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Vasorelaxant Effect of Trapidil on Human Basilar Artery

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Abstract—We have investigated the vasorelaxant effect of trapidil on human isolated basilar artery. Trapidil (10^{-5}–10^{-4} M) dose-dependently caused relaxation in vascular strips with or without endothelium, with no significant difference between the two types of strips. The relaxation responses were not inhibited by atropine, propranolol or methylene blue. Trapidil increased the concentration of 6-keto-$PGF_{1\alpha}$, a prostacyclin degradation product, released from an artery ring in the incubation medium, but trapidil-induced relaxation was not inhibited by indomethacin. Pretreatment of vascular strips with 10^{-5} M trapidil increased the relaxation responses to forskolin and dibutyryladenosine cyclic monophosphate but not to sodium nitroprusside or 8-bromoguanosine cyclic monophosphate. Trapidil induced a significant increase in the cAMP concentration but not in the cGMP concentration in artery strips. These results suggest that the relaxation response to trapidil is not caused by prostacyclin release or an increase in cGMP in the smooth muscle, but possibly by an increase in the cAMP levels, probably via an inhibitory effect on cAMP phosphodiesterase.

Trapidil (5-methyl-7-diethylamino-s-triazolo [1,5-*a*]pyrimidine, Rocornal), a drug that has been developed as a dilating agent of coronary arteries, is known to increase coronary blood flow (Noguchi et al 1981, 1984). It has been used clinically in the treatment of ischaemic heart disease (Dittrich et al 1971; Weser et al 1972). The protective effect of trapidil against cerebral vasospasm after subarachnoid haemorrhage has also been reported (Suzuki et al 1981). Thus, trapidil may also have a dilator effect on the intracranial vessels. Furthermore, this agent is known to inhibit platelet aggregation (Suzuki et al 1982; Mazurov et al 1984) and increase production of prostacyclin of the arterial wall (Kawamura et al 1980). Despite the various effects of trapidil, no detailed research has been undertaken on the mechanism of its dilator effect on blood vessels.

The aim of the present study was to determine whether trapidil has a dilator effect on human isolated basilar artery, and if it does, to elucidate the mechanism.

Materials and Methods

Tissue preparation

Basilar arteries of 16 men, aged 21 to 45 years, and five women, aged 20 to 40 years, were obtained during autopsy 2–15 h after death. The vessels of subjects who died of cerebrovascular diseases, such as cerebral haemorrhage and cerebral infarction, were not used. The basilar arteries obtained were immediately placed in Krebs-Ringer solution (KRS) and carefully dissected free of arachnoid tissue.

Measurement of isometric tension

The basilar arteries were cut helically into vascular strips (15 mm long and 2 mm wide). These strips were fixed vertically between hooks in a 10 mL tissue bath containing KRS, which was maintained at 37°C, pH 7·4, and aerated with a mixture of 95% O_2–5% CO_2. KRS was (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. Isometric tension, monitored with a force-displacement transducer (Nihon Kohden Kogyo Co., Tokyo, Japan) to which the upper ends of the strips were connected, was recorded with a pen recorder (Nihon Kohden Kogyo Co.), as previously reported (Altura & Altura 1970). The artery strips were equilibrated for approximately 2 h, during which time the medium was replaced every 15 min, and were adjusted to obtain a resting tension of 1·5 g. The endothelium was removed from some strips by gently rubbing the intimal surface with a filter paper (Furchgott & Zawadzki 1980). The removal was confirmed by loss of the relaxation induced by thrombin (0·5 units mL^{-1}), which causes endothelium-dependent relaxation in the human basilar artery (Hatake et al 1990). After precontraction with 10^{-6} M phenylephrine had reached the peak tension, relaxant drugs were added to the organ bath. In experiments with various inhibitors, intact arterial strips were first incubated for 10 min with 10^{-6} M propranolol or 10^{-6} M atropine or for 1 h with 10^{-5} M indomethacin or 10^{-5} M methylene blue before the precontraction with phenylephrine. We had confirmed in a preliminary experiment that concentrations of inhibitors used were those which completely blocked the maximal relaxations induced by 10^{-6} M isoprenaline and 5×10^{-5} M acetylcholine, and caused 60% inhibition of 10^{-6} M sodium nitroprusside-induced maximal relaxation. Indomethacin at 10^{-5} M almost completely inhibited the formation of vascular 6-keto-$PGF_{1\alpha}$, a degradation product of prostacyclin, observed after stimulation by 0·5 units mL^{-1} thrombin and 10^{-6} M bradykinin (95 and 98% inhibition, respectively). When the contraction had reached a plateau, trapidil (10^{-5}–10^{-4} M) was cumulatively administered. In a separate experiment, intact arterial strips were preincubated for 15 min with 10^{-5} M trapidil, and after precontraction with phenylephrine, the following substances were cumulatively administered: N^6-2′-*O*-dibutyryladenosine 3′:5′-cyclic monophosphate (db-cAMP, 10^{-5}–10^{-3} M), 8-bromoguano-

