induced contractions in rat saphenous vein (Cheung, 1985).

Previously, we have shown that although the Ca^{2+} channel inhibitors selectively inhibit KCl-induced contractions in rabbit aorta, these inhibitors prevent the contractions induced by either KCl or receptor agonists in rat aorta and guinea-pig taenia (Karaki *et al.*, 1984a; 1985; 1986b; Karaki & Weiss, 1984). In the present paper, the effects of Ca^{2+} channel facilitator, CGP 28,392, on different types of contractile responses in rabbit aorta, rat aorta and guinea-pig taenia were examined in order to ascertain whether this Ca^{2+} channel facilitator selectively modifies either type of contraction in each of these smooth muscle preparations.

Some of the results has been described previously (Karaki *et al.*, 1986a).

Methods

Tissue preparations

Three different 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 medial layer as described by Karaki & Urakawa (1977) in order to avoid possible involvement of release of endogenous catecholamines (Karaki *et al.*, 1984b), and muscle strips (4–6 mm long) were prepared. (2) Male Wistar rats, weighing about 200 g, were decapitated and bled. 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 bled and a section of taenia (5–10 mm in length) was dissected from the caecum. Each muscle strip was 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.

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. High K^+ solution was prepared by increasing the concentration of KCl to 55.4 mM, and Ca^{2+}-free solution was prepared by removing $CaCl_2$ from the normal solution. 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. Contractions were induced either by cumulative application of KCl, noradrenaline or histamine, or by Ca^{2+} to the Ca^{2+}-depleted muscle in the presence of high concentration of K^+, noradrenaline or histamine. Ca^{2+}-depleted muscle was obtained by incubating the muscle with a Ca^{2+}-free solution containing 1.0 mM EGTA for 10 min followed by incubation with a Ca^{2+}-free solution (without EGTA) containing a stimulant for 10 min. CGP 28,392 or verapamil was added 10 min before the application of either stimulant (in normal muscle) or Ca^{2+} (in Ca^{2+}-depleted muscle). Inhibitory effects of verapamil were examined by cumulative application of verapamil during sustained contractions induced by a simulant. CGP 28,392 was added 10 min before the addition of stimulant.

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 of less than 0.01 was taken as significant.

Drugs and chemicals

CGP 28,392 (4-[2- (difluormethoxy)phenyl] -1,4,5,7-

tetrahydro-2-methyl-5-oxofuro [3,4-b]pyridine-3-carboxylic acid ester) donated by Ciba-Geigy), (±)-verapamil hydrochloride (Sigma), (−)-noradrenaline bitartrate (Wako Pure Chemicals), histamine dihydrochloride (Wako) and ethyleneglycol bis-(β aminoethylether) N,N,N′,N′-tetraacetic acid (EGTA, Sigma) were used. CGP 28,392 was dissolved in dimethylsulphoxide (Wako). The final concentration of dimethylsulphoxide was less than 0.01%. Control experiments indicated that this concentration of dimethylsulphoxide did not have any effect on the responses of the smooth muscle to the agents tested.

Results

Effects of CGP 28,392 on the resting tone

Addition of CGP 28,392 in concentrations up to 10^{-6} M had little effect on the resting tone of rabbit and rat aortae, whereas 10^{-6} M CGP 28,392 sometimes increased the spontaneous rhythmic contraction in guinea-pig taenia. CGP 28,392 (10^{-5} M) slightly increased the resting tone of these smooth muscle preparations.

Effects of CGP 28,392 on contractions induced by KCl, noradrenaline and histamine

Effects of CGP 28,392 on contractions induced by cumulative applications of KCl, noradrenaline and histamine are shown in Figure 1. The concentration-response curves for K^+ were shifted to the left by 10^{-6} M CGP 28,392 in rabbit and rat aortae and in guinea-pig taenia. The maximum response also increased in the latter two preparations. The concentration-response curves for noradrenaline in rabbit aorta was not affected by 10^{-6} M CGP 28,392 whereas the corresponding curves for noradrenaline in rat aorta and for histamine in guinea-pig taenia were shifted to the left and the maximum responses increased. As reported previously (Truog *et al.*, 1984; Loutzenihiser *et al.*, 1984), 10^{-6} M CGP 28,392 was maximally effective (10^{-5} M CGP 28,392 had similar or smaller effects). Thus, a concentration of 10^{-6} M was used in subsequent experiments.

