Effects of isocarbacyclin, a stable prostacyclin analogue, on monkey isolated cerebral and peripheral arteries

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- 1 The effects of isocarbacyclin (TEI 7165), a stable prostacyclin analogue, were examined in monkey isolated cerebral and peripheral arteries.
- 2 Addition of TEI 7165 (0.1 nM–10 μ M) produced a dose-dependent relaxation in cerebral arteries pre-contracted with 1 μ M 5-hydroxytryptamine (5-HT). High concentrations (more than 1 μ M) of TEI 7165 elicited a transient contraction followed by a sustained relaxation.
- 3 TEI 7165 also elicited a dose-dependent relaxation in the peripheral (except popliteal) arteries. The maximum relaxation induced by 10 μ M TEI 7165 was greater ($P < 0.05$) in the mesenteric artery than in the cerebral artery. The negative logarithm of the EC₅₀ value for the mesenteric, 7.6 ± 0.3 , was greater ($P < 0.05$) than that for the cerebral artery, 6.4 ± 0.3 . The decreasing order of potency for the TEI 7165-induced relaxation was as follows: mesenteric > renal > cerebral > coronary > popliteal.
- 4 Removal of the endothelium did not significantly affect TEI 7165-induced relaxations.
- 5 The transient contraction produced by high concentrations of TEI 7165 was not observed in cerebral arteries precontracted with 1 nM U46619, a stable analogue of thromboxane A₂ (TXA₂). Furthermore, the TEI 7165-induced contraction was markedly suppressed ($P < 0.05$) by treatment with 10 nM S1452, a TXA₂ blocking agent.
- 6 These results suggest that TEI 7165 causes an endothelium-independent relaxation in monkey cerebral and peripheral arteries, and that there is a marked regional difference in the TEI 7165-induced relaxations. A high concentration of TEI 7165 also produces a transient contraction which is probably through activation of TXA₂ (TP-) receptors.

Keywords: Cerebral artery; isocarbacyclin; prostacyclin; vascular smooth muscle; thromboxane A₂

Introduction

Prostacyclin (PGI₂) causes a relaxation of vascular smooth muscles of cerebral arteries (Chapleau & White, 1979) and increases cerebral blood flow (Pickard *et al.*, 1980). PGI₂ also inhibits aggregation of platelets (Moncada & Vane, 1978). These effects of PGI₂ suggest a possible therapeutic use in ischaemic cerebral disorders (Hoshi & Mizushima, 1990). Isocarbacyclin, (+)-9(0)-methano- $\Delta^{9(0)}$ -PGI₁, has recently been synthesized as a stable analogue of PGI₂ (Shibasaki *et al.*, 1983). This compound shows antithrombotic (Mizushima *et al.*, 1987) and angiogenic activities (Ohtsu *et al.*, 1988). Kawai & Ohhashi (1991) reported that isocarbacyclin produced a relaxation of vascular smooth muscles in monkey cerebral arteries. Since PGI₂ also dilates peripheral arteries (Toda, 1980), however, it is important to compare the relative potency of isocarbacyclin between cerebral and other arteries.

It is known that high concentrations of PGI₂ produce contractile responses in cat (Uski *et al.*, 1983), dog (Chapleau & White, 1979), and human (Uski *et al.*, 1983) cerebral arteries. Other prostanoids also cause marked contraction of isolated cerebral arteries, TXA₂ showing the highest potency (Uski & Andersson, 1984). To the best of our knowledge, however, a possible role of TP-receptors in PGI₂-induced contraction of vascular smooth muscles has not been examined in detail.

The purpose of the present study was (1) to compare the potency for isocarbacyclin-induced relaxation between monkey isolated cerebral and extracerebral arteries and (2) to investigate the mechanism of contraction produced by high concentrations of isocarbacyclin with special reference to the TP-receptor.

