British Journal of Pharmacology (1996) 118, 233–236

Rhythmic relaxations of active tension in the rabbit large arteries induced by a combination of cyclopiazonic acid and Bay K 8644

Masayuki Omote & [1]Hideo Mizusawa

Marion Merrell Dow, 3-11 Shodai-Tajika, Hirakata, Osaka 573, Japan

1 We previously demonstrated that cyclopiazonic acid (CPA), an inhibitor of Ca^{2+}-ATPase in the sarcoplasmic reticulum, induced rhythmic relaxations of active tension in the endothelium-denuded small arteries of the mesentery and the ear of the rabbit, but that this agent failed to induce rhythmic responses in the endothelium-denuded rabbit femoral artery.

2 In the present study, an attempt was made to induce rhythmic relaxations of active tension in the endothelium-denuded rabbit femoral artery and the thoracic aorta, both of which were suspended in organ chambers for isometric tension recordings, by using CPA plus Bay K 8644, an L-type Ca^{2+} channel agonist, to induce an excessive increase in cytosolic Ca^{2+}.

3 CPA or Bay K 8644 alone failed to produce rhythmic relaxations in the femoral artery that had been contracted with phenylephrine. In contrast, rhythmic responses were induced by the sequential treatment of the femoral artery with CPA and Bay K 8644.

4 The rhythmic relaxations of active tension in the femoral artery induced by CPA plus Bay K 8644 were inhibited by charybdotoxin and by iberiotoxin, both of which are antagonists of the Ca^{2+}-activated K^+ channel, but not by glibenclamide, a blocker of the ATP-sensitive K^+ channel.

5 The endothelium-denuded rabbit aorta also exhibited rhythmic responses by the sequential addition of CPA and Bay K 8644. These responses were sensitive to charybdotoxin.

6 These findings indicate that, like small arteries, the large femoral and aortic arteries of the rabbit are also capable of displaying rhythmic relaxations of active tension; these relaxations may be in part attributed to the activation of the Ca^{2+}-activated K^+ channel as a result of the Ca^{2+} overload caused by CPA and Bay K 8644.

Keywords: Bay K 8644; cyclopiazonic acid; K^+ channels; rhythmic relaxations; sarcoplasmic reticulum; vascular smooth muscle

Introduction

Cyclopiazonic acid (CPA), a mycotoxin derived from *Aspergillus* and *Penicillium*, has been shown to be a highly selective inhibitor of Ca^{2+}-ATPase (Ca^{2+}-pump) in the sarcoplasmic reticulum (SR) of rabbit skeletal muscle (Seidler *et al.*, 1989). CPA has also been implicated in the inhibition of the SR Ca^{2+}-pump in vascular smooth muscle of the rat aorta (Deng & Kwan, 1991) and the canine mesenteric artery (Low *et al.*, 1992).

In smooth muscle, the superficial SR functions as a buffer barrier by sequestering part of the Ca^{2+} entering from the extracellular space (Van Bremen & Saida, 1989; Van Breeman *et al.*, 1995). We previously reported that CPA induced rhythmic relaxations of active tension in endothelium-denuded rabbit mesenteric and ear arteries; these relaxations were thought to be associated with the activation of the Ca^{2+}-activated K^+ channel (K_{Ca}) (Omote & Mizusawa, 1993; 1994). We speculated that an increase in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$), as a result of the CPA-induced impairment of the superficial buffer function of the SR, led to the activation of the K_{Ca}. When tested on endothelium-denuded rabbit femoral artery, in contrast, CPA did not induced rhythmic relaxations of tension (Omote & Mizusawa, 1995). We postulated that the failure of CPA to produce rhythmic responses in the femoral artery may have been partly due to the increase in $[Ca^{2+}]_i$ being too small to affect the K_{Ca}. Thus, in the present study we introduced an excessive increase in $[Ca^{2+}]_i$ by using CPA plus Bay K 8644, an L-type Ca^{2+} channel agonist (Schramm *et al.*, 1983), to see whether the endothelium-denuded rabbit femoral artery is capable of exhibiting rhythmic relaxations of active tension like those seen in the ear and the mesenteric arteries. The effects of CPA and Bay K 8644 were also examined in rabbit aortae.