Effects of CGP 28,392 on the relaxation induced by verapamil

Cumulative application of verapamil inhibited the contractions induced by high K^+ in rabbit aorta, rat aorta and guinea-pig taenia. Noradrenaline-induced contractions in rat aorta and histamine-induced contractions in taenia were also inhibited by verapamil, although at higher concentrations of verapamil than those needed to inhibit K^+-induced contractions in the respective preparations. The inhibitory effects of verapamil on noradrenaline-induced contractile responses in rabbit aorta were small. In the presence of 10^{-6} M CGP 28,392, the inhibitory effects of verapamil on K^+-induced contractions were greatly reduced, the effects on noradrenaline-induced contractions in rat aorta and on histamine-induced contractions in taenia were slightly reduced, and the effects on noradrenaline-induced contractions in rabbit aorta were not affected. The concentrations of verapamil needed to induce half-maximum inhibition (IC_{50}) in the presence or absence of CGP 28,392 are listed in Table 1.

Table 1 Concentration of verapamil needed to induce half-maximum inhibition of the contractions in the presence or absence of CGP 28,392

	Control (A)	CGP 28,392 (B)	*B/A*
	× 10^{-7} M		
Rabbit aorta			
KCl	0.50 ± 0.05	6.65 ± 0.17*	13.3
Noradrenaline	89.0 ± 11.5	57.0 ± 13.0	0.6
Rat aorta			
KCl	1.03 ± 0.33	6.50 ± 0.72*	6.3
Noradrenaline	6.10 ± 0.55	12.7 ± 1.10*	2.3
Guinea-pig taenia			
KCl	0.19 ± 0.02	2.15 ± 0.15*	11.1
Histamine	1.33 ± 0.15	4.47 ± 0.39*	4.5

Verapamil was cumulatively added during the sustained contraction induced by 55.4 mM K^+, 10^{-6} M noradrenaline or 10^{-6} M histamine. CGP 28,392 (10^{-6} M) was applied 10 min before the addition of stimulant. Each value represents the mean ± s.e.mean for 4 to 8 experiments. Verapamil (10^{-5} M) induced 100% inhibition of these contractions except in noradrenaline-induced contraction in rabbit aorta (51.0 ± 2.1% inhibition). *Significantly different from control ($P < 0.01$). Concentration ratio of verapamil needed to induce half-maximum inhibition in the presence (B) to in the absence (A) of CGP 28,392 is also shown (B/A).

Effects of CGP 28,392 and verapamil on Ca^{2+}-induced contractions

Cumulative addition of Ca^{2+} induced concentration-dependent contractions in smooth muscle preparations pretreated with Ca^{2+}-free solutions containing KCl, noradrenaline or histamine. As shown in Figure 2, all of the concentration-response curves were shifted to the left by 10^{-6} M CGP 28,392. The maximum contractile responses in K^+-depolarized muscles and in noradrenaline-treated rat aorta were also