Methods

Eighteen adult macaque monkeys of either sex, weighing 6.5 ± 0.6 kg, were anaesthetized with ketamine hydrochloride (50 mg kg⁻¹, i.m.) and killed by bleeding from the common carotid artery. Cerebral (basilar and intracranial vertebral), coronary, mesenteric, renal, and popliteal arteries (outer diameter, 1.5–2.0 mm for popliteal arteries and 0.8–1.3 mm for other arteries) were rapidly dissected. The arterial segments were cut into helical strips, approximately 15 mm long and 1.5 mm wide. Each strip was fixed vertically between hooks in a 10 ml organ bath which was perfused with Krebs solution at a constant rate of 4 ml min⁻¹. The hook anchoring the lower end of the strip was fixed to the bottom of the bath, and the upper end was connected to the lever of a force-displacement transducer (Shinko Tsushin UL-10). The composition of the Krebs solution (mM) was as follows: NaCl 120.0, KCl 5.9, NaHCO₃ 25.0, NaH₂PO₄ 1.2, CaCl₂ 2.5, MgCl₂ 1.2 and glucose 5.5. The solution was maintained at $36.5 \pm 0.5^\circ\text{C}$ and aerated with a gas mixture of 95% O₂ and 5% CO₂ to give a pH of 7.3–7.4. The resting tension was adjusted to the optimal level for obtaining maximum contractile response to 80 mM KCl solution in each arterial preparation. Changes in the isometric tension were recorded on a direct writing oscillograph (Sanei Sokki 8K 20).

Following a 90 min equilibration, the preparations were contracted by perfusion with Krebs solution containing 1 μ M 5-hydroxytryptamine (5-HT). In the previous study, TEI 7165-induced relaxation was observed in the preparations without precontraction because isolated monkey cerebral arteries developed a high myogenic tone in an organ bath perfused with Krebs solution (Kawai & Ohhashi, 1991). Since isolated peripheral arteries failed to show sufficient myogenic tone in Krebs solution in the current study, these arteries were precontracted when the relaxant responses to TEI 7165 were examined. Therefore, cerebral arteries were also contracted before addition of TEI 7165 in order to compare their responses with those of peripheral arteries. The

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precontraction level produced by $1\ \mu\text{M}$ 5-HT solution seems to be most suitable for elucidating the dose-response relationship for relaxant agents (Kawai & Ohhashi, 1989). The basal tension of the preparations did not change significantly throughout the experiment. After the precontraction reached a plateau, the response to isocarbacyclin (TEI 7165) was evaluated by cumulative addition of the agent to the bathing medium with a microsyringe. The concentration of TEI 7165 was increased by a factor of 10 as soon as a steady response to the previous administration had been achieved. At the end of each experiment, $10\ \mu\text{M}$ sodium nitroprusside (SNP) was added to obtain maximum relaxation in each preparation. The extent of TEI 7165-induced relaxation is expressed as a percentage of the SNP-induced relaxation.

In order to examine the possibility that TEI 7165 acts by releasing nitric oxide from endothelium, the responses to TEI 7165 were also examined in preparations from which endothelial cells had been removed by mechanically rubbing the intimal surface with a filter paper following Furchgott's technique (Furchgott & Zawadzki, 1980). The absence or presence of endothelium was confirmed histologically by the silver staining procedure (Poole *et al.*, 1958).

The amount of contraction induced by isocarbacyclin was expressed as a percentage of the $1\ \mu\text{M}$ 5-HT-induced contraction. In some experiments, the effect of isocarbacyclin was examined in cerebral and mesenteric arteries which were contracted by $1\ \text{nM}$ – $10\ \text{nM}$ U46619, a stable analogue of TXA_2 . When the effect of S1452 (the calcium salt of (+)-S-145), an antagonist of the TXA_2 receptor (Arita *et al.*, 1992), on isocarbacyclin-induced contraction was tested, the preparations were treated for 30 min with S1452 and contracted with $1\ \mu\text{M}$ 5-HT before the responses to isocarbacyclin were determined.