[1] Author for correspondence.

Methods

Tension recordings

Male albino rabbits (2.4–3.2 kg) were anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, i.v.) and exsanguinated. The femoral arteries and the thoracic aorta were immediately removed from each rabbit, cleaned of adherent connective tissue, and cut into rings approximately 2 mm in length. The endothelium was then removed by rubbing the intimal surface of the artery with a roughened syringe needle. Each arterial ring was suspended for isometric tension recordings in a 5-ml organ bath containing Krebs-Henseleit solution (composition, mM: $CaCl_2$ 2.5, glucose 10, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, NaCl 118, and $NaHCO_3$ 25; pH 7.2–7.4) at 37°C and oxygenated (95% O_2, 5% CO_2). The arterial rings were equilibrated for more than 90 min at a resting tension of 0.6 g for the femoral artery and 2.0 g for the aorta.

After equilibration, the rings were stimulated with phenylephrine (30 μM). The successful removal of the endothelium was confirmed by the lack of any relaxant response to substance P (1 μM), which is known to induce endothelium-dependent relaxation, during phenylephrine-induced contractions. The arteries were then washed and equilibrated for another 60 min before the subsequent experiment. The effects of CPA and Bay K 8644 were then examined both in the femoral artery and in the aorta that had been contracted with phenylephrine. The concentration of phenylephrine was fixed

to induce a contraction of approximately 50% of the maximum contraction induced by 30 μM phenylephrine. This concentration was chosen because (i) a high concentration of phenylephrine has been shown to inhibit the L-type Ca^{2+} channel in the vascular smooth muscle (Declerck *et al.*, 1990), and (ii) no rhythmic responses were observed in the rabbit small arteries after the inhibition of the Ca^{2+} channel (Omote & Mizusawa, 1993; 1994).

Drugs

Iberiotoxin, (-)-phenylephrine hydrochloride and substance P were purchased from Sigma Chemical Co. (St. Louis, MO., U.S.A.). (±)-Bay K 8644 (methyl-1,4-dihydro-2, 6-dimethyl-3-nitro-4-(2-trifluoro-methyl-phenyl)-pyridine-5-carboxylate), charybdotoxin and glibenclamide were purchased from Research Biochemicals Inc. (Natick, MA., U.S.A.).

Results

As we previously demonstrated (Omote & Mizusawa, 1995), CPA (10 μM) caused a slight increase in tension without inducing rhythmic relaxations in the endothelium-denuded rabbit femoral artery that had been contracted with phenylephrine (0.3 μM) (Figure 1a). Similar results were obtained when we used Bay K 8644 (0.1 μM) instead of CPA (Figure 1b). By contrast, rhythmic relaxations of the tension were elicited when CPA (10 μM) and Bay K 8644 (0.1 μM) were sequentially added to the femoral artery precontracted with phenylephrine (Figure 1c). These rhythmic relaxations induced by CPA and Bay K 8644 were observed in 27 of the 29 rings isolated from 7 rabbits.

The rhythmic relaxations of active tension in the endothelium-denuded rabbit femoral artery induced by sequential additions of CPA and Bay K 8644 were abolished by charybdotoxin (50 nM) (Figure 2a) and iberiotoxin (50 nM) (Figure 2b), both of which are antagonists of the K_{Ca} (Miller *et al.*, 1985; Galvez *et al.*, 1990). On the other hand, glibenclamide (10 μM), an antagonist of the ATP-sensitive K^+ channel (Nelson *et al.*, 1990), hardly affected the CPA plus Bay K 8644-induced rhythmic relaxations in the femoral artery (Figure 2c). Glibenclamide has, however, been shown to be effective in preventing ATP-sensitive K^+ channel-mediated responses at the concentration used in the present study (Nelson *et al.*, 1990).