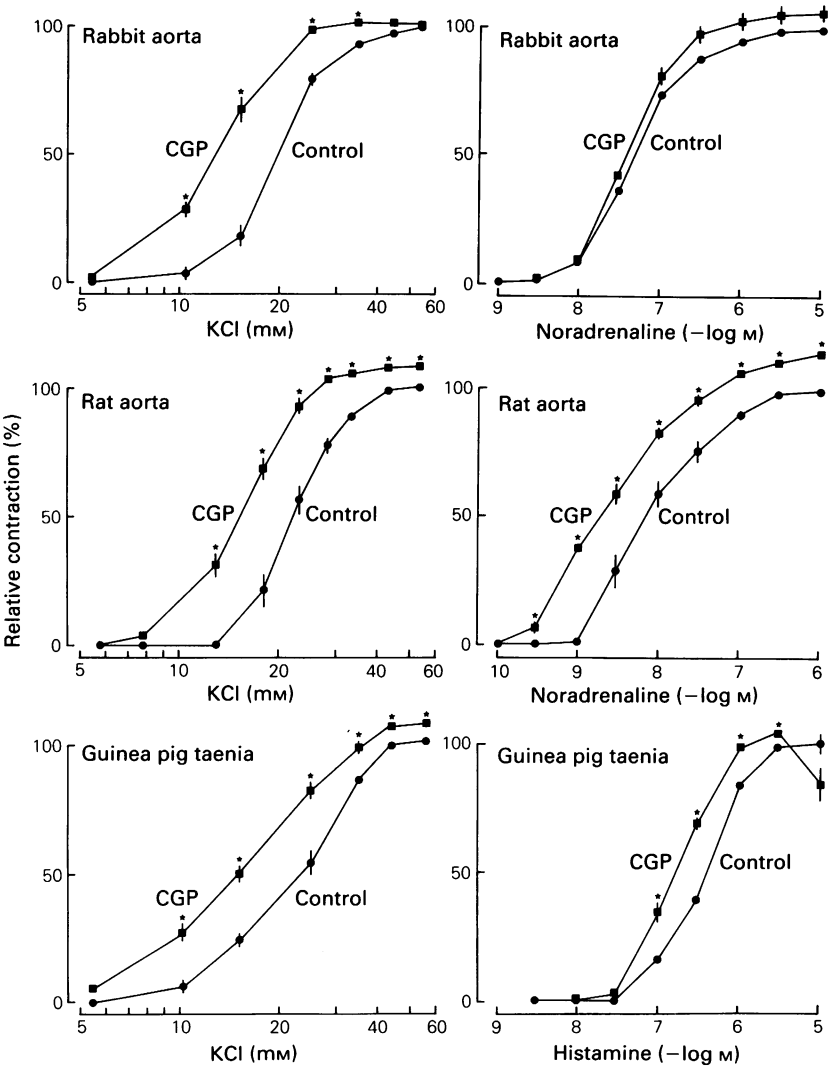

Figure 1 Effects of CGP 28,392 on the contractions induced by cumulative application of KCl, noradrenaline or histamine in rabbit aorta, rat aorta and guinea-pig taenia caeci. Application of a stimulant was repeated three times in one muscle strip, and since the second and the third applications produced similar contractions, the second contraction was taken as control. CGP 28,392 (10^{-6} M) was added 10 min before the third cumulative addition of stimulant. 100% represents the maximum contractile tension of control contraction. Each curve represents the mean of 4 to 8 experiments and the s.e. of the mean is shown by vertical bars when it is greater than the symbol. *Significantly different from control ($P < 0.01$).

increased. Conversely, verapamil (at IC_{50} concentrations for K^+-induced contractions as in Table 1) shifted these curves to the right. The maximum contractile response in K^+-depolarized rabbit and rat aortae, noradrenaline-treated rat aorta and histamine-treated guinea-pig taenia were also decreased. However, in the presence of 10^{-6} M CGP 28,392, verapamil did not inhibit these contractions.

Discussion

Smooth muscle contraction is due either to release of cellular Ca^{2+} or influx of external Ca^{2+}. Since the larger portion of the sustained contraction is inhibited in Ca^{2+}-free solution and this effect can be correlated with the observance of an increase in $^{45}Ca^{2+}$ uptake during the sustained contraction, it seems likely that

