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Interaction Between Cicletanine and the Eicosanoid System in Human Subcutaneous Resistance Arteries

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Abstract—In human subcutaneous resistance arteries in-vitro, the relaxation produced by the novel furopyridine compound cicletanine (30 μM) was inhibited by 51% ($P < 0.0001$) in the presence of 20 μM of the cyclo-oxygenase inhibitor indomethacin. Maximal cicletanine-induced relaxation was reduced by both the cyclo-oxygenase inhibitor mefenamic acid and the relatively specific blocker of prostacyclin synthetase, tranylcypromine by 55% ($P < 0.0005$) and 43% ($P < 0.01$), respectively. The potassium channel blocker, glibenclamide (100 μM), did not affect the relaxation produced by cicletanine but did inhibit the relaxation produced by the potassium channel blocker cromakalim ($P < 0.0001$). Cromakalim-induced relaxation was inhibited in the presence of indomethacin; relaxation induced by cromakalim (30 μM) was reduced by 22% ($P < 0.02$). The relaxation produced by the hypotensive agonists sodium nitroprusside, hydrochlorothiazide, bumetanide and nicardipine was unaffected by incubation with indomethacin. The results suggest that the vascular eicosanoid system, specifically prostacyclin, may be involved in the mechanism of the acute vasodilator action of cicletanine. Although at high doses, cicletanine is a diuretic, in this model it did not act like either a thiazide or a loop diuretic. The acute vasodilator action of the thiazide diuretic, hydrochlorothiazide was a novel finding of this study. Cromakalim showed a reduced response in the presence of indomethacin suggesting an involvement of the eicosanoid system in its mechanism of action.

Cicletanine is an antihypertensive agent with a novel chemical structure containing a furopyridine group (Chabrier et al 1988). At doses in excess of those required for its antihypertensive effect, cicletanine exhibits diuretic properties and can cause an acute increase in plasma renin activity (Bippi & Guinot 1988). The mechanism of action for cicletanine's antihypertensive effects is not known, although several hypotheses have been put forward, including an interaction with the vascular eicosanoid system, specifically prostacyclin (Castro et al 1989) or an action on potassium channels (Koltai et al 1990).

Previous studies (Bourgain et al 1989; Dorian et al 1988) using in-vivo and in-vitro animal models have shown that cicletanine enhances the production of vasodilator prostaglandins (especially prostacyclin) and we examined this hypothesis in a human in-vitro model. The aim of the study was to investigate the proposed interaction betwen cicletanine and the eicosanoid system and to compare these findings with results obtained with different classes of antihypertensive drugs: namely a guanylate cyclase activator (sodium nitroprusside), a potassium channel opener (cromakalim), a thiazide diuretic (hydrochlorothiazide), a loop diuretic (bumetanide) and a calcium channel blocker (nicardipine).

The potent non-steroidal anti-inflammatory drug (NSAID) indomethacin was used in these experiments. It has been well established that NSAIDs inhibit prostaglandin (PG) synthesis (Vane 1971). However these agents' actions may not be entirely PG-mediated and recent data have suggested their action may involve effects on calcium mobilization at the level of the plasma membrane (Jeremy et al 1989; Gill et al 1990). Indomethacin can inhibit the binding of calcium to isolated smooth muscle cell plasma membranes (Northover 1973). Since indomethacin may not be a typical NSAID we repeated our cicletanine experiments with another NSAID, mefenamic acid. As several researchers have reported a direct association between cicletanine and generation of prostacyclin (Hajjar & Pomerantz 1989) we also conducted a series of experiments with cicletanine using the relatively specific prostacyclin synthetase inhibitor tranylcypromine.

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Materials and Methods

Materials

Cicletanine was a gift from Ipsen Pharmaceuticals (France). Cromakalim was obtained from Beecham Pharmaceuticals (UK) and glibenclamide from Roussel Laboratories (UK). All other drugs were from Sigma Chemical Co.

