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Femoral Vasodilation to Cromakalim is Blocked by U37883A, a Non-sulphonylurea that Selectively Inhibits K_{ATP} Channels

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Abstract

The purpose of the present study was to determine the effects of U37883A, a non-sulphonylurea inhibitor of K_{ATP} channels, in the femoral vascular bed of the anaesthetized dog.

Administration of U37883A, 4-morpholinecarboxamidine-*N*-1-adamentyl-*N'*-cyclohexyl hydrochloride (2·5 mg kg^{-1}, i.v.), significantly inhibited the femoral vasodilator response to intra-femoral arterial injection of cromakalim, an activator of K_{ATP} channels. In contrast, U37883A had no effect on the femoral vasodilator responses to nitroglycerin, isoprenaline, 5-HT, or 5-carboxamidotryptamine, suggesting this agent is a novel and selective inhibitor of hindlimb vasodilation induced by K_{ATP}-channel activation.

Since U37883A did not significantly alter baseline femoral blood flow and femoral vascular resistance, the present data suggest that K_{ATP} channels do not contribute, in large measure, to regulating the canine femoral vascular bed under resting conditions in-vivo.

K_{ATP} channels have been identified in arterial smooth muscle (Cook & Hales 1984), neurons (Ashford et al 1988), skeletal muscle (Spruce et al 1984), and cardiac muscle (Noma 1983). Cromakalim, pinacidil and minoxidil are known to stimulate K_{ATP} channels, promote intracellular hyperpolarization, relax vascular smooth muscle, and dilate the systemic vascular bed (Quast & Cook 1989; Hamilton & Weston 1989; Edwards & Weston 1990). Oral sulphonylureas including glibenclamide inhibit the systemic vasodepressor and systemic vasodilator response to cromakalim and pinacidil (Cavero et al 1989). U37883A (4-morpholinecarboxamidine-*N*-l-adamentyl-*N'*-cyclohexyl hydrochloride) a non-sulphonylurea (Fig. 1), is a novel inhibitor of the K_{ATP} channel and is a reversible and competitive inhibitor of the vasorelaxant response to cromakalim in rabbit mesenteric artery (Ohrnberger et al 1993). U37883A has recently been reported to inhibit systemic vasodepressor responses to cromakalim, pinacidil and minoxidil in the rat, cat and dog without altering the systemic vasodepressor responses to nitroglycerin, isoprenaline, and nifedipine (Meisheri et al 1993). U37883A acts as a selective inhibitor of K_{ATP} channels in the systemic circulation (Meisheri et al 1993); however, the effects of U37883A in regional vascular beds in-vivo are unknown. Although a role for K_{ATP}-channel activation has been proposed for ischaemic states of skeletal muscle (Hatton et al 1991), the contribution of K_{ATP}-channel activation as a vascular control mechanism in skeletal muscle under resting conditions has received little attention.

The purpose of this study was to determine the ability of U37883A to influence vasodilator responses mediated by K_{ATP} channels in the femoral vascular bed of the adult dog. Results of the present study suggest that U37883A is a novel and selective inhibitor of femoral vasodilation induced by activation of K_{ATP} channels. The present data also suggest that K_{ATP} channels do not serve to regulate the canine femoral vascular bed in-vivo under resting conditions.

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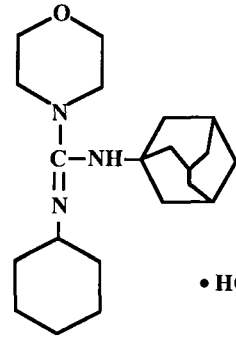
Materials and Methods

Surgical and experimental protocols

Anaesthetized, mechanically ventilated adult dogs (20–25 kg, n = 7) underwent cannulation of a brachial artery to record mean systemic arterial pressure. A foreleg vein was cannulated for administration of additional anaesthetic agents, maintenance fluid (2–3 mL kg^{-1} h^{-1} lactated Ringer's), and test agents. The common femoral artery was isolated through a groin incision, and a side-branch cannulated to



U-37883A

FIG. 1. The structure of U37883A.

Table 1. Effect of U37883A on baseline systemic arterial pressure, femoral blood flow and femoral vascular resistance.