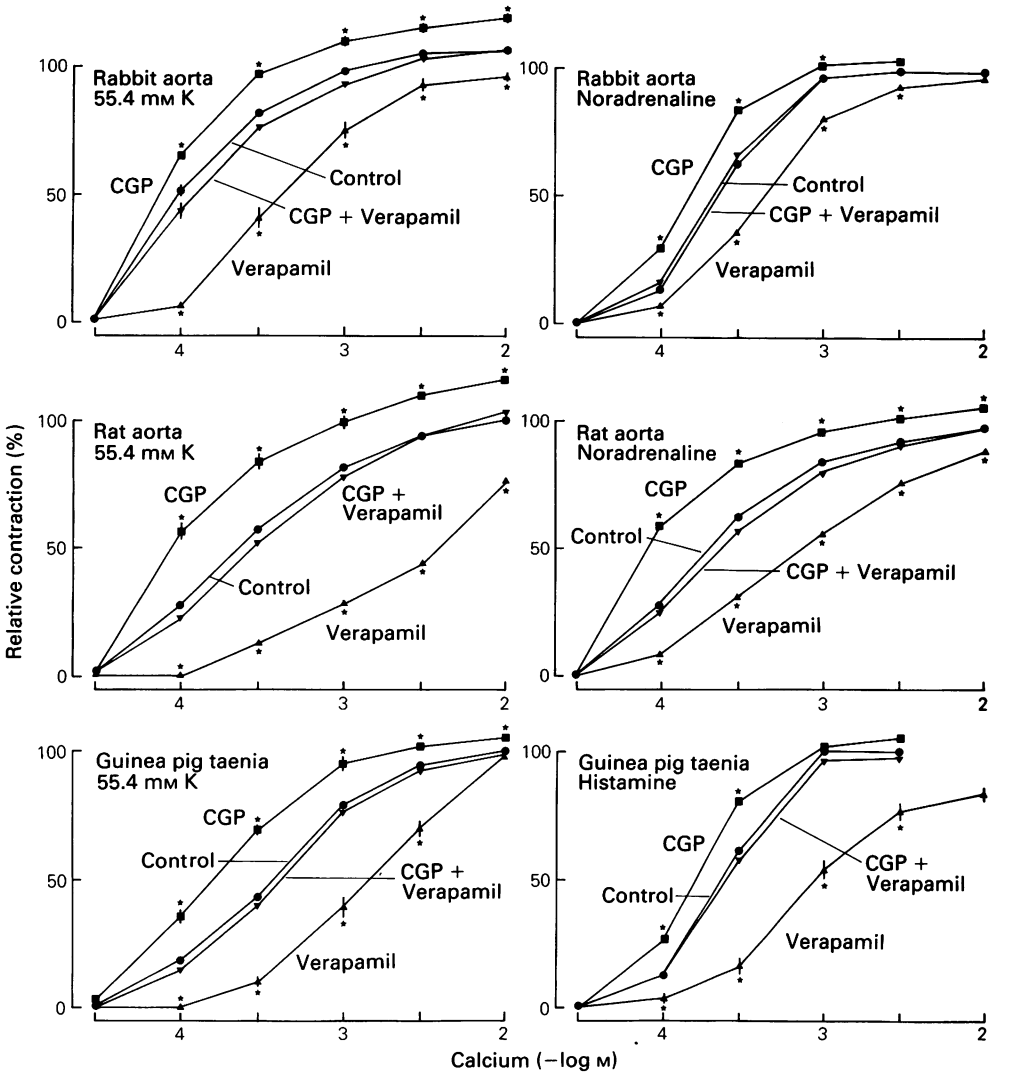

Figure 2 Effects of CGP 28,392 and verapamil on the contractions induced by cumulative application of Ca^{2+} in rabbit aorta, rat aorta and guinea-pig taenia caeci. Muscle strips were treated with Ca^{2+}-free solution containing 55.4 mM KCl, 10^{-6} M noradrenaline or 10^{-6} M histamine and Ca^{2+} was cumulatively added. CGP 28,392 (10^{-6} M) and/or verapamil (5×10^{-8} M for rabbit aorta, 10^{-7} M for rat aorta and 2×10^{-8} M for taenia) were added 10 min before the addition of Ca^{2+}. 100% represents the maximum contractile tension of control contraction. Actual contractile tension was as follows: Rabbit aorta: K^+, 1.6 ± 0.1 g, noradrenaline, 1.9 ± 0.2 g, rat aorta: K^+, 1.1 ± 0.1 g, noradrenaline, 1.5 ± 0.2 g; and guinea-pig taenia: K^+, 9.5 ± 0.3 g, histamine, 7.1 ± 0.3 g. For further details see Methods. Each curve represents the mean of 4 to 8 experiments and the s.e. of the mean is shown by vertical bars when it is greater than the symbol. *Significantly different from control ($P < 0.01$).