The following drugs were used: 5-hydroxytryptamine creatinine sulphate (5-HT, Sigma, St. Louis, MO, U.S.A.), isocarbacyclin (TEI 7165, Teijin, Tokyo, Japan), sodium nitroprusside (SNP, Merk, Darmstadt, Germany), S1452 (Calcium 5(Z)-1R, 2S, 3S, 4S-7-[3-phenylsulphonylamino]bicyclo [2.2.1] hept-2-yl]-5-heptenoate hydrate) (Shionogi, Osaka, Japan), and U46619 (9,11-dideoxy-11 α , 9 α -epoxymethano-prostaglandin $\text{F}_{2\alpha}$) (Sigma, St. Louis, MO, U.S.A.). TEI 7165, S1452 and U46619 were dissolved in ethanol if not otherwise stated and diluted with distilled water just before use. 5-HT and SNP were diluted in fresh stock Krebs solution. The doses of drugs are expressed as a final organ bath concentration.

Experimental values shown in the text and tables are means $\pm$ s.e. The n values represent the number of animals used. The concentration of TEI 7165 causing a 50% relaxation of $10\ \mu\text{M}$ TEI 7165-induced relaxation is expressed as the EC_{50} value. For multiple comparisons, one-way analysis of variance was used. The differences between two groups were analysed by Student's paired or unpaired t test. The differences in means were considered significant at $P < 0.05$.

Results

Effects of TEI 7165 on monkey isolated cerebral arteries

Perfusion with $1\ \mu\text{M}$ 5-HT caused a contraction of cerebral arteries (Figure 1a and Table 1). TEI 7165 ($0.1\ \text{nM}$ – $10\ \mu\text{M}$) produced a dose-dependent relaxation in a monkey vertebral artery (Figure 1a). High concentrations of TEI 7165 ($1\ \mu\text{M}$ and $10\ \mu\text{M}$) elicited a transient contraction followed by a sustained relaxation. This transient contraction was also induced by TEI 7165 when it was dissolved in dimethyl sulphoxide (DMSO, Sigma, St. Louis, MO, U.S.A.) but was not produced by solvent only e.g. either ethanol or DMSO. No significant difference was observed between the responses of basilar and vertebral arteries. The threshold concentration for relaxant response to TEI 7165 was $1\ \text{nM}$ (Figure 2).

Figure 1 Typical responses to isocarbacyclin (TEI 7165) in concentrations ranging from $0.1\ \text{nM}$ to $10\ \mu\text{M}$ and sodium nitroprusside (SNP) $10\ \mu\text{M}$ in a monkey vertebral artery which was contracted by (a) $1\ \mu\text{M}$ 5-hydroxytryptamine 5-HT and (b) $1\ \text{nM}$ U46619.

Figure 2 Cumulative dose-response curves for isocarbacyclin (TEI 7165) in monkey mesenteric ($\circ$, $n = 8$), renal (∇ , $n = 5$), cerebral ($\bullet$, $n = 5$), coronary ($\diamond$, $n = 5$), and popliteal arteries ($\square$, $n = 6$) which had been contracted by $1\ \mu\text{M}$ 5-hydroxytryptamine.

Figure 3 Contraction induced by $10\ \mu\text{M}$ isocarbacyclin (TEI 7165) in the monkey cerebral ($n = 5$), mesenteric ($n = 8$), coronary ($n = 5$), renal ($n = 5$), and popliteal arteries ($n = 6$) treated with $1\ \mu\text{M}$ 5-hydroxytryptamine.

Effects of TEI 7165 on monkey isolated peripheral arteries

The degree of contraction produced by 5-HT in coronary and popliteal arteries was significantly greater than that in cerebral arteries (Table 1). In all peripheral arteries, addition of 10 μ M SNP produced a remarkable relaxation which was almost 100% (90–112%) of the 5-HT-induced precontraction.