As with the femoral artery, CPA (10 μM) alone failed to induce rhythmic relaxations in the endothelium-denuded aorta precontracted with phenylephrine (0.1 μM) (Figure 3a), whereas the sequential addition of CPA (10 μM) and Bay K 8644 (10 μM) induced rhythmic relaxations in this artery (Figure 3b). These rhythmic responses induced by CPA plus Bay K 8644 in the aorta were greatly inhibited by charybdotoxin (50 nM) (Figure 3b).

The rhythmic relaxations of active tension in the aorta induced by CPA plus Bay K 8644 were observed in 32 of the 47 rings isolated from 14 rabbits. With the lower concentrations of Bay K 8644, however, the incidence of the rhythmic relaxations in the aortae pretreated with CPA (10 μM) was greatly decreased: none of six rings treated with 0.1 μM Bay K 8644, and three of 18 rings treated with 1 μM Bay K 8644 showed rhythmic relaxations.

Discussion

Rhythmic responses of tension in smooth muscle may be associated with oscillations of $[Ca^{2+}]_i$. It has been suggested that the $[Ca^{2+}]_i$ oscillations may be induced either by the periodical release of Ca^{2+} from cytosolic Ca^{2+} stores by a process of

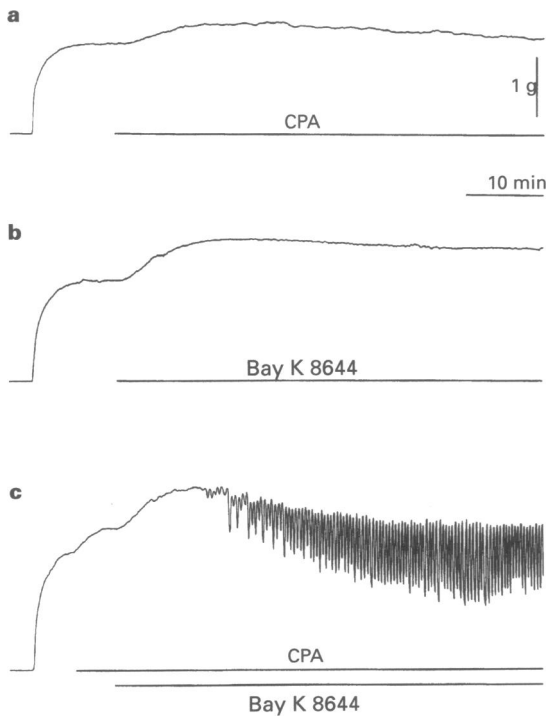

Figure 1 Typical recordings of the changes in tension produced by (a) cyclopiazonic acid (CPA, 10 μM), (b) Bay K 8644 (0.1 μM), and (c) CPA (10 μM) plus Bay K 8644 (0.1 μM) in endothelium-denuded rabbit femoral arteries that had previously been contracted with phenylephrine (0.3 μM).

