The potassium channel opening drug cromakalim produces arterioselective vasodilation in the upper limbs of healthy volunteers

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1 The effect of the K⁺ channel opening drug cromakalim on forearm blood flow during direct infusion into the brachial artery, and on the size of noradrenaline precontracted hand veins during infusion directly into the vein, was studied in eight healthy volunteers.

2 Cromakalim (0.01–10.0 µg min⁻¹) produced a dose-dependent increase in blood flow in the infused forearm, from 5.4 ± 2.5 to 15.1 ± 7.3 ml 100 ml⁻¹ forearm min⁻¹ at 10 µg min⁻¹ ($P < 0.001$). The half-time of offset of its action was 30 min. There was no change in blood flow in the non-infused forearm.

3 There was no increase in the size of noradrenaline pre-constricted dorsal veins during local infusion of cromakalim (0.001–1.0 µg min⁻¹). Glyceryl trinitrate (0.4 µg min⁻¹) however, completely reversed the constriction to noradrenaline ($P < 0.001$).

4 The clear arterioselectivity of cromakalim, as with other members of this new class of drug, accords with the dependency of venoconstriction on receptor-operated, rather than potential-operated mechanisms which are of importance in resistance vessels. With this haemodynamic profile cromakalim may prove of value in the treatment of arterial hypertension.

Keywords potassium ionic channel vascular smooth muscle plethysmography hypertension

Introduction

Cromakalim is a recently developed member of a new class of agents, the K⁺ channel opening drugs. It produces vasodilatation by opening plasmalemmal K⁺ channels in vascular smooth muscle cells, allowing increased K⁺ efflux (Hamilton *et al.*, 1986; Weir & Weston, 1986; Cook, 1988) with consequent membrane hyperpolarisation. This leads to a reduction in the availability of calcium for the contractile process, and reduced sensitivity of blood vessels to depolarising stimuli (Clapham & Wilson, 1987; Cook, 1988; Cook *et al.*, 1988). Preliminary studies have suggested that cromakalim lowers

blood pressure in patients with hypertension, producing a reduction in both supine and standing blood pressure together with moderate reflex tachycardia (Vanden Burg *et al.*, 1987).

Another drug, minoxidil, which appears to produce vasodilatation in vascular smooth muscle by increasing K⁺ permeability (Meisheri *et al.*, 1988), acts predominantly as a vasodilator of arterial vessels, producing a marked reduction in systemic but not pulmonary vascular resistance (Hall *et al.*, 1980). Such selective arteriolar vasodilatation may be of value in the treatment of arterial hypertension, where the primary ab-

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normality is an increase in peripheral vascular resistance.

The present studies were designed to examine the actions of cromakalim on resistance and capacitance vessels in the upper limbs of healthy volunteers. These studies, employing local drug infusion, allow examination of the direct action of cromakalim on blood vessels, with minimal interference from reflex effects associated with the haemodynamic changes consequent on systemic administration.

Methods

Eight healthy male volunteers, aged 20 to 34 years (mean 27 years), each participated in two studies, which were performed with the approval of the hospital Ethics Committee and the informed consent of each volunteer, in the Clinical Pharmacology Laboratory at St George's Hospital Medical School. The first study involved measurement of forearm blood flow during local infusion of cromakalim into the left brachial artery; the second involved local infusion of cromakalim into a noradrenaline pre-constricted dorsal hand vein. On the morning of

each study subjects were admitted to the laboratory where they rested supine, at a constant room temperature of 25–26°C, the study starting when the subject had rested supine for a minimum of 45 min. Arterial and venous studies were separated by at least 3 weeks.

Local arterial infusion

Forearm blood flow was measured in both arms using venous occlusion plethysmography coupled with mercury-in-silastic strain gauges (Whitney, 1953). Collecting cuff pressure was 40 mm Hg, and wrist occlusion cuff pressure 200 mm Hg. A 27 gauge unmounted steel cannula was inserted into the left brachial artery under 1% lignocaine local anaesthesia for infusion of saline and cromakalim (Beechams, Harlow, Essex) by means of a constant-rate infusion pump (Harvard 944A; rate 0.5 ml min⁻¹).

