British Journal of Pharmacology (1995) 115, 607-612

The involvement of ATP-sensitive potassium channels in β-adrenoceptor-mediated vasorelaxation in the rat isolated mesenteric arterial bed

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1 We have used the isolated buffer-perfused superior mesenteric arterial bed of the rat to assess the involvement of ATP-sensitive potassium (K_{ATP}) channels in the vasorelaxant responses to β-adrenoceptor agonists.

2 The vasorelaxant potencies of the non-selective β-adrenoceptor agonist, isoprenaline, the β_1-adrenoceptor agonist, dobutamine and the β_2-adrenoceptor agonist, terbutaline were all significantly ($P<0.05$) reduced (isoprenaline, $ED_{50}=265\pm31$ pmol v. 1.05 ± 0.42 nmol; dobutamine, $ED_{50}=294\pm67$ pmol v. 497 ± 115 pmol; terbutaline, $ED_{50}=157\pm26$ nmol v. 452 ± 120 nmol) in the presence of the K_{ATP}-channel blocker, glibenclamide.

3 The presence of glibenclamide only weakly influenced the vasorelaxant properties of salbutamol, a β_2-adrenoceptor agonist, while those of verapamil, a β-adrenoceptor-independent vasorelaxant, were unaffected.

4 In radioligand binding experiments, glibenclamide (1 nM-100 μM) did not displace any specific [^{3}H]-dihydroalprenolol binding from rat β-adrenoceptors. Therefore, glibenclamide does not bind to β-adrenoceptors at the concentration used in the present investigation.

5 Vasorelaxant responses to dibutyryl cyclic AMP, the cell permeable analogue of cyclic AMP, were also unaffected by glibenclamide, indicating that the coupling of β-adrenoceptors to K_{ATP}-channels occurs independently of the elevation of intracellular cyclic AMP.

6 We have shown that a significant element of the vasorelaxant responses to both β_1- and β_2-adrenoceptor activation involves the opening of K_{ATP}-channels. In conclusion, K_{ATP}-channels may play a physiological role in β-adrenoceptor-mediated vasodilatation.

Keywords: ATP-sensitive potassium channels (K_{ATP}-channels); glibenclamide; mesenteric arterial bed; β-adrenoceptors; isoprenaline; salbutamol; dobutamine; terbutaline; dibutyryl cyclic AMP

Introduction

The movement of potassium ions across the cytoplasmic membrane is an important determinant of membrane potential and consequently, cellular activity in excitable tissue such as vascular smooth muscle. Recently, interest has focused on ATP-sensitive potassium channels (K_{ATP}-channels) which are regulated by purine derivatives associated with cellular metabolism (Nichols & Lederer, 1991). Pharmacologically, these channels are known to be the site of action of a novel class of vasodilators, known as potassium channel activators (Edwards & Weston, 1990). These channels are also the site of action of endogenous vasoactive mediators including calcitonin gene-related peptide (Nelson *et al.*, 1990), prostanoids (Bouchard *et al.*, 1994) and adenosine (Kirsch *et al.*, 1990; Merkel *et al.*, 1992; Dart & Standen, 1993). Recent interest has focused on the regulation of vascular tone by K_{ATP}-channels, and in this respect Jackson (1993) has reported that these channels are important regulators of basal microvascular tone in both the hamster cheek pouch and cremaster muscle. Furthermore, Jackson found that, in the cheek pouch, the blockade of K_{ATP}-channels with the sulphonylurea, glibenclamide results in reduced vasodilator responses to adenosine, a prostacyclin analogue and also the non-selective β-adrenoceptors agonist isoprenaline. This raises the possibility that K_{ATP}-channels are involved, at least in part, in mediating vasodilatation to a diverse range of agents. These findings may accord with recent reports that glibenclamide antagonizes relaxant responses to isoprenaline in the mouse ileum (Yeung *et al.*, 1994) and rat aortic rings (Hüsken *et al.*, 1994), while recent electrophysiological evidence has demonstrated that isoprenaline causes hyperpolarization of the canine saphenous vein which is sensitive to glibenclamide (Nakashima & Vanhoutte, 1995). Furthermore, studies on both guinea-pig and bovine isolated trachealis muscle indicate that β-adrenoceptor agonists may activate potassium channels leading to hyperpolarization, which contributes towards the relaxant effects of these agents (Cook *et al.*, 1993; Chiu *et al.*, 1993). Such a hyperpolarizing action of isoprenaline, via potassium channels, has previously been identified in rat myometrial muscle (Kroeger & Marshall, 1973). Furthermore, recent electrophysiological evidence from cat ventricular myocytes has demonstrated that β-adrenoceptors are coupled to the activation of K_{ATP}-channels (Schackow & Ten Eick, 1994). Such findings have challenged the traditional view that β-adrenoceptors are solely coupled to adenylate cyclase and adenosine 3':5'-cyclic monophosphate (cyclic AMP) formation (Torphy, 1994). In addition Gardiner *et al.* (1991a,b) have reported that in the conscious rat, β-adrenoceptor activation is linked to the release of nitric oxide, indicating an alternative, endothelium-dependent, pathway of vasodilatation.