There was a marked regional difference in the TEI 7165-induced relaxation, expressed as percentage of the SNP-induced relaxation, among various monkey arteries which had been contracted by 1 μ M 5-HT (Figure 2). TEI 7165 elicited the greatest relaxation in mesenteric arteries. The threshold concentration was less than 0.1 nM, and maximum relaxation induced by 10 μ M TEI 7165 was $89.6 \pm 3.5\%$ in the mesenteric arteries. On the other hand, popliteal arteries were much less sensitive to TEI 7165, the threshold concentration and maximum relaxation being 1 μ M and $2.9 \pm 1.4\%$ (statistically not significant comparing with time-control), respectively. Table 2 demonstrates the EC_{50} values and E_{max} values (maximum relaxations induced by 10 μ M TEI 7165). The decreasing order of potency for the TEI 7165-induced relaxation in monkey isolated arteries contracted by 5-HT was as follows: mesenteric > renal > cerebral > coronary > popliteal. A high concentration (10 μ M) of TEI 7165 produced transient contraction not only in cerebral but also in coronary and mesenteric arteries (Figure 3). In renal and popliteal arteries, the contraction was statistically not significant because of variability between preparations.

Effects of endothelium removal on TEI 7165-induced relaxation

The responses to TEI 7165 were also examined in cerebral and mesenteric arteries from which endothelial cells had been removed. The amount of contraction produced by 1 μ M 5-HT was not significantly different from that obtained in the endothelium-intact preparations. Addition of 10 μ M SNP relaxed the endothelium-denuded preparations almost to the same extent as in endothelium-intact preparations (Table 1). There was no significant difference in EC_{50} and E_{max} values between preparations with and without endothelium in both cerebral and mesenteric arteries (Table 2).

Contractile responses to high concentration of TEI 7165

The transient contraction of cerebral artery preparations induced by high concentrations of TEI 7165 was almost abolished when the preparation was contracted with 1 nM U46619 (Figure 1b). Addition of U46619 (1 nM–10 nM) increased the tension by 730 ± 110 mg in five cerebral arteries. The amount of contraction elicited by 10 μ M TEI 7165 was only $8.1 \pm 4.8\%$ of the U46619-induced contraction ($n = 5$).

Figure 4 Effects of S1452 on isocarbacyclin (TEI 7165)-induced contraction of monkey (a) cerebral ($n = 6$), and (b) mesenteric arteries ($n = 6$): (●) and (○) indicate the results from control and 1 μ M S1452-treated preparations, respectively. ** $P < 0.05$; * $P < 0.01$.

Treatment with 10 nM S1452 did not significantly affect contraction produced by 1 μ M 5-HT; 710 ± 70 mg in control vs 580 ± 90 mg after treatment with S1452 ($n = 6$). On the other hand, S1452 attenuated the TEI 7165-induced contraction of cerebral (Figure 4a) and mesenteric arteries (Figure 4b).

Table 1 5-Hydroxytryptamine (5-HT)-induced precontraction and sodium nitroprusside (SNP)-induced relaxation of monkey isolated arteries

Artery	n	5-HT (mg)	SNP (mg)	% of precontraction ^a (%)
Cerebral	5	520 ± 90	1240 ± 120	258 ± 32
Cerebral E(-) ^b	5	420 ± 60	1040 ± 160	251 ± 28
Coronary	5	$1010 \pm 130^*$	1150 ± 130	$112 \pm 7^*$
Mesenteric	8	880 ± 160	930 ± 160	$107 \pm 3^*$
Mesenteric E(-) ^b	6	$1230 \pm 170^*$	1400 ± 150	$119 \pm 10^*$
Renal	5	580 ± 100	$620 \pm 90^*$	$110 \pm 4^*$
Popliteal	6	$2740 \pm 480^*$	$2550 \pm 480^*$	$90 \pm 5^*$

*Significantly different from results obtained in endothelium-intact cerebral artery, $P < 0.05$.

^aPercentage of SNP-induced relaxation relative to 5-HT-induced contraction.

^bE(-): preparations without endothelium.

