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Vasorelaxant Effects of Oxpentifylline and Theophylline on Rat Isolated Aorta

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Abstract—The mechanism of the relaxation response of rat aorta to the phosphodiesterase inhibitors oxpentifylline and theophylline was studied. Oxpentifylline induced a greater vasorelaxation response in the intact strips than in those without endothelium. The endothelium-dependent relaxation response to oxpentifylline was inhibited by nitro-L-arginine but not by indomethacin, and the endothelium-independent relaxation response was potentiated by the combination with isoprenaline but not sodium nitroprusside. Theophylline induced a similar relaxation response in vascular strips with and without endothelium. The relaxation response to theophylline was not inhibited by indomethacin or nitro-L-arginine in intact strips, but was potentiated by combination with isoprenaline or sodium nitroprusside in the denuded strips. These results suggest that the two phosphodiesterase inhibitors oxpentifylline and theophylline induce vasorelaxation by different mechanisms. Oxpentifylline can induce both endothelium-dependent relaxation, which is probably mediated by an endothelium-derived relaxing factor, and endothelium-independent relaxation, which may be due to an inhibitory action on phosphodiesterase of vascular smooth muscle. In contrast, theophylline can induce endothelium-independent relaxation alone, without modulation by the endothelium.

Oxpentifylline (3,7-dimethyl-1-(5-oxo-hexyl)-xanthine) is an orally active haemorrheological agent for the treatment of peripheral vascular disease and cerebrovascular disease (Ward & Clissold 1987). Oxpentifylline has also been reported to have a vasodilator effect on vascular tissue by its ability to increase intracellular cAMP (Stefanovich 1973). Oxpentifylline can stimulate the production of prostacyclin in vascular smooth muscle (Sinzinger 1983) and has been shown to inhibit phosphodiesterase (PDE) isolated from animal and human cells, including platelets (Stefanovich 1973; Stefanovich et al 1974; Nenci et al 1981). Thus, the vasorelaxant effect of the compound appears to be due to the prostacyclin production and the inhibitory action of PDE. Recently, PDE was classified into five major families, i.e. PDE I, II, III, IV and V (Beavo & Reifsnyder 1990). The mode of the relaxation response appears to differ with the PDE type, i.e. some PDE inhibitors produce endothelium-dependent relaxation (Komas et al 1991). An intact vascular endothelium has been shown to play an obligatory role in producing relaxation via endothelium-derived relaxing factor (EDRF), which is thought to be nitric oxide (NO), in response to a number of agonists (Moncada et al 1991). However, there are few papers regarding the mechanism underlying the vasorelaxation caused by oxpentifylline.

The aim of the present study was to clarify the relaxation mechanism of oxpentifylline by comparing the mode of the relaxation response to oxpentifylline with that of theophylline, a well-known PDE inhibitor.

Materials and Methods

Tissue preparation

Thoracic aortae were obtained from male Wistar rats, 250–300 g, after decapitation. The aortae were cut helically into vascular strips (2·0 mm wide, 15 mm long) and set up isometrically in-vitro, as previously described (Altura & Altura 1970). The strips were fixed vertically between hooks in a 10-mL organ-bath containing Krebs–Ringer solution, which was maintained at 37°C, pH 7·4, and aerated with 95% O_2–5% CO_2. The Krebs–Ringer solution was composed of (mM): NaCl 118, KCl 4·7, $CaCl_2$ 2·5, KH_2PO_4 1·2, $MgSO_4 \cdot 7H_2O$ 1·2, glucose 10, $NaHCO_3$ 25.

Measurement of tension

Isometric tension, monitored with a force-displacement transducer (Nihon Kohden Kohgyo Co., Tokyo, Japan) to which the upper ends of the strips were connected, was recorded with a pen recorder (Nihon Kohden Kohgyo Co.). The artery strips were equilibrated for approximately 2 h, during which time the medium was replaced every 15 min, and were adjusted for a resting tension of 1·0 g. The endothelium was removed from some strips by rubbing the intimal surface with filter paper (Furchgott & Zawadzki 1980). Denudation of the endothelium was confirmed by the loss of relaxation induced by 10^{-6} M acetylcholine. When the precontraction with 10^{-7} M noradrenaline or 10^{-6} M phenylephrine had stabilized, oxpentifylline or theophylline was added to the organ bath. In an experiment with inhibitors, intact arterial strips were first incubated with 10^{-5} M indomethacin or 10^{-4} M N^G-nitro-L-arginine (L-NNA) for 60 min before the precontraction. In an experiment with sodium nitroprusside or isoprenaline, when precontraction had reached a peak tension, 10^{-9} M isoprenaline or 10^{-9} M sodium nitroprusside was then added to decrease the tension to 90% of the initial contraction. Ten minutes later, a concentration-response curve to oxpentifylline or theophylline was obtained. Noradrenaline at 10^{-7} M was used to evoke the precontractions except in an experiment with isoprenaline, a stimulant of the β-adrenoceptor, in which 10^{-6} M phenyl-

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ephrine was used to avoid interference with the β-adrenoceptor. Relaxation was expressed as a percentage of the precontraction to noradrenaline or phenylephrine. The concentrations of the drugs are expressed as the final molar concentrations in the bath solution.