The present investigation was intended to determine whether K_{ATP}-channels contribute towards β-adrenoceptor-mediated vasodilator responses in the isolated perfused superior mesenteric arterial bed of the rat. Specifically, the vasodilator responses to various β-adrenoceptor agonists have been compared in the absence and presence of the K_{ATP}-channel blocker glibenclamide (Sturgess *et al.*, 1985).

A preliminary account of part of this work was communicated to the December 1994 meeting of the British Pharmacological Society (Randall, 1995).

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Methods

Preparation of the isolated buffer-perfused superior mesenteric arterial bed

Male Wistar rats (200–350 g; Bantin & Kingman, Hull, Humberside) were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.: Sagatal, Rhône Mérieux, Harlow, Essex) and following a mid-line incision the superior mesenteric artery was cannulated. The arterial vasculature was dissected away from the guts and placed in a jacketed organ bath as previously described (Randall & Hiley, 1988) and perfused at 5 ml min^{-1} with gassed (95% O_2/5% CO_2) Krebs-Henseleit solution (containing (mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, $CaCl_2$ 2 and D-glucose 10).

Experimental protocol

Perfusion pressure in the superior mesenteric arterial bed was continuously monitored by a pressure transducer coupled to a MacLab 4e recording system (ADInstruments, New South Wales, Australia). Following a 30 min equilibration period, methoxamine (10 μM) was added to the perfusion fluid to increase vascular tone. Once stable tone had been established, bolus doses of the vasodilator agents were administered close-arterially in random order and in volumes less than 100 μl. In order to assess the influence of K_{ATP}-channels on the vasodilator responses, glibenclamide was added to the perfusion fluid to achieve a concentration of 10 μM, in order to block these channels selectively (Randall & Griffith, 1993). Following a further 30 min it was found necessary to add more methoxamine (20–100 μM) to restore vascular tone to a level comparable to that found in the absence of the sulphonylurea. The vasodilator activities of the agents were then assessed in the same preparation.

Radioligand binding studies

Rat cerebral cortex was homogenized in 10 volumes of ice-cold 50 mM Tris-HCl buffer (pH 8.0 at 25°C) with a polytron disruptor for 30 s on ice. The homogenate was centrifuged at 38,000 *g* (MSE Europa 24) for 10 min at 4°C. The supernatant was discarded and the pellet resuspended and re-centrifuged. The washing procedure was repeated once more and the final pellet was re-suspended to a concentration of 75 mg ml^{-1} (original wet weight of tissue) in 50 mM Tris-HCl buffer (pH 8.0 at 25°C). The homogenate was stored in 2 ml aliquots at −30°C and was thawed immediately prior to the binding assay.