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sine 3′:5′-cyclic monophosphate (8-bromo-cGMP, 10^{-5}–10^{-3} M), forskolin (10^{-9}–10^{-6} M) and sodium nitroprusside (10^{-9}–10^{-6} M). The relaxation response was determined in terms of the percentage of the contraction induced by phenylephrine.

Determination of prostacyclin

The basilar arteries were cut into 5 mm rings. The rings were incubated in 1 mL of KRS for 3 h at 37°C and then were stimulated with various drugs (0·5 units mL^{-1} thrombin, 10^{-6} M bradykinin and 5×10^{-5} M trapidil) at 37°C for 15 min. 6-Keto-$PGF_{1\alpha}$ in a 0·1 mL portion of the incubation medium was determined with a ^{125}I radioimmunoassay kit (New England Nuclear, Boston, MA, USA) as previously described (Busse et al 1984). Anti-serum against 6-keto $PGF_{1\alpha}$ had the following cross-reactivity characteristics (compared with 6-keto-$PGF_{1\alpha}$ of 100%): $PGF_{1\alpha}$, 0·4%; $PGF_{2\alpha}$, 0·6%; PGE_1, 0·8%; PGE_2, 0·4%; TXB_2, 0·4%; PGA_1, <0·02%; PGA_2, <0·02%; PGD_2, 0·01%.

Determination of cyclic nucleotides

Cyclic nucleotide measurements were carried out using vascular strips which had been mounted in the organ chamber and equilibrated for 2 h, as described above. At 3 min after exposure to 5×10^{-5} M trapidil, the strips were immediately frozen in liquid nitrogen, and the cyclic nucleotide levels were assayed. The strips were preincubated for 1 h with 10^{-5} M indomethacin before exposure to trapidil for cAMP measurement, because trapidil induces the production of prostacyclin (Kawamura et al 1980), which elevates the intravascular cAMP level. Briefly, frozen strips were homogenized in 6% trichloroacetic acid and the samples were centrifuged for 15 min at 2000 and 1200 *g* for cGMP and cAMP, respectively. Supernatant fractions were extracted with ether and radioimmunoassayed using New England Nuclear Kits (Boston, MA, USA) for cGMP and cAMP as described previously (Ignarro et al 1981). The tissue residue was dissolved in 2 M NaOH and protein content was determined using the Biorad assay technique, with bovine serum albumin as the standard.

Drugs used

1-Phenylephrine, atropine, (±)-propranolol hydrochloride, methylene blue, indomethacin, forskolin, sodium nitroprusside, db-cAMP and 8-bromo-cGMP were all obtained from Sigma Chemical Co. (St Louis, MO, USA). Trapidil and bovine α-thrombin (1280 NIH units mg^{-1}) were obtained from Mochida Pharmaceutical Co. Ltd (Tokyo, Japan).

Calculations and statistics

The data are expressed as mean ± s.e. Student's unpaired *t*-test was used for statistical comparisons. Differences were accepted as significant for $P < 0.05$.

Results

Effect of trapidil on prostacyclin production

Like bradykinin and thrombin, which are known to increase prostacyclin production (Pearson et al 1983), trapidil increased the concentration of 6-keto-$PGF_{1\alpha}$ in KRS when compared with the control value (in the absence of agonists, 120·8 ± 5·2 pg mL^{-1} n = 8): 220·4 ± 7·6 pg mL^{-1} (n = 8, $P < 0.01$), 320·8 ± 10·8 pg mL^{-1} (n = 8, $P < 0.01$), and 250·8 ± 8·2, pg mL^{-1} (n = 8, $P < 0.01$) for thrombin (0·5 units mL^{-1}), bradykinin (10^{-6} M) and trapidil (5×10^{-5} M), respectively.