the sustained contraction results from Ca^{2+} influx and that release of cellular Ca^{2+} is responsible only for an initial transient contraction (Weiss, 1977; Cauvin *et al.*, 1983; Karaki & Weiss, 1984).

In rabbit aorta (Mekata, 1974; 1976) and main pulmonary artery (Haeusler & Thorens, 1980), high K^+ induces contraction which correlates which membrane depolarization whereas noradrenaline induces similar contraction without or with a small depolarization. Addition of both high K^+ and noradrenaline elicits additive contractions (Karaki *et al.*, 1985) as well as additive increases in $^{45}Ca^{2+}$ uptake (Karaki &

Weiss, 1980; Meisheri et al., 1981). These additional changes cannot depend only upon membrane depolarization. Furthermore, in a Ca^{2+}-free and Sr^{2+}-substituted solution, high K^+ induced both contractions and subsequent increases in $^{89}Sr^{2+}$ uptake whereas noradrenaline is almost ineffective on both muscle tension and $^{89}Sr^{2+}$ uptake (Karaki et al., 1986b). Ba^{2+} also enters the high K^+-depolarized but not the noradrenaline-treated rabbit aorta (Karaki et al., 1986c). Thus, Sr^{2+} and Ba^{2+} can penetrate the high K^+-activated channel more than the noradrenaline-activated channel. These results support the idea that voltage dependent and receptor-linked Ca^{2+} channels exist in this smooth muscle (Bolton, 1979; van Breeman et al., 1979; Karaki & Weiss, 1984).

The results in Figure 1 and Table 1 confirmed previous observations that verapamil can selectively inhibit (Karaki et al., 1984a) and CGP 28,392 can selectively potentiate the K^+-induced contraction in rabbit aorta (Loutzenhiser et al., 1984). The slight inhibition or noradrenaline-induced contraction observed with higher concentrations of verapamil was not antagonized by CGP 28,392 (Table 1). Thus, this effect of verapamil may not result from a specific inhibitory effect on Ca^{2+} channels, as suggested by Golenhofen (1981). However, the Ca^{2+}-induced contraction in Ca^{2+}-depleted and noradrenaline-treated rabbit aorta was potentiated by CGP 28,392 and inhibited by verapamil (Figure 2). This discrepancy may be attributable to the difference in the concentration of external Ca^{2+}. In the presence of 1.5 mM Ca^{2+}, neither CGP 28,392 (Figure 1) nor verapamil (Table 1) affected the noradrenaline-induced contraction whereas, when the Ca^{2+} concentration was lower than 1.5 mM (Figure 2), both CGP 28,392 and verapamil modified the noradrenaline-induced contraction.

In contrast to responses of the rabbit aorta, the contractions induced by high K^+ and receptor agonists in rat aorta and guinea-pig taenia were potentiated by CGP 28,392 even in the presence of 1.5 mM Ca^{2+}. Furthermore, all of these contractions were inhibited by verapamil and the inhibition was antagonized by CGP 28,392. If CGP 28,392 and verapamil are assumed to affect selectively the voltage-dependent Ca^{2+} channel, the contractions induced by high K^+ as well as by noradrenaline and histamine can be attributed to activation of this Ca^{2+} channel and the receptor-linked Ca^{2+} channel does not appear to function as separate entity. In fact, the receptor activation may induce membrane depolarization resulting in opening of the voltage-dependent Ca^{2+} channel (Bolton, 1979). However, receptor agonists induce greater increase in membrane conductance than the matched membrane depolarization due to electrical stimuli (Benham & Bolton, 1983). Additivity in high K^+- and receptor agonist-induced contractions (Brading & Sneddon, 1980; Lincoln, 1983), Sr^{2+} permeability only in K^+-depolarized cells (Karaki et al., 1986b), and higher sensitivity of high K^+-induced contraction to verapamil and CGP 28,392 (Table 1) suggest that at least a portion of the contractions elicited with receptor agonists depends upon different Ca^{2+} channels from those involved in K^+-induced contractions in rat aorta and guinea-pig taenia.