Drugs used

Acetylcholine chloride, phenylephrine hydrochloride, L-noradrenaline hydrochloride, theophylline, indomethacin, L-isoprenaline, sodium nitroprusside and oxpentifylline were obtained from Sigma Chemical Co. N^G-Nitro-L-arginine was obtained from Aldrich Chemical Co. (Milwaukee, WI). Drugs were prepared daily in distilled water, except noradrenaline which was dissolved in ascorbic acid (0·1 mM). The drugs were added to the organ bath in volumes of 100 μL. A stock solution of indomethacin was prepared immediately before use with equimolar amounts of Na_2CO_3.

Statistical analysis

The data are expressed as means ± s.e. Student's unpaired *t*-test was used for statistical comparisons. The concentrations needed to reach 50% maximal relaxation (ED50 values) were determined graphically after linear regression of the 20–80% region of the log concentration-response curves. Differences were accepted as significant for $P < 0{\cdot}05$.

Results

Effect of endothelium removal on oxpentifylline- or theophylline-induced vasorelaxation

Oxpentifylline dose-dependently induced a slowly developing vasorelaxation in both intact and denuded strips, with a greater response for the strips with than without endothelium (Fig. 1). However, theophylline dose-dependently induced a similar relaxation response in two types of strips (Fig. 2). Relaxation responses in intact strips were greater for oxpentifylline (ED50, $4{\cdot}5 \pm 0{\cdot}2 \times 10^{-5}$ M; maximal relaxation, 98·6 ± 2·3%) than theophylline (ED50, $1{\cdot}2 \pm 0{\cdot}3 \times 10^{-4}$ M; 94·3 ± 2·3%, $P < 0{\cdot}01$ for ED50, n = 7).

Effect of pretreatment with indomethacin or L-NNA on oxpentifylline- or theophylline-induced relaxation in vascular strips with endothelium

L-NNA, but not indomethacin, significantly inhibited oxpentifylline-induced relaxation in the vascular strips with endothelium (ED50, $1{\cdot}3 \pm 0{\cdot}2 \times 10^{-4}$ M; maximal relaxation, 71·9 ± 1·9%) to a similar degree to the relaxation response in the vascular strips without endothelium (ED50, $1{\cdot}8 \pm 0{\cdot}3 \times 10^{-4}$ M; maximal relaxation, 78·6 ± 5·8%, n = 7) (Fig. 3A, C). Theophylline-induced relaxation was not inhibited by these two compounds (Fig. 3B, D).

Effect of oxpentifylline or theophylline on vascular relaxation in combination with sodium nitroprusside or isoprenaline in vascular strips without endothelium

Oxpentifylline-induced relaxations were potentiated by pretreatment with isoprenaline but not sodium nitroprusside (Fig. 4A, C). Theophylline-induced relaxations were potentiated by pretreatments with the two compounds (Fig. 4B, D).

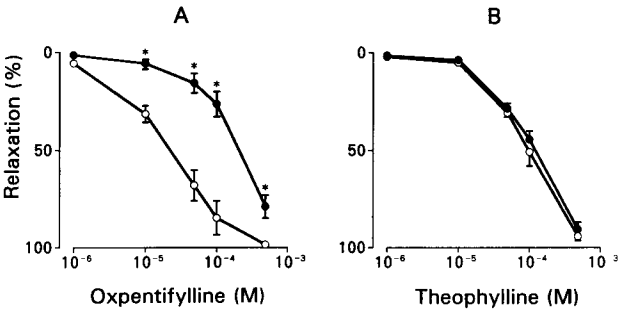

FIG. 2. Effect of endothelium removal on (A) oxpentifylline- or (B) theophylline-induced vasorelaxation in rat aorta. Results are expressed as means ± s.e. of seven observations. *$P < 0{\cdot}01$, significantly different from the results in the presence of endothelium. ○ With endothelium, ● without endothelium.