Vasorelaxant effect of trapidil

Trapidil dose-dependently induced a slowly developing relaxation in both intact and denuded artery strips without any significant difference in the relaxation between the two types of strips (Fig. 1A). This relaxation response in the strips with endothelium was not inhibited by indomethacin or methylene blue (Fig. 1B). Furthermore, the relaxations were not inhibited by propranolol or atropine (data not shown). The relaxation response to sodium nitroprusside or 8-bromo-cGMP was not affected by preincubation with 10^{-5} M trapidil (Fig. 2A, B). However, the relaxation response induced by forskolin and db-cAMP was significantly increased by preincubation with trapidil at 10^{-5} M, a concentration which has only a small relaxant effect (Fig. 2C, D).

Effects of trapidil on cyclic nucleotides

A significant increase in cAMP level was induced by 5×10^{-5} M trapidil, but the cGMP level did not change in the presence of trapidil (Table 1).

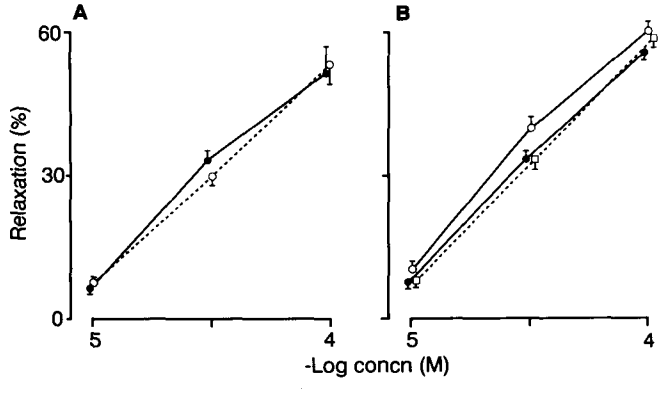

FIG. 1. Endothelium-independent relaxation induced by trapidil (A), and effect of indomethacin and methylene blue on the relaxation induced by trapidil (B) in human basilar artery. Results are expressed as means ± s.e. of 5 observations in A and 11 observations in B. For A, ● with endothelium, ○ without endothelium; for B, ● control, ○ indomethacin, □ methylene blue.

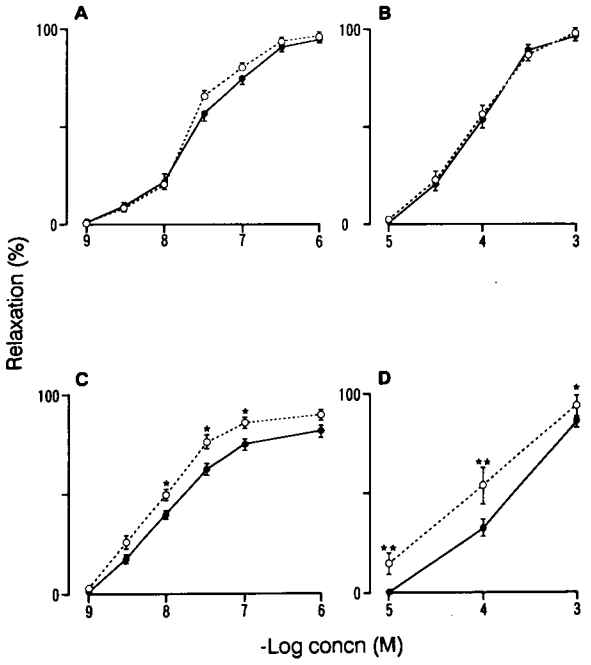

FIG. 2. Effect of pretreatment of human basilar arteries with 10^{-5} M trapidil (TRP) on the relaxation induced by sodium nitroprusside (SNP) (A), 8-bromo-cGMP (B), forskolin (C) and db-cAMP (D). Results are expressed as means ± s.e. of 9–11 observations in A and B, and 7 observations in C and D. $*P < 0.05$, $**P < 0.01$, significantly different from the results in the absence of TRP. For A, ● SNP, ○ SNP+TRP; for B, ● 8-bromo-cGMP, ○ 8-bromo-cGMP+TRP; for C, ● forskolin, ○ forskolin+TRP; for D, ● db-cAMP, ○ db-cAMP+TRP.