Table 2 Relaxant response of monkey isolated arteries to isocarbacyclin (TEI 7165)

Artery	n	$-\log EC_{50}$	E_{max} (%)
Mesenteric	8	$7.6 \pm 0.3^*$	$89.6 \pm 3.5^*$
Mesenteric E(-) ^b	6	$7.3 \pm 0.1^*$	$94.9 \pm 1.4^*$
Renal	5	7.6 ± 0.6	51.4 ± 12.6
Cerebral	5	6.4 ± 0.3	43.4 ± 6.0
Cerebral E(-) ^b	5	6.3 ± 0.2	39.3 ± 5.4
Coronary	5	6.1 ± 0.2	28.5 ± 6.2
Popliteal	6	— ^a	$2.9 \pm 1.4^*$

EC₅₀: concentration of TEI 7165 causing 50% of each maximum response.

E_{max} : maximum relaxation expressed as percentage of SNP-induced relaxation.

^aNot evaluated.

^bE(-): preparation without endothelium.

*Significantly different from results obtained with endothelium-intact cerebral artery, $P < 0.05$.

Discussion

PGI₂ causes a marked relaxation in human cerebral arteries (Brandt *et al.*, 1981; Uski *et al.*, 1983), and in cerebral arteries of dogs (Chapleau & White, 1979; Toda, 1980), cats (Uski *et al.*, 1983), and baboons (Hayashi *et al.*, 1985). Isocarbacyclin (TEI 7165) is a stable analogue of PGI₂ (Shibasaki *et al.*, 1983; Mizuno-Yagyu *et al.*, 1987; Mizushima *et al.*, 1987). However, there is little information about its vasorelaxant activity except in our recent report which examined the effect of TEI 7165 on monkey isolated cerebral arteries (Kawai & Ohhashi, 1991). The present results demonstrate that TEI 7165 produces a dose-dependent relaxation in monkey isolated cerebral and peripheral (except popliteal) arteries. High concentrations of TEI 7165 (1 μ M and 10 μ M) caused a transient contraction followed by a sustained relaxation (Figure 1a). Although the dose-response curves for TEI 7165 did not reach a plateau at concentrations up to 10 μ M, higher concentrations of TEI 7165 were not examined in the present study because preliminary experiments indicated that such concentrations of TEI 7165 elicited sustained contractions of monkey cerebral arteries.

There was a marked regional difference in the TEI 7165-induced relaxation. In preparations contracted by 5-HT, TEI 7165 produced the greatest relaxation in mesenteric arteries followed by renal, cerebral, coronary, and popliteal arteries in decreasing order (Figure 2). The maximum relaxation induced by 10 μ M TEI 7165 was significantly greater in the mesenteric artery and significantly smaller in the popliteal artery compared with responses obtained in the cerebral artery (Table 2). Although the dose-response curves for TEI 7165 did not reach a plateau for the reasons mentioned above, we calculated apparent EC₅₀ values on the assumption that TEI 7165 exerts its maximum relaxant effect at a concentration of 10 μ M. The negative logarithm of the EC₅₀ for the mesenteric artery, 7.6 ± 0.3 , was significantly greater than that for the cerebral artery, 6.4 ± 0.3 (Table 1). The EC₅₀ value for the popliteal artery was not evaluated because TEI 7165 produced no relaxation in some of the preparations. Toda (1980) reported that PGI₂ Na or PGI₂ methyl-ester produced a dose-dependent relaxation in canine isolated arteries contracted by PGF_{2 α} or high K⁺ with a decreasing order of effect on mesenteric > renal > coronary > cerebral. Our result is consistent with those of Toda (1980) except that the cerebral artery is more sensitive to PGI₂ than is the coronary artery. Our results also show that monkey popliteal artery is much less sensitive to isocarbacyclin than the cerebral or other peripheral arteries.