Cicletanine, indomethacin, nicardipine and glibenclamide were dissolved in dimethylsulphoxide (DMSO). Ethanol was used in order to dissolve hydrochlorothiazide and bumetanide. Mefenamic acid was dissolved in a small amount of 0·2 M sodium hydroxide. All other agents and all serial dilutions were made either in distilled water or in physiological saline solution (PSS: NaCl 118 mM, KCl 4·7 mM, $CaCl_2 \cdot 6H_2O$ 2·5 mM, $MgSO_4 \cdot 7H_2O$ 1·2 mM, $NaHCO_3$ 25·0 mM, $NaH_2PO_4 \cdot 2H_2O$ 1·0 mM, Na_2EDTA 0·03 mM, glucose 5·5 mM).

Methods

Human subcutaneous resistance arteries (internal diameter 150–740 μm, mean 346 μm) were dissected free and mounted as a ring segment on a microvascular myograph (Mulvany & Halpern 1977) for the measurement of isometric tension. The arteries were normalized to a standard resting tension (this being the tension at which the vessel is under a transmural pressure of 90 mmHg since it has been shown that under these conditions the active force production of the vessel is maximal) in PSS maintained at 37°C and constantly aerated

with 95% O_2–5% CO_2. After 1 h the vessels were tested for viability using depolarizing K^+ (118 mM) in PSS (KPSS) and noradrenaline (1 and 10 μM); those vessels failing to produce a tension equivalent to an active pressure of 90 mmHg to both KPSS and both concentrations of noradrenaline were discarded. The functional integrity of the endothelium was investigated by application of 10 μM acetylcholine to a vessel preconstricted by noradrenaline (10 μM): relaxation of the vessel was assumed to indicate the presence of functional endothelium.

Each experiment involved the construction of two concentration curves to one of the drugs after preconstriction with noradrenaline (10 μM): cicletanine (1 nM–30 μM), sodium nitroprusside (10 nM–10 μM), cromakalim (1 nM–30 μM), hydrochlorothiazide (10 nM–30 μM), bumetanide (1 nM–30 μM) or nicardipine (100 pM–3 μM). One curve was constructed after 30 min incubation with indomethacin (20 μM) and the other after 30 min exposure to vehicle. The order in which the two relaxation curves were constructed was randomly varied for each experiment.

At the end of the experiment, the vessels were again challenged with KPSS in a final test for viability; vessels which failed to produce a tension equivalent to at least 80% of the original tension produced by KPSS were discarded.

Following the same protocol, but using only cicletanine, the experiment was repeated, replacing the incubation with indomethacin by a similar incubation with the alternative cyclo-oxygenase inhibitor mefenamic acid (100 μM). The experiment was also performed utilizing the prostacyclin synthetase inhibitor tranylcypromine (100 μM) in place of indomethacin in conjunction with cicletanine. In order to test the possibility that cicletanine may open potassium channels, a cicletanine concentration response curve was constructed in the presence and absence of glibenclamide (100 μM). Glibenclamide, a sulphonylurea, is a potent blocker of ATP-sensitive K^+ channels (Standen et al 1989). Concentration response curves to cromakalim were also constructed in the presence and absence of glibenclamide.

The experimental data were analysed statistically using both the Student's *t*-test (parametric test) and the Mann-Whitney test (non-parametric test). All the results found to be statistically different were so in both tests.

Results

In total, 50 viable artery segments (mean diam. 346 ± 18 μm) from 38 patients were used in these experiments. The vessels were obtained from patients undergoing cardiac artery bypass operations. These patients were under general anaesthesia and a sample of fat was taken from tissue routinely removed during excision of saphenous vein for grafting and was utilized within 24 h of removal.

Effects of drugs on the noradrenaline-induced contracture

All the compounds used produced concentration-dependent relaxations of the arteries following preconstriction with noradrenaline (10 μM). The vasorelaxation produced by the drugs was found not to be dependent upon the presence or absence of endothelium as determined by acetylcholine-induced relaxation. The pretreatment drugs (indomethacin, mefenamic acid, tranylcypromine and glibenclamide) had no effects on the subsequent contractions to noradrenaline. There was no difference between the agonist-induced relaxations in experiments regardless of whether the inhibitor (indomethacin, mefenamic acid, tranylcypromine or glibenclamide) was present during the first concentration-response curve or the second.

Table 1. Log EC50 (M) values of agonists in the absence (control) and presence of indomethacin (20 μM).