Table 1. Effect of trapidil on the cyclic nucleotide level of human basilar arteries.

Treatment	cGMP (pmol (mg protein)$^{-1}$)	cAMP (pmol (mg protein)$^{-1}$)
None (control)	0·52 ± 0·08	2·42 ± 0·30
Trapidil (5×10^{-5} M)	0·47 ± 0·09	7·20 ± 0·64*

Data are the mean ± s.e. of 7 observations. $*P < 0.01$, significantly different from the cyclic nucleotide level of the control.

Discussion

The present study showed that trapidil causes a dose-dependent relaxation of human basilar artery. The relaxation did not significantly differ between strips with and without endothelium, suggesting that trapidil produces endothelium-independent relaxation. Thus, the relaxation response to trapidil is not mediated by relaxing factors, such as endothelium-derived relaxing factor (Furchgott 1983, 1984) or endothelium-derived hyperpolarizing factor (Chen & Suzuki 1989; Chen et al 1989) released from the endothelial cells. Trapidil has been reported to stimulate the production of prostacyclin in rat aorta (Suzuki et al 1982) and to elevate the level of prostacyclin in human blood after oral administration (Okumura 1990). The present study also showed that trapidil has a stimulatory effect on prostacyclin production in the human basilar artery. Prostacyclin is known to be synthesized not only in endothelial cells, but also in smooth muscle cells (Jeremy & Dandona 1989). However, the increase in prostacyclin caused by trapidil is inhibited by pretreatment with indomethacin, a cyclo-oxygenase inhibitor (Kawamura et al 1980), but indomethacin did not inhibit the trapidil-induced relaxation. This indicates that the relaxation is not mediated by prostacyclin released from smooth muscle cells.

The relaxation response to trapidil was not inhibited by methylene blue, a guanylate cyclase inhibitor, suggesting that the relaxation is not mediated via activation of guanylate cyclase in smooth muscle cells. Pretreatment with trapidil did not affect the relaxation response to sodium nitroprusside, a soluble guanylate cyclase activator, or 8-bromo-cGMP, a membrane permeant analogue of cGMP. Thus, trapidil does not modify the relaxation caused by increase of cGMP. This result suggests that trapidil-induced relaxation does not occur as a result of the inhibitory action of cGMP degradation enzyme, cGMP phosphodiesterase. This interpretation is also supported by the fact that the intravascular cGMP concentration did not rise in the presence of 5×10^{-5} M trapidil, a concentration which shows a significant relaxing effect on the human basilar artery.

On the other hand, trapidil caused an increase in the intravascular level of cAMP. However, the increase in cAMP was not due to trapidil-induced prostacyclin release, since cAMP increased even in the presence of indomethacin. Therefore, other mechanisms causing the increase of cAMP need to be considered. Since the relaxing effect of trapidil was not inhibited by propranolol or atropine, it can be concluded the effect was not mediated by the β-adrenoceptor or cholinergic receptor in the smooth muscle, thus confirming the findings of a previous report (Sakanashi et al 1980). Trapidil also inhibits platelet aggregation via inhibitory action of cAMP phosphodiesterase (Mazurov et al 1984) and the cAMP phosphodiesterase activity of dog ventricular muscle (Satoh et al 1980). In the present study, the pretreatment of vascular strips with trapidil increased the relaxation via an increase in cAMP due to forskolin, which stimulates adenylate cyclase, and to db-cAMP, which can penetrate the cell membrane. Thus, pretreatment with trapidil can modify the relaxation by an increase in cAMP. Together with previous reports (Satoh et al 1980; Mazurov et al 1984), these results suggest that the rise in cAMP induced by trapidil is due to its inhibitory action on cAMP degradation, that is, on cAMP phosphodiesterase. Of course, the possibility that the relaxation response to trapidil takes place through adenylate cyclase activation in the smooth muscle cannot be excluded.

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