Previously, it was suggested that the receptor-linked Ca^{2+} channels in rat aorta and guinea-pig taenia are sensitive to verapamil (see Introduction). Present results suggest that these types of receptor-linked Ca^{2+} channels are also sensitive to CGP 28,392. In contrast to this, the receptor-linked Ca^{2+} channels in rabbit aorta may be less sensitive to either CGP 28,392 or verapamil in the presence of the normal concentration (1.5 mM) of Ca^{2+} and they become sensitive when the Ca^{2+} concentration is lowered.

CGP 28,392 increased the maximum contractile tension induced by high K^+ and receptor agonists in some experiments (Figures 1 and 2). Bay K 8644 also potentiated the maximum contraction induced by Ba^{2+} in rabbit aorta (Karaki et al., 1986c). The mechanism for this effect of Ca^{2+} channel facilitators was not clarified in the present experiments.

This work was supported by a research grant (No. 60480085) from the Ministry of Education, Science and Culture of Japan.

References

BENHAM, C.D. & BOLTON, T.B. (1983). Comparison of the excitatory actions of substance P, carbachol, histamine, and prostaglandin $F_{2\alpha}$ on the smooth muscle of the taenia of the guinea-pig caecum. *Br. J. Pharmac.*, **80**, 409–420.

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

BRADING, A.F. & SNEDDON, P. (1980). Evidence for multiple sources of calcium for activation of the contractile mechanism of guinea-pig taenia coli on stimulation with carbachol. *Br. J. Pharmac.*, **70**, 229–240.

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

CAUVIN, C., LOUTZENHISER, R. & VAN BREEMEN, C. (1983). Mechanisms of calcium antagonist-induced vasodilation. *A. Rev. Pharmac.*, **23**, 373–396.

CHEUNG, D.W. (1985). The effect of Bay K 8644 on contraction mediated by α-adrenoceptors in rat saphenous vein. *Br. J. Pharmac.*, **85**, 317–319.

DUBE, G.P., BAIK, Y.H. & SCHWARTZ, A. (1985). Effects of novel calcium channel agonist dihydropyridine analogue,

Bay k 8644, on pig coronary artery: Biphasic mechanical response and paradoxical potentiation of contraction by diltiazem and nimodipine. *J. cardiovasc. Pharmac.*, **7**, 377–389.

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

GOLENHOFEN, G. (1981). Differentiation of calcium activation processes in smooth muscle using selective antagonists. In *Smooth Muscle*. ed. Bülbring, E., Brading, A.F., Jones, A.W. & Tomita, T. pp. 157–170. London: Edward Arnold.

HAEUSLER, G. & THORENS, S. (1980). Effects of tetraethylammonium chloride on contractile, membrane and cable properties of rabbit artery muscle. *J. Physiol.*, **303**, 203–224.

KANMURA, Y., ITOH, T. & KURIYAMA, H. (1984). Agonist action of Bay k8644, a dihydropyridine derivative, on the voltage-dependent calcium influx in smooth muscle cells of the rabbit mesenteric artery. *J. Pharmac. exp. Ther.*, **231**, 717–723.

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

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.

KARAKI, H. & WEISS, G.B. (1984). Calcium channels in smooth muscle. *Gastroenterology*, **87**, 960–970.