	Control	Indomethacin
Sodium nitroprusside	6·5	6·8
Cicletanine	6·4	6·9
Cromakalim	8·2	7·8
Hydrochlorothiazide	7·3	7·3
Bumetanide	7·8	8·1
Nicardipine	6·5	7·0

Effect of indomethacin on the drug-induced relaxation of preconstricted vessels

The relaxation produced by sodium nitroprusside (n = 3), hydrochlorothiazide (n = 7), bumetanide (n = 4) and nicardi-

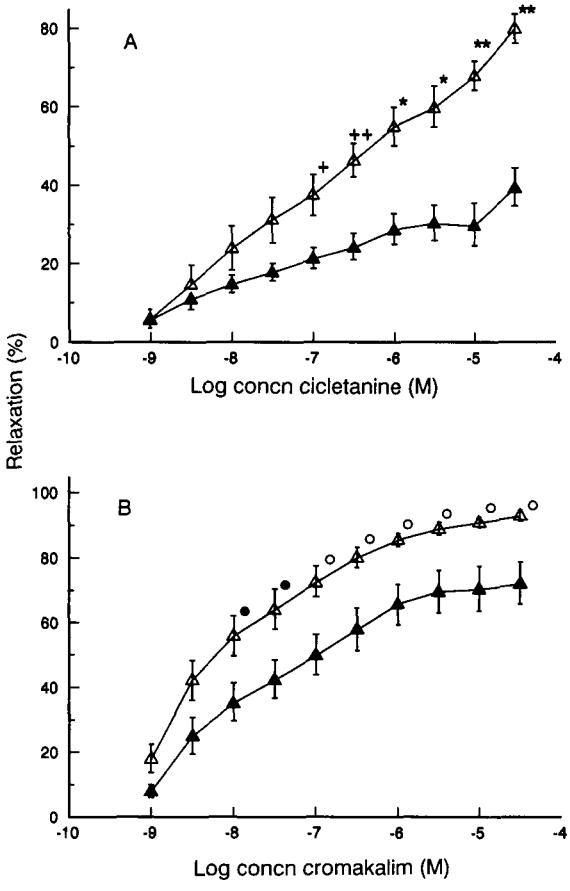

FIG. 1.A. Dose/response effect of increasing doses of cicletanine on the relaxation of a preconstricted resistance vessel in the presence (▲) and absence (△) of indomethacin (20 μM). Each value represents the mean ± s.e. of seven artery segments. $+P<0.02$, $++P<0.001$, $*P<0.0002$, $**P<0.0001$ compared with control. B. Dose/response effect of cromakalim on vasorelaxation of human subcutaneous vessels in the presence (▲) and absence (△) of indomethacin (20 μM). Each value represents the mean ± s.e. of seven artery segments. $\bullet P<0.05$, $\circ P<0.02$ compared with control.

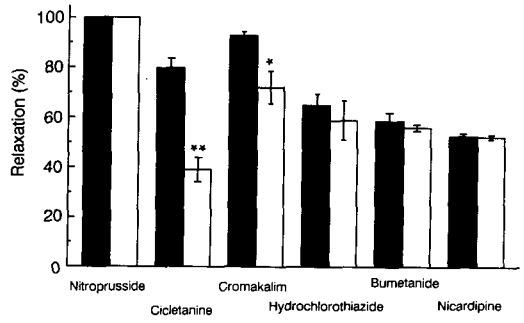

FIG. 2. Comparison of the effects of indomethacin on the vasorelaxation produced by agonists at their maximum concentrations. ■ Represents relaxation produced in control, □ represents relaxation in the presence of indomethacin. Bar height shows mean maximal relaxation, error bars represent s.e. $*P<0.02$, $**P<0.0001$ compared with control.

pine (n = 4) was unaffected by incubation with indomethacin. Cicletanine (n = 7) and cromakalim (n = 7) produced less relaxation in the presence of indomethacin (Fig. 1). Fig. 2 summarizes all the findings for the drugs at their maximum concentrations in the presence and absence of indomethacin. The EC50 values were calculated (Table 1) and no significant differences were found between the values in the presence and absence of indomethacin.