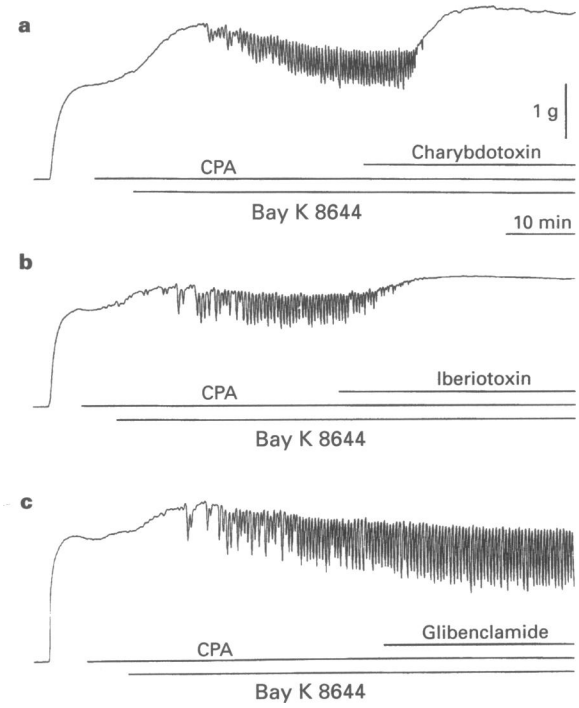

Figure 2 Effects of (a) charybdotoxin (50 nM), (b) iberiotoxin (50 nM) and (c) glibenclamide (10 nM) on rhythmic relaxations of tensions induced by the sequential addition of CPA (10 μM) and Bay K 8644 (0.1 μM) in endothelium-denuded rabbit femoral arteries that had been contracted with phenylephrine (0.3 μM). For each of these K^+ channel antagonists, similar results were obtained with 4 other endothelium-denuded femoral arteries from different rabbits.

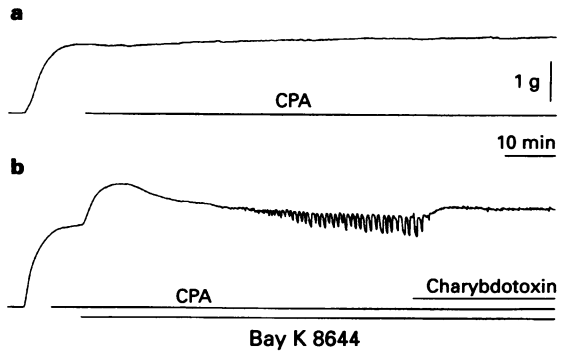

Figure 3 Typical responses produced by (a) CPA (10 μM) and (b) CPA (10 μM) plus Bay K 8644 (10 μM) in endothelium-denuded rabbit aortae that had previously been contracted with phenylephrine (0.1 μM). Effects of charybdotoxin (50 nM) were also examined in an endothelium-denuded aorta that displayed rhythmic relaxations after the sequential addition of CPA and Bay K 8644 (b). Similar responses to charybdotoxin were obtained with 4 other aortae isolated from different rabbits.

Ca^{2+}-induced Ca^{2+} release, or the periodical Ca^{2+} influx via the L-type Ca^{2+} channel, which is regulated by the K_{Ca} (Berridge & Galione, 1988).

We previously demonstrated that CPA induced rhythmic relaxations of active tension in the endothelium-denuded rabbit mesenteric and ear arteries, and suggested that these rhythmic responses were associated with the periodical decrease in Ca^{2+} influx via the L-type Ca^{2+} channel regulated by the K_{Ca} (Omote & Mizusawa, 1993; 1994). Our proposal was as follows: the CPA-induced impairment of the superficial buffer barrier function of the SR would result in an increase in $[Ca^{2+}]_i$, which would then affect the K_{Ca}. When the K_{Ca} was activated, the Ca^{2+} influx via the L-type Ca^{2+} channel activated by phenylephrine would be decreased by hyperpolarization, leading to relaxation. Contractions would then be restored when the K_{Ca} was closed as a result of the decreased $[Ca^{2+}]_i$ and the subsequent depolarization. The consequent increase in $[Ca^{2+}]_i$ might then allow the next cycle of relaxation.