KARAKI, H., NAGASE, H., OZAKI, H. & URAKAWA, N. (1986a). Effects of calcium channel agonist, CGP 28,392, on the smooth muscle contractions. *Jap. J. Pharmac.*, **40** (suppl), 200P.

KARAKI, H., NAKAGAWA, H. & URAKAWA, N. (1984a). 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. *Br. J. Pharmac.*, **81**, 393–400.

KARAKI, H., NAKAGAWA, H. & URAKAWA, N. (1984b). Effects of calcium antagonists on release of [^{3}H] noradrenaline in rabbit aorta. *Eur. J. Pharmac.*, **101**, 177–183.

KARAKI, H., NAKAGAWA, H. & URAKAWA, N. (1985). Age-related changes in the sensitivity to verapamil and sodium nitroprusside of vascular smooth muscle of rabbit aorta. *Br. J. Pharmac.*, **85**, 223–228.

KARAKI, H., NAKAGAWA, H. & URAKAWA, N. (1986b). Strontium uptake during the different modes of contraction in the smooth muscle of rabbit aorta, rat aorta and guinea pig taenia coli. *Archs. int. Pharmacodyn.*, (in press).

KARAKI, H., SATAKE, N. & SHIBATA, S. (1986c). Mechanism of barium-induced contraction in the vascular smooth muscle of rabbit aorta. *Br. J. Pharmac.*, (in press).

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

LOUTZENHISER, R., RÜEGG, U.T., HOF, A. & HOF, R.P. (1984). Studies on the mechanism of action of the vasoconstrictive dihydropyridine, CGP 28392. *Eur. J. Pharmac.*, **105**, 229–237.

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

MEKATA, F. (1974). Current spread in the smooth muscle of rabbit aorta. *J. Physiol.*, **242**, 143–155.

MEKATA, F. (1976). Rectification in the smooth muscle cell membrane of rabbit aorta. *J. Physiol.*, **258**, 269–278.

SCHRAMM, M., THOMAS, G., TOWART, R. & FRANCKOWIAK, G. (1983). Novel dihydropyridines with positive inotropic action through activation of Ca^{2+}-channels. *Nature*, **303**, 535–537.

SCHRAMM, M., TOWART, R., LAMP, B. & THOMAS, G. (1985). Modulation of calcium ion influx by the 1,4-dihydropyridines nifedipine and Bay k 8644. *J. cardiovasc. Pharmac.*, **7**, 493–496.

SPEDDING, M. & BERG, C. (1984). Interactions between a "calcium channel agonist", Bay k 8644, and calcium antagonists differentiate calcium antagonist subgroup in K^{+}-depolarized smooth muscle. *Naunyn-Schmiedebergs Arch. Pharmac.*, **328**, 69–75.

SU, C., SWAMY, N.C. & TRIGGLE, D.J. (1984). Calcium channel activation in vascular smooth muscle by Bay k 8644. *Can. J. Physiol. Pharmac.*, **62**, 1401–1410.

TAKENAKA, T. & MAENO, H. (1982). A new vasoconstrictor 1,4-dihydropyridine derivative, YC-170. *Jap. J. Pharmac.*, **32** (suppl), 139P.

TRUOG, A.G., BRUNNER, H., CRISCIONE, L., FALLERT, M., KUHNIS, H., MEIER, M. & ROGG, H. (1984). CGP 28392, a dihydropyridine Ca^{2+} entry stimulator. In *Calcium in Biological Systems*. ed. Rubin, R.P., Weiss, G.B. & Putney, J.W. Jr. pp. 441–449. New York: Plenum.

WEISS, G.B. (1977). Calcium and contractility in vascular smooth muscle. *Adv. gen. Cell. Pharmac.*, **2**, 71–154.

YAMAMOTO, H., HWANG, O. & VAN BREEMEN, C. (1984). Bay k 8644 differentiates between potential and receptor operated Ca^{2+} channels. *Eur. J. Pharmac.*, **102**, 555–557.

(Received May 7, 1986.
Revised June 6, 1986.
Accepted June 10, 1986.)