Subjects received intra-arterial infusion of saline for 15 min, followed by cromakalim at sequential doses of 0.01, 0.1, 1.0 and 10 µg min⁻¹, each for 15 min, followed by further saline alone for 45 min (see Figure 1). Forearm blood flow was recorded for the last 5 min of each 15 min period. Flows were recorded for 10 s

Figure 1 Blood flow (ml 100 ml⁻¹ min⁻¹) in both the infused (■) and non-infused forearms (□) of eight subjects during intra-brachial artery infusion of incremental doses of cromakalim (0.01–10 µg min⁻¹), preceded, and followed by saline infusion. Values shown are mean ± s.d.

in every 15 s, and the final five measurements of each period used in analysis. Results are shown as mean $\pm$ s.d. Venous blood was drawn from an antecubital vein in the non-infused right arm, on completion of infusion of cromakalim (total dose 167 μ g), for assay of the (+) and (-) enantiomers of cromakalim (BRL 34915), BRL 38226 and BRL 38277 respectively, as described by Gill *et al.* (1988a). The biological activity of cromakalim resides primarily in the (-) enantiomer (Buckingham *et al.*, 1986).

Local venous infusion

The size of a vein on the dorsum of a hand which rested above heart level was recorded by a modification of the technique of Collier & Robinson (1974), and using a constant congesting pressure of 40 mm Hg from a cuff around the upper arm. Changes in size of the congested vein were recorded continuously by means of a light-weight lever, the tip of which was resting on the summit of the vein, and the fulcrum of which was connected to an electronic transducer (Harvard 386 smooth muscle transducer) and recorder (Electromed MX216). Measurements were made with the vein congested and, after cuff deflation, with the vein fully collapsed. The difference between these two measurements was taken as vein size. Saline or cromakalim were infused into the vein (rate 0.25 ml infusate min^{-1}) via a 23 standard wire gauge needle linked to a constant-rate infusion pump (Harvard 994A). Measurements of vein size were made between 10 and 15 mm downstream of the tip of the needle, and before the development of vein branching.

Three baseline measurements of vein size were made, separated by at least 2 min, after which noradrenaline (8–64 ng min^{-1} ; Levophed, Winthrop) was infused at a dose sufficient to cause approximately 50% constriction. This dose was then continued throughout the experiment (rate 0.25 ml infusate min^{-1}). Incremental doses of cromakalim of 0.001, 0.01, 0.1 and 1.0 μ g min^{-1} were then added, each given for 15 min. This was followed by glyceryl trinitrate (Tridil, American Hospital Supply) at 0.4 μ g min^{-1} (a dose shown previously to cause full dilatation), again for 15 min. Measurements of vein size were made at 13 and 15 min after starting each infusion, and the average value used for analysis. Results are shown as mean $\pm$ s.d.

Venous blood was drawn from an ante-cubital vein in the non-infused arm, on completion of infusion of cromakalim (total dose 17 μ g),

for assay of the (+) and (-) enantiomers of cromakalim, in one subject.

Results

Forearm study

There was a dose-related increase in blood flow in the infused forearm during local infusion of cromakalim ($P < 0.001$; Figure 1), with blood flow increasing three-fold, from 5.4 ± 2.5 ml 100 ml^{-1} forearm min^{-1} during saline infusion, to 15.1 ± 7.3 ml 100 ml^{-1} forearm min^{-1} during infusion of cromakalim at the highest dose (10 μ g min^{-1}). The lowest dose of cromakalim at which there was a significant increase in blood flow (compared with the control forearm) was 0.1 μ g min^{-1} ($P < 0.05$; Wilcoxon signed rank test). The increase in flow in the infused forearm was sustained after drug infusion was stopped, with half-time for offset of action of the order of 30 min. There was no consistent change in blood flow in the control arm during the study (Figure 1).

Plasma concentrations of BRL enantiomers 38226 and 38227, from samples taken at the end of the period of drug infusion, were 1.5 ± 0.6 and 1.3 ± 0.5 ng ml^{-1} respectively. These compare with peak plasma concentrations of 6.0 ± 5.1 and 10.2 ± 7.6 ng ml^{-1} following single daily oral cromakalim (1 mg) for 28 days.