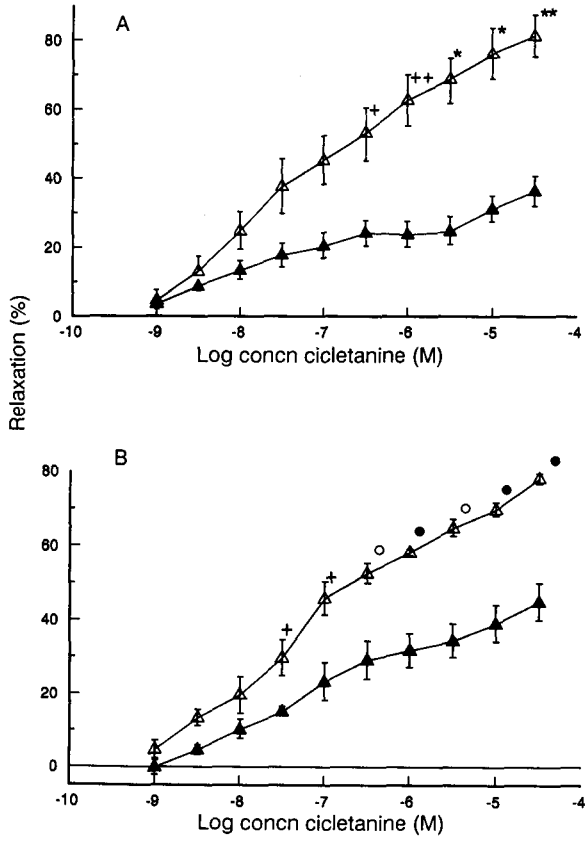

FIG. 3.A. Dose/response curve for the effect of cicletanine on resistance vessels in the presence (▲) and absence (△) of mefenamic acid (100 μM). Each value represents the mean ± s.e. of five artery segments. $+P<0.05$, $++P<0.02$, $*P<0.001$, $**P<0.0005$ compared with control. B. Dose/response curve showing relaxant effect of cicletanine on resistance arteries in the presence (▲) and absence of (△) of tranylcypromine (100 μM). Each value represents the mean ± s.e. of four artery segments. $+P<0.05$, $\bullet P<0.01$, $\circ P<0.005$ compared with control.

Effects of mefenamic acid and tranylcypromine on the cicletanine-induced relaxation of preconstricted vessels

Maximal cicletanine-induced relaxation was inhibited in the presence of either mefenamic acid (n = 5) or tranylcypromine (n = 4) (Fig. 3). The EC50 values were calculated for cicletanine in the presence and absence of either mefenamic acid (6·9 and 7·1, respectively) or tranylcypromine (7·0 and 7·1, respectively) and no significant differences were observed.

Interaction between cicletanine and potassium channels

The relaxation produced by cicletanine (n = 4) was unaffected by preincubation with the potassium channel blocker glibenclamide. The cromakalim-induced relaxation (30 μM) (n = 5) was inhibited by 70% ($P<0.0001$) in the presence of glibenclamide.

Discussion

Cicletanine is an antihypertensive agent with potentially beneficial properties: it is cardioprotective (Jouve et al 1986; Tosaki et al 1990) and may also protect against hypertensive retinopathy (Millerin et al 1989). The mechanism of action of cicletanine is unknown although several theories have been proposed. In addition to those already mentioned it has been reported that cicletanine exerts H_1-receptor antagonistic effects (Schoeffter & Godfraind 1988; Ebeigbe et al 1989) and interacts with vascular α_1-receptors (Chabrier et al 1988), but these mechanisms are not thought to be important in determining cicletanine's vasodilator properties. We were unable to confirm the α_1 inhibitory action of cicletanine in this tissue. Pre-incubation with 1 and 10 μM concentrations of cicletanine had no effect on subsequent noradrenaline dose-response curves (unpublished data). We were able to confirm other researchers' findings (Ebeigbe & Cabanie 1991) that relaxation responses to cicletanine are uninfluenced by the presence or absence of endothelium. The two leading hypotheses for the mechanism of cicletanine's antihypertensive efficacy are an interaction with the vascular eicosanoid system (specifically prostacyclin) or an action on ion fluxes in particular via potassium channels. The results of our study suggest that the vascular eicosanoid system may be involved in the mechanism of the hypotensive action of cicletanine. The finding that the relatively specific prostacyclin synthetase inhibitor tranylcypromine exhibited significant inhibition of the cicletanine-induced relaxation, may demonstrate the importance of prostacyclin, at least in acute vasodilatation. Prostacyclin (1 μM: n = 2) completely relaxed human vessels (data not shown).