There are several possibilities which account for the regional difference in TEI 7165-induced relaxation: (1) the contractile effect of TEI 7165 may decrease its relaxant effects in some arteries, (2) TEI 7165 may release nitric oxide from the endothelium to cause smooth muscle relaxation and (3) receptor and/or intracellular signal transduction system for TEI 7165 are different. The first and second possibilities are,

however, unlikely for the following reasons: (1) contraction induced by TEI 7165 was lowest in popliteal arteries in which the relaxant response was not observed, whereas the contraction was greatest in the mesenteric arteries where the relaxation response was also greatest. (2) TEI 7165 caused almost the same relaxation response in endothelium-denuded cerebral and mesenteric arteries as in the endothelium-intact preparations (Table 2). Therefore, a difference in the ability of the endothelium to produce nitric oxide cannot explain the regional difference in TEI 7165-induced relaxation. Thus, differences in receptor and/or the intracellular signal transduction system seem to be the most likely reason for the regional difference. PGI₂ relaxes vascular smooth muscle through stimulation of the PGI₂ receptor, increasing the intracellular concentration of adenosine 3':5'-cyclic monophosphate (cyclic AMP) (Oliva *et al.*, 1984) which, in turn, activates protein kinase A in smooth muscle cells. Further studies are needed to clarify the heterogeneity of receptor and signal transduction mechanisms for TEI 7165-induced relaxation.

According to standard nomenclature (see TIPS, 1993) prostanoid receptors are classified into five main classes; DP, EP (including EP₁, EP₂ and EP₃), FP, IP and TP. Activation of EP (except EP₂), FP and TP-receptors leads to the contraction of smooth muscle cells (Kennedy *et al.*, 1982). Several findings suggest that PGI₂ may act on EP and FP as well as IP-receptors. For example, Dong *et al.* (1986) showed that isocarbacyclin contracted the guinea-pig trachea through activation of EP₁-receptors. Uski *et al.* (1983) showed that PGI₂ relaxed isolated feline cerebral arteries contracted with PGF_{2 α} , but not when contracted with noradrenaline or 5-HT, and suggested that the relaxant effect was due to PGI₂ competing with PGF_{2 α} for the same contraction-inducing prostanoid receptors (probably FP-receptors). On the other hand, a role of TP-receptors in PGI₂-induced contraction has not been examined in detail. In the present study, high concentrations of TEI 7165 (1 μ M and 10 μ M) elicited a transient contraction of cerebral, coronary, and mesenteric arteries (Figure 3). This is consistent with the observations that PGI₂ causes a contraction in cerebral arteries (Chapleau & White, 1979; Uski *et al.*, 1983), coronary artery (Dusting *et al.*, 1977) and various veins (Levy, 1978). In this study, the TEI 7165-induced contraction seems to be produced by stimulation of TP-receptors on vascular smooth muscle cells since a TXA₂ antagonist, S1452, attenuated the contraction observed (Figure 4). S1452 is a highly potent and selective antagonist for platelet TP-receptors with no ability to displace the specific binding of [³H]-PGD₂ and [³H]-PGF_{2 α} (Hanasaki & Arita, 1988). However, since we used a relatively high concentration of S1452 to observe its maximum effect the involvement of EP- and/or FP-receptors in the mechanism of TEI 7165-induced blood vessel contraction cannot be excluded. That TEI 7165 failed to contract monkey cerebral arteries when precontracted with U46619 may support the above hypothesis i.e. that high concentrations of TEI 7165 contract monkey

arteries through activation of TP-receptors. Although the mechanism of this effect remains somewhat obscure the following two possibilities may be suggested: (1) U46619 may produce a partial desensitization of the preparation and/or (2) TEI 7165 may compete with U46619 at the same TP-receptors.

The possibility that high concentrations of TEI 7165 stimulate the synthesis and release of endogenous TXA₂ in the vascular wall also cannot be excluded. However, Hadhazy *et al.* (1984) showed that high concentrations of PGI₂ cause a contraction of rabbit isolated arteries in the presence of indomethacin. Thus, the contractile effect of TEI 7165 in monkey isolated arteries is likely to be due, at least in part, to its direct action on TP-receptors in vascular smooth muscle.

In conclusion, TEI 7165 produces an endothelium-independent relaxation in monkey isolated cerebral and peripheral (except popliteal) arteries. A marked regional difference in TEI 7165-induced relaxation of monkey isolated arteries was observed. High concentrations of TEI 7165 cause a transient contraction of these blood vessels probably due to stimulation of TP-receptors on vascular smooth muscle.

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