	Mean (± s.e.) baseline systemic arterial pressure (mmHg)	Mean (± s.e.) baseline femoral blood flow (mL min^{-1})	Mean (± s.e.) baseline femoral vascular resistance (mmHg mL^{-1} min^{-1})
Before U37883A	128·1 ± 3·2	57·9 ± 4·3	2·7 ± 0·2
After U37883A	135·0 ± 2·9	66·4 ± 4·0	2·4 ± 0·2

provide access for intra-arterial injections. An ultrasonic flow probe was placed around the femoral artery to record blood flow (mL min^{-1}). Once stable baseline haemodynamic parameters were obtained, the dogs were given, in random order, bolus intra-femoral arterial injections of vasodilator agents: nitroglycerin (0·003 $\mu g\,kg^{-1}$, n = 7); cromakalim (3 $\mu g\,kg^{-1}$, n = 6); isoprenaline (1 $ng\,kg^{-1}$, n = 4); 5-HT (10 $ng\,kg^{-1}$, n = 4); and 5-carboxamidotryptamine (5-CT, 30 $ng\,kg^{-1}$, n = 7), a selective 5-HT-receptor agonist (Connor et al 1986). The maximal changes in femoral blood flow and systemic arterial pressure were recorded after each injection, and were subsequently allowed to return to baseline before the next drug was injected. U37883A (2·5 $mg\,kg^{-1}$, i.v.) was then administered over a 5–10-min interval. Five minutes later, the aforementioned agents were again injected, in the same order, into the femoral artery, and the maximal changes in femoral blood flow and mean systemic arterial pressure were recorded using the same protocol.

Drugs

Nitroglycerin (Parke-Davis, Morris Plains, NJ), isoprenaline and 5-HT (Sigma, St Louis, MO), 5-CT (Research Biochemicals International, Natick, MA) and U37883A (Upjohn, Kalamazzo, MI) were dissolved in saline. Cromakalim (SmithKline-Beecham, Pittsburg, PA) was initially dissolved in 0·3 mL ethyl alcohol and 0·3 mL 5 M hydrochloric acid. Saline was then added to make a final working solution of 1 $mg\,mL^{-1}$.

Data expression and statistical analysis

Femoral blood flow (mL min^{-1}) is expressed as the mean (± s.e.) increase in flow from baseline. Femoral vascular resistance (mmHg mL^{-1} min^{-1}), is expressed as the mean (± s.e.) decrease in resistance from baseline. Data were subjected to one way analysis of variance, and $P < 0·05$ was accepted as a significant difference.

Results

Before the administration of U37883A, baseline femoral blood flow and systemic arterial pressure values recorded immediately before the intra-femoral arterial administration of each known vasodilator agent were averaged, then compared with their respective mean baseline values obtained after giving U37883A. As seen in Table 1, while there was a slight increase in each of these parameters following peripheral intravenous administration of U37883A, these differences were not statistically significant.

Data showing the effects of U37883A on vascular responses to vasodilator agents are depicted in Table 2. Although none of these agents significantly altered mean systemic arterial pressure, cromakalim, nitroglycerin, isoprenaline, 5-CT, and 5-HT each increased femoral blood flow concomitant with a decrease in femoral vascular resistance. After peripheral intravenous administration of U37883A, the femoral vasodilator response to cromakalim was significantly reduced (from a mean increase in femoral blood flow of 165·8 ± 15·1 mL min^{-1} following cromakalim

Table 2. Effect of U37883A on regional vascular responses to known vasodilator agents.

	Change from baseline values	
	Femoral artery blood flow (mL min^{-1})	Femoral vascular resistance (mmHg mL^{-1} min^{-1})
Nitroglycerin (n = 7)		
pre-U37883A	189 ± 21·9 (223 ± 42)	2·14 ± 0·49
post-U37883A	140 ± 17·8 (238 ± 51)	1·88 ± 0·63
Isoprenaline (n = 4)		
pre-U37883A	193 ± 17·6 (88 ± 10)	2·05 ± 0·49
post-U37883A	133 ± 9·7 (111 ± 26)	1·87 ± 0·60
5-Hydroxytryptamine (n = 4)		
pre-U37883A	98·8 ± 5·2 (158 ± 29)	1·97 ± 0·41
post-U37883A	61·5 ± 9·7 (148 ± 3)	1·30 ± 0·56
5-Carboxamidotryptamine (n = 7)		
pre-U37883A	126·6 ± 15·5 (213 ± 98)	1·90 ± 0·56
post-U37883A	87·3 ± 11·8 (225 ± 814)	1·19 ± 0·27
Cromakalim (n = 6)		
pre-U37883A1	165·8 ± 15·1 (91 ± 4)	2·01 ± 0·55
post-U37883A	23·7 ± 5·9** (98 ± 7)	0·69 ± 0·31*