The relaxation response to cicletanine was unaffected by the presence of the potassium channel blocker glibenclamide. This suggests that the ATP-sensitive K^+ channel is not involved in the vasorelaxant activity of cicletanine. This is also supported by our observations that cicletanine relaxes a vessel preconstricted with potassium depolarizing solution (118 mM K^+) (Calder et al 1992).

We compared the results obtained with cicletanine with those obtained with cromakalim. Cromakalim is a new

antihypertensive, smooth muscle relaxant drug thought to produce its vasodilator actions by opening potassium channels, although the exact nature of the drug/cell interactions are unclear (McPherson & Angus 1990). We have confirmed other researchers' findings (Yanagisawa et al 1990; Brayden et al 1991) that glibenclamide-sensitive mechanisms are involved in the mechanism of the vasodilator action of cromakalim. Our results indicate that the relaxation produced by cromakalim is in part affected by the vascular eicosanoid system since the vasorelaxant response was attenuated after incubation with indomethacin. This response with cromakalim was much less striking than that seen with cicletanine but it does suggest some similarity of action between the two drugs.

As cicletanine in doses in excess of those usually required for the hypotensive effect demonstrates a diuretic action, it was of interest to compare the responses with cicletanine to those with a thiazide diuretic (hydrochlorothiazide) and a loop diuretic (bumetanide). In our experiments we found that the relaxation produced by hydrochlorothiazide was unaffected by incubation with indomethacin. It should be noted that the acute vasodilatory action of thiazide diuretics in human resistance vessels was a novel finding of this study. Numabe et al (1989) had found that the thiazide diuretic trichloromethiazide significantly decreased vascular vasodepressor prostacyclin generation and Gerber et al (1990) showed that the antihypertensive efficacy of hydrochlorothiazide is not dependent on prostacyclin. This strengthens the argument that, at least with regard to the vascular eicosanoid system, the actions of cicletanine do not resemble those of a thiazide diuretic. The loop diuretic bumetanide also failed to show inhibition of relaxation in the presence of indomethacin. It has been shown (Copello et al 1989) that the vascular relaxant effect of this drug does not require the integrity of the endothelium but is abolished by methylene blue, suggesting that increased synthesis of cGMP secondary to guanylate cyclase activation may be directly involved. It is therefore not surprising that the relaxation produced by the guanylate cyclase activator sodium nitroprusside is also unaffected by incubation with indomethacin.

Nicardipine is a member of the dihydropyridine family of drugs. These drugs share a basic mechanism of action as vasodilators, namely the inhibition of transmembrane Ca^{2+} influx through L-type channels during plasma membrane depolarization (Ohtsuka et al 1989). The relaxation produced by nicardipine was unaffected by incubation with indomethacin. This suggests that there is no involvement of the vascular system in its mechanism of action. As it has been proposed that indomethacin may also exert part of its actions via an inhibition of calcium binding we might have expected to see an additive or synergistic interaction between the two drugs: this was not the case.

In conclusion, these findings suggest that in human subcutaneous resistance arteries in-vitro there is an involvement of the eicosanoid system in the acute vasodilator action of cicletanine. In particular, prostacyclin may be implicated in mediating this relaxation. This may also be relevant to the chronic antihypertensive properties of the drug. The eicosanoid system accounted for approximately 50% of the relaxant activity of cicletanine and we do not know what accounts for the remaining 50%, although glibenclamide-sensitive mechanisms do not appear to be involved. Although cicletanine may have diuretic properties at high concentrations it does not, in this model, act like a thiazide or a loop diuretic. The potassium channel opener, cromakalim, also demonstrated a reduction of its vasorelaxant response in the presence of indomethacin which suggests involvement of the vascular eicosanoid system in its mechanism of action.

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