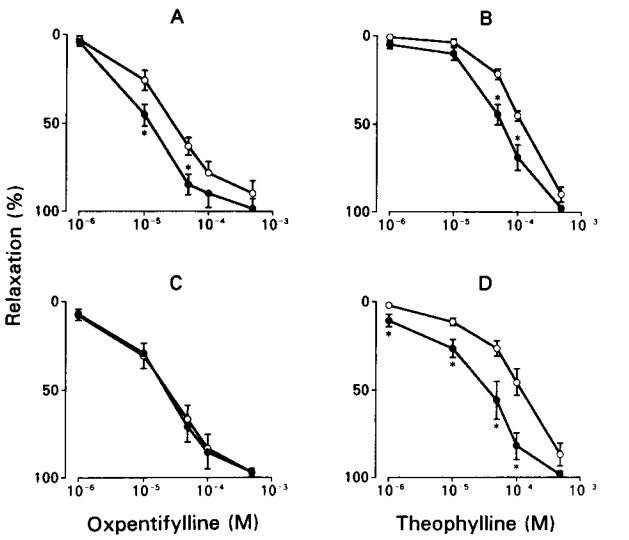

FIG. 3. Effects of (A, B) indomethacin or (C, D) nitro-L-arginine on oxpentifylline- or theophylline-induced vasorelaxation in the vascular strips with endothelium. Results are expressed as means ± s.e. of seven observations. *$P < 0{\cdot}01$, significantly different from the results without indomethacin- or nitro-L-arginine-pretreatment (control). For A and B, ○ control, ● indomethacin. For C and D, ○ control, ● nitro-L-arginine.

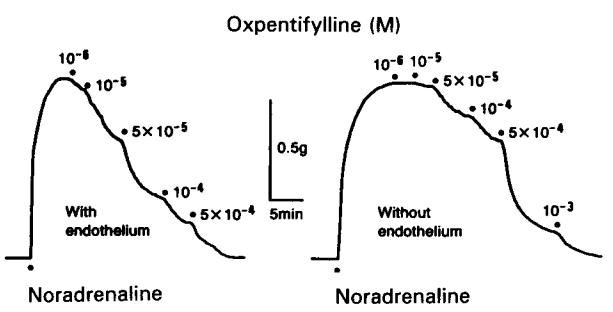

FIG. 1. Representative recordings of tension show the relaxation response to oxpentifylline in rat aortic strips with and without endothelium. Vascular strips were precontracted with 10^{-7} M noradrenaline.

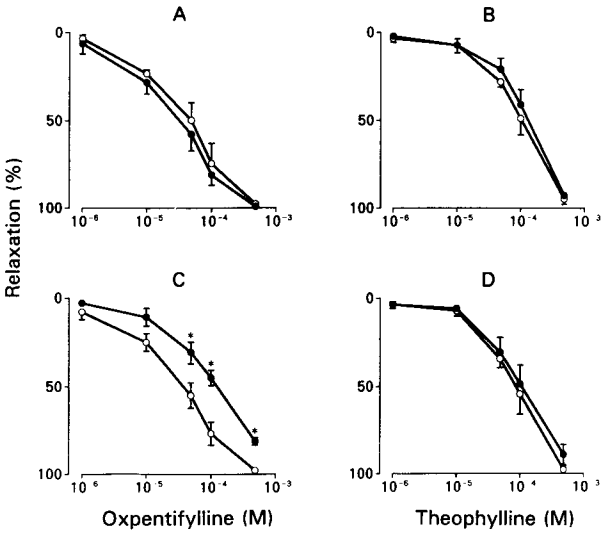

FIG. 4. Effects of the combination with (A, B) isoprenaline or (C, D) sodium nitroprusside on oxpentifylline- or theophylline-induced relaxation in the vascular strips without endothelium. Results are expressed as means ± s.e. of seven observations. *$P < 0.01$, significantly different from the results without combination with isoprenaline or sodium nitroprusside (control). For A and B, ○ control, ● isoprenaline. For C and D, ○ control, ● sodium nitroprusside.