Unlike the mesenteric and the ear arteries, the endothelium-denuded rabbit femoral artery has been shown to display no rhythmic relaxations of active tension when CPA is added (Omote & Mizusawa, 1995). For many years, smooth muscle has often been classified according to its functional characteristics as either single-unit or multi-unit (Bozler, 1948). Large arteries are considered to be multi-unit smooth muscles which are distinguished from the single-unit type by their lack of myogenic and rhythmic activity as well as their inability to propagate electrical activity (Somlyo & Somlyo, 1968). Thus, the lack of the rhythmic activity of the femoral artery might be due to the multi-unit nature of the artery. However, another explanation for the lack of the rhythmic responses in the rabbit femoral artery could be that the rise in $[Ca^{2+}]_i$, as a result of the impairment of the SR buffering function by CPA, was not large enough to activate the K_{Ca}. Thus, in the present study Bay K 8644 was added to the femoral artery, which had previously been treated with CPA, to induce a further increase in $[Ca^{2+}]_i$. Under these conditions, it was found that the femoral artery successfully exhibited rhythmic relaxations that were sensitive to K_{Ca} antagonists. Like the femoral artery, the endothelium-denuded rabbit aorta also exhibited the K_{Ca}-associated rhythmic relaxations of active tension after the addition of CPA plus Bay K 8644. These findings may provide evidence that even multi-unit large arteries of the rabbit are capable of exhibiting rhythmic relaxations of active tension, presumably as a result of an excessive increase in $[Ca^{2+}]_i$. These rhythmic relaxations may be attributed to the periodical decrease in Ca^{2+} entry regulated by the K_{Ca}, as has been suggested to occur in the rabbit small arteries (Omote & Mizusawa, 1993; 1994).

Although we postulated that the CPA-induced increase in the $[Ca^{2+}]_i$ may be smaller in the large femoral and aortic arteries of the rabbit than in the small arteries of the ear and the mesentery, the reason for this difference cannot be determined from the results of the present study. It has been reported, however, that vascular smooth muscle cells in large arteries have a significantly more extensive SR than do those in small arteries (Ashida *et al.*, 1988). In addition, the SR in vascular smooth muscle has been shown to consist of two different types: one CPA-sensitive and the other CPA-insensitive (Low *et al.*, 1992; Tribe *et al.*, 1994). Thus, the SR in the large arteries of the rabbit may constitute a more potent buffer barrier against Ca^{2+} influx than does the SR in the small arteries, partly because of the extensive contribution of the CPA-insensitive SR. Alternatively, Ca^{2+} extrusion through the plasmalemma might be more efficient in the large arteries than in the small ones. Our finding that the concentration of Bay K 8644 required to induce rhythmic relaxations in the aorta, the largest artery, was much higher than that required in the femoral artery is consistent with this hypothesis.

The physiological significance of the rhythmic relaxations of active tension in the rabbit arteries induced by an introduction of the Ca^{2+} overload cannot be determined from the results of the present study. It has been reported, however, that an excessive increase in $[Ca^{2+}]_i$, due to failure of the Ca^{2+} extrusion or to excessive Ca^{2+} influx, causes cell death (Ganote & Nayler, 1985; Pauwels *et al.*, 1991). Thus, the rhythmic relaxations which may be related to periodical decreases in $[Ca^{2+}]_i$ could protect the vascular smooth muscle against the toxicity of the Ca^{2+} overload.

In conclusion, this study provides the first evidence that the endothelium-denuded rabbit large femoral and aortic arteries are capable of exhibiting the K_{Ca}-associated rhythmic relaxations of active tension as has previously been reported in the endothelium-denuded rabbit small ear and mesenteric arteries, presumably as a result of an excessive increase in $[Ca^{2+}]_i$. The rhythmic relaxations in the small arteries were induced by CPA alone, whereas Bay K 8644 was additionally required to induce the rhythmic responses in the femoral and aortic arteries. We postulate that the reason for this could be that Ca^{2+} extrusion into the SR or out of the plasmalemma is more potent in the large arteries than in the small arteries.

References

ASHIDA, T., SCHAEFFER, J., GOLDMAN, W.F., WADE, J.B. & BLAUSTEIN, M.P. (1988). Role of sarcoplasmic reticulum in arterial contraction: comparison of ryanodine's effect in a conduit and a muscular artery. *Circ. Res.*, **62**, 854–863.

BERRIDGE, M.J. & GALIONE, A. (1988). Cytosolic calcium oscillators. *FASEB J.*, **2**, 3074–3082.

BOZLER, E. (1948). Conduction automaticity and tonus of visceral smooth muscle. *Experientia*, **4**, 213–218.

DECLERCK, I., HIMPENS, B., DROOGMANS, G. & CASTEELS, R. (1990). The α_1-agonist phenylephrine inhibits voltage-gated Ca^{2+}-channel in vascular smooth muscle cells of rabbit ear artery. *Pflügers Arch.*, **417**, 117–119.