Values in parenthesis are from similar experiments in dogs receiving vehicle only in place of U37883A.
*$P < 0·006$, **$P < 0·0001$ compared with pre-dose values.

before U37883A infusion, to a mean increase in femoral blood flow of $23{\cdot}7 \pm 5{\cdot}9\,mL\,min^{-1}$ in response to cromakalim after U37883A infusion), femoral vascular resistance was significantly increased, and mean systemic arterial pressure was not altered. In contrast, the increased femoral blood flow and decreased femoral vascular resistance observed following intra-femoral arterial injections of nitroglycerin, isoprenaline, 5-CT, and 5-HT were not significantly altered by U37883A.

Discussion

Results of the present study demonstrate that U37883A inhibits the femoral vasodilator response to cromakalim in the anaesthetized dog. Since the femoral vasodilator responses to nitroglycerin, isoprenaline, 5-HT and 5-CT were not altered by U37883A, the present data suggest that U37883A acted in a selective manner to inhibit vasodilation in skeletal muscle induced by activation of K_{ATP} channels. The present data are consistent with recent studies demonstrating that U37883A is a novel inhibitor of vasorelaxation induced by activators of K_{ATP} channels including cromakalim (Meisheri et al 1993; Ohrnberger et al 1993). Release of endothelium-derived relaxing factor (EDRF) has been reported to be the common mediator for vasodilation to K_{ATP} channel activation in-vivo (Chang et al 1992). Since the femoral vasodilator response to 5-CT, a 5-HT agonist purported to promote vasorelaxation via EDRF release, was not altered by U37883A, the present data suggest K_{ATP} channel activation does not serve as a common intermediate step for EDRF-dependent vasodilation in skeletal muscle in-vivo. K_{ATP} channels have been reported to mediate changes in blood flow and vascular resistance in skeletal muscle (Jackson 1993).

Glibenclamide, an oral sulphonylurea which blocks K_{ATP} channels, has been reported to increase baseline arteriolar vascular tone in the hamster cheek pouch and cremaster muscles (Jackson 1993). In contrast, tetraethylammonium, an inhibitor of calcium-dependent K^+ channels, did not alter baseline arteriolar vascular tone in hamster preparations (Jackson 1993). Although this earlier work suggests that K_{ATP} channels specifically contribute to resting tone in the vascular bed of skeletal muscle, the present data do not support a role for K_{ATP} channels in the femoral vascular bed. Since U37883A did not alter baseline femoral blood flow and femoral vascular resistance at a dose that selectively inhibited the femoral vasodilator response to cromakalim, the present data suggest that K_{ATP} channels are not activated under resting conditions in the femoral vascular bed of the dog in-vivo. It is well established that under resting conditions the sympathetic tone of the blood vessels supplying skeletal muscle is typically high. Although recent data have accumulated demonstrating that activation of K_{ATP} channels modulates the release of adrenergic transmitter (Cai et al 1994), the present data suggest this action of K_{ATP} channels does not contribute, in large measure, to the regulation of femoral vasoconstrictor tone in dogs, under resting conditions. Further studies are necessary to confirm a therapeutic role of K_{ATP} channels in the femoral vascular bed under pathologic conditions, including peripheral vascular disease as suggested by others (Hatton et al 1991). However, reversal of skeletal muscle ischaemia in and of itself has been reported not to improve muscle function (Trezise et al 1993).

In conclusion, U37883A is a novel and selective inhibitor of the femoral vasodilator response to activation of K_{ATP} channels in dogs. The present data suggest that, under resting conditions, K_{ATP} channels do not contribute to the regulation of femoral haemodynamics in the dog.

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