Discussion

Oxpentifylline produced a greater relaxation response in the vascular strips with than without endothelium, suggesting that the relaxation induced by oxpentifylline is partially mediated by the endothelium. The endothelium can produce and release prostacyclin (Moncada 1982) and EDRF (Furchgott 1983), both of which have a vasorelaxant effect. Therefore, prostacyclin or EDRF may contribute to the endothelium-dependent relaxation response to oxpentifylline. As oxpentifylline-induced relaxation was not inhibited by indomethacin, a cyclo-oxygenase inhibitor, and prostacyclin has no vasorelaxant effect on the rat isolated aorta (Levy 1980), the relaxation is not likely to be mediated by cyclo-oxygenase products, such as prostacyclin. However, oxpentifylline-induced endothelium-dependent relaxation was inhibited by L-NNA, an EDRF/NO synthesis inhibitor. This result suggests that the endothelium-dependent relaxation to oxpentifylline is mediated by EDRF/NO, thus confirming a previous report (Berkenboom et al 1991). The relaxation may be due to an increase in the sensitivity of the vascular smooth muscle cells to spontaneously released EDRF/NO, or oxpentifylline might reduce the breakdown of EDRF/NO by impeding the superoxide anion. However, these two possibilities are unlikely, since oxpentifylline-induced relaxation was not potentiated by the combination with sodium nitroprusside which releases NO spontaneously, activates soluble guanylate cyclase in smooth muscle cells and produces relaxation. Martin et al (1986) suggested that the cGMP-PDE-specific inhibitor M&B 22948 elicited the endothelium-dependent component of relaxation by inhibiting the hydrolysis of cGMP formed in response to EDRF released spontaneously from endothelial cells. Similarly, oxpentifylline is also a likely cGMP-PDE inhibitor. However, M&B 22948 potentiated the relaxation of endothelium-denuded rings induced by glyceryl nitrate, which increases cGMP, but did not potentiate the relaxation induced by isoprenaline, which can activate adenylate cyclase to result in an increase in cAMP. In contrast, in the present study, a oxpentifylline-induced relaxation response was potentiated by the combination with isoprenaline but not sodium nitroprusside, which causes an increase in cGMP. Therefore, oxpentifylline is likely to be a cAMP-PDE inhibitor but not a cGMP-PDE inhibitor. Oxpentifylline may also cause an increase in the production or release of EDRF/NO. It has recently been reported (Gray & Marshall 1992) that isoprenaline can elicit endothelium-dependent relaxation and an increase in cGMP, effects which are attenuated by L-NNA. The endothelium-relaxation induced by isoprenaline may act via an increase in cAMP in endothelial cells; i.e. an increase in cAMP either directly or indirectly may activate the synthesis of EDRF/NO. Thus, in view of the likelihood that oxpentifylline inhibits cAMP degradation in endothelial cells, this is a possible mechanism for oxpentifylline-induced endothelium-dependent relaxation.

On the other hand, oxpentifylline produced relaxation even in the vascular strips without endothelium. The endothelium-independent relaxation is probably due to inhibition of cAMP-PDE, as, together with previous reports of oxpentifylline being a cAMP-PDE inhibitor in bovine platelets (Stefanovich 1975), rat brain (Hayashi & Ozawa 1974) and guinea-pig aorta (Stefanovich et al 1974), the relaxation response to oxpentifylline was potentiated by pretreatment with isoprenaline but not by pretreatment with sodium nitroprusside. Thus, oxpentifylline is likely to inhibit breakdown of cAMP but not cGMP. In a previous study (Berkenboom et al 1991), oxpentifylline induced a completely endothelium-dependent relaxation without causing relaxation in rat aortic preparations deprived of endothelium. The difference between that study (Berkenboom et al 1991) and the present one may be due to experimental conditions; Berkenboom et al used rat aortic ring strips, 10^{-7} M phenylephrine as a precontractile agent, and oxpentifylline at 10^{-8}–10^{-4} M, while we used rat aortic helical strips, 10^{-7} M noradrenaline as the agent, and oxpentifylline at 10^{-6}–10^{-3} M. In contrast, theophylline induced a similar relaxation response in the presence and absence of endothelium, suggesting that the relaxation induced by theophylline is independent of endothelium. The relaxation responses to theophylline were potentiated by pretreatment with isoprenaline or sodium nitroprusside. This result suggests that theophylline may inhibit both cAMP- and cGMP-PDE.

Four PDE forms (i.e. PDE I, III, IV and V) have been isolated from rat aorta by Komas et al (1991) who found that PDE IV (a cAMP-specific enzyme) inhibitors, such as rolipram and debufylline, or PDE V (a cGMP-specific enzyme) inhibitors, such as zaprinast, produce partially endothelium-dependent relaxation, but PDE III inhibitors, such as milrinone or SK&F 94120, produce endothelium-independent relaxation. Furthermore, the endothelium-dependent relaxations induced by PDE IV and PDE V inhibitors were inhibited by L-NNA. Thus, the mode of relaxation response differs depending on type of PDE inhibitor. In the present study, the mode of the relaxation response to oxpentifylline differed from that of theophylline. Compared with theophylline, oxpentifylline can induce a greater relaxation response due to endothelial modulation.

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