DENG, H.W. & KWAN, C.Y. (1991). Cyclopiazonic acid is a sarcoplasmic reticulum Ca^{2+}-pump inhibitor of rat aortic muscle. *Acta Pharmacol. Sin.*, **12**, 53–58.

GALVEZ, A., GIMENEZ-GALLEGO, G., REUBEN, J.P., ROY-CONTANCIN, L., FEIGENBAUM, P., KACZOROWSKI, G.J. & GARCIA, M.L. (1990). Purification and characterization of a unique, potent, peptidyl probe for the high conductance calcium-activated potassium channel from venom of the scorpion. *Buthus tamulus*. *J. Biol. Chem.*, **265**, 11083–11090.

GANOTE, C.E. & NAYLER, W.G. (1985). Contracture and the calcium paradox. *J. Mol. Cell Cardiol.*, **17**, 733–745.

LOW, A.M., KWAN, C.Y. & DANIEL, E.E. (1992). Evidence for two types of internal Ca^{2+} stores in canine mesenteric artery with different refilling mechanisms. *Am. J. Physiol.*, **262**, H31–H37.

MILLER, C., MOCZYDLOWSKI, E., LATORRE, R. & PHILIPS, M. (1985). Charybdotoxin, a protein inhibitor of single Ca^{2+}-activated K^+ channels from skeletal muscle. *Nature*, **313**, 316–318.

NELSON, M.T., PATLAK, J.B., WORLEY, J.F. & STANDEN, N.B. (1990). Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle tone. *Am. J. Physiol.*, **259**, C3–C18.

OMOTE, M. & MIZUSAWA, H. (1993). The role of sarcoplasmic reticulum in endothelium-dependent and endothelium-independent rhythmic contractions in the rabbit mesenteric artery. *Acta Physiol. Scand.*, **149**, 15–21.

OMOTE, M. & MIZUSAWA, H. (1994). Effects of cyclopiazonic acid on phenylephrine-induced contractions in the rabbit ear artery. *Br. J. Pharmacol.*, **111**, 233–237.

OMOTE, M. & MIZUSAWA, H. (1995). Endothelium-dependent rhythmic contractions induced by cyclopiazonic acid, a sarcoplasmic reticulum Ca^{2+}-pump inhibitor, in the rabbit femoral artery. *Acta Physiol. Scand.*, **154**, 113–119.

PAUWELS, P.J., LEYSEN, J.E. & JANSSEN, P.A.J. (1991). Ca^{2+} and Na^{2+} channels involved in neuronal cell death, protection by flunarizine. *Life Sci.*, **48**, 1881–1893.

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.

SEIDLER, N.W., JONA, I., VEGH, M. & MARTONOSI, A. (1989). Cyclopiazonic acid is a specific inhibitor of the Ca^{2+}-ATPase of sarcoplasmic reticulum. *J. Biol. Chem.*, **264**, 17816–17823.

SOMLYO, A.P. & SOMLYO, A.V. (1968). Electromechanical pharmacomechanical coupling in vascular smooth muscle. *J. Pharmacol. Exp. Ther.*, **159**, 129–145.

TRIBE, R.M., BORIN, M.L. & BLAUSTEIN, M.P. (1994). Functionally and spatially distinct Ca^{2+} stores are revealed in cultured vascular smooth muscle. *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 5908–5912.

VAN BREEMEN, C., CHEN, Q. & LAHER, I. (1995). Superficial buffer barrier function of smooth muscle sarcoplasmic reticulum. *Trends Pharmacol. Sci.*, **16**, 98–105.

VAN BREEMEN, C. & SAIDA, K. (1989). Cellular mechanisms regulating $[Ca^{2+}]_i$ smooth muscle. *Annu. Rev. Physiol.*, **51**, 315–329.

(Received July 7, 1995
Revised November 13, 1995
Accepted January 22, 1996)