Hand vein study

Local noradrenaline infusion (8–64 ng min^{-1}) reduced hand vein size by $56 \pm 14\%$, from 32 ± 8.5 a.u. (arbitrary units) to 15 ± 5.7 a.u. Thereafter increasing doses of cromakalim, up to a maximum of 1.0 μ g min^{-1} , produced no consistent change in size of the infused vein (Figure 2). However local glyceryl trinitrate infusion (0.4 μ g min^{-1}) produced a clear increase in vein size to 34 ± 5.7 a.u., completely reversing the effect of infused noradrenaline. This increase in vein size was highly significant, both when compared with the initial vein size during noradrenaline infusion alone, and when compared with vein size at the highest dose of cromakalim ($P < 0.001$; paired *t*-tests).

Plasma levels of the (+) and (-) enantiomers of cromakalim, obtained in one subject, were less than the lower limit of detectability of the assay (< 0.60 ng ml^{-1}).

Figure 2 Measurement of dorsal hand vein size (arbitrary units) in eight subjects. Subjects received local infusion of saline, followed by infusion of noradrenaline at the lowest dose producing close to 50% constriction ($8\text{--}64\text{ ng min}^{-1}$). Thereafter incremental doses of cromakalim ($0.001\text{--}1.0\text{ }\mu\text{g min}^{-1}$), followed by glyceryl trinitrate (GTN; $0.4\text{ }\mu\text{g min}^{-1}$), were infused simultaneous with noradrenaline. Values shown are mean $\pm$ s.d.

Discussion

The present studies show that, in man, cromakalim produces dose-dependent dilatation of forearm resistance vessels, but unlike glyceryl trinitrate, produces no dilatation in dorsal hand veins precontracted with noradrenaline. The increase in forearm blood flow was produced using a maximum dose of locally infused cromakalim of $10\text{ }\mu\text{g min}^{-1}$ (total dose $167\text{ }\mu\text{g}$). This compares with an effective oral hypotensive dose of $0.5\text{--}1.5\text{ mg}$. Doses infused in the vein study were 10-fold lower, though taking into account the blood flow in a single hand vein, which is less than a tenth of that through the forearm, the amount of cromakalim delivered to the blood vessel wall is probably greater in the venous than in the arterial study (Robinson, 1978). There were no effects on blood flow in the non-infused forearm during the arterial study, and circulating plasma concentrations of cromakalim were lower than those measured (Gill *et al.*, 1988b) during chronic systemic administration of cromakalim (1 mg). These findings appear to confirm that cromakalim, in the doses employed, produces local effects within the forearm without interference from indirect or reflex effects resulting from a systemic action of the drug.

Superficial limb veins differ from forearm resistance vessels in having no intrinsic tone (Robinson, 1983), and contraction of vascular smooth muscle in such capacitance vessels appears to depend on a rise in intracellular free calcium mediated by activation of receptor-operated rather than voltage-operated events (Bolton, 1979). In the resting state, and provided that the subject is warm, supine and relaxed, dorsal hand veins exhibit no background sympathetic tone. In this state these vessels show no response to venodilators or sympatholytics (Robinson & Collier, 1979). In order to study vasodilatation in these vessels it is necessary to induce tone. Here, using locally infused noradrenaline to produce venoconstriction (Robinson, 1978), we have shown that cromakalim, as with most other drugs shown to open potassium channels, produces no venodilatation. An exception is nicorandil, but this agent possesses nitro vasodilator properties in addition to opening K^+ channels (Coldwell & Howlett, 1987; Clapham & Wilson, 1987). This is in accord with an action of cromakalim on voltage-operated mechanisms, on the same basis that drugs which act on voltage-operated calcium-channels, such as nifedipine and verapamil, are also markedly arterioselective in their vascular activity (Robinson *et al.*, 1980).

With the assumption that responses in dorsal hand veins are representative of capacitance vessels in other vascular beds it would appear likely that cromakalim will be effective in the treatment of hypertension. In view of its arterioselective action stimulation of sympathetic activity may lead to reflex tachycardia, and it remains to be seen whether this drug will differ significantly from other agents of the same class, such as minoxidil sulphate and pinacidil, use of which has been associated with haemodynamic actions suggesting a selective arteriolar action

(Cook, 1988). It is also at present unclear whether opening of potassium channels in other tissues, such as in bronchial or uterine smooth muscle, may lead to specific advantages in the use of these agents (Cook, 1988; Hamilton & Weston, 1989; Weston & Abbot, 1987).

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