Portions (50 μl) of the homogenate were added, in triplicate, to polystyrene tubes containing 0.2 nM [^{3}H]-dihydroalprenolol ([^{3}H]-DHA) in 50 mM Tris-HCl (pH 8.0 at 25°C). The total volume of the assay was 1 ml. Non-specific binding was defined by the presence of 10 μM (±)-propranolol. Stock solutions (0.1 mM) of glibenclamide were prepared in dimethylsulphoxide. These were diluted in assay buffer immediately before use such that 50 μl aliquots were added to the assay. Isoprenaline was diluted in Tris-HCl buffer. The assay was conducted by incubating the membrane suspension at room temperature for 30 min. The reaction was terminated by rapid filtration over glass fibre filter using a Brandel cell harvesting apparatus. Scintillation cocktail (5 ml) (Packard Scintillator Plus) was added to the filters and after soaking overnight at room temperature, radioactivity was quantified in a LKB rack beta scintillation counter.

Data and statistical analysis

All data are given as the mean ± s.e.mean and were compared by either paired or unpaired Student's *t* tests as appropriate. ED_{50} values for vasodilator responses were obtained from individual dose-response curves as the dose at which the half-maximal relaxant response occurred. These variables were determined by fitting the data to the logistic equation:

$$R = \frac{R_{max} \times A^{n_H}}{ED_{50}^{n_H} + A^{n_H}}$$

where R is the reduction in tone, A the dose of vasorelaxant, R_{max} the maximum reduction of established tone, n_H the slope function and ED_{50} the dose of the vasorelaxant giving half the maximal relaxation. The curve fitting was carried out using KaleidaGraph software (Synergy, Reading, PA, U.S.A.) running on a Macintosh LC III computer. The ED_{50} values were converted to the logarithmic values for statistical analysis.

Drugs

All solutions were prepared on the day of the experiment. (−)-Isoprenaline bitartrate, salbutamol, terbutaline hemisulphate, (±)-propranolol, (±)-verapamil hydrochloride, dibutyryl cyclic AMP and methoxamine hydrochloride were obtained from the Sigma Chemical Company, Poole, U.K., glibenclamide was obtained from Research Biochemicals Incorporated, Natick, MA, U.S.A., dobutamine hydrochloride ('Dobutrex') was obtained from Ely Lilly and Co, Basingstoke U.K. and [^{3}H]-dihydroalprenolol (specific activity 55 Ci $mmol^{-1}$) was obtained from Amersham PLC, U.K. Isoprenaline and salbutamol were dissolved in 0.1 M HCl and then diluted in 0.5 mM ascorbic acid, while terbutaline was dissolved, and further diluted, in 0.5 mM ascorbate. Verapamil and dibutyryl cyclic AMP were dissolved in absolute ethanol and then diluted in 0.9% saline. Glibenclamide was dissolved in dimethylsulphoxide (DMSO) as a 0.2 M stock solution and the final concentration of DMSO in the perfusion fluid was <0.005% (v/v). All other drugs were then diluted to the required concentrations in Krebs-Henseleit solution.

Results

Basal perfusion pressures and established tone

In the 49 preparations used in the present investigation basal perfusion pressure was 22.2 ± 1.8 mmHg. Following the addition of 10 μM methoxamine, tone was raised by 98.6 ± 3.9 mmHg. After the addition of 10 μM glibenclamide, tone was significantly reduced by 23.6 ± 2.2 mmHg but supplementary addition of methoxamine 20–100 μM restored the level of established tone to 78.3 ± 4.1 mmHg above basal perfusion pressure.

Effect of glibenclamide on vasorelaxant responses to isoprenaline

The dose-response curve for the vasodilator effects of isoprenaline (8.3 pmol–27.7 nmol) under control conditions is shown in Figure 1 and is described by an ED_{50} of 265 ± 31 pmol and a maximum relaxation of tone (R_{max}) of 73.7 ± 4.1% (n = 8). In the presence of glibenclamide the dose-response curve for the vasorelaxant effects of isoprenaline was significantly (P < 0.05) shifted to the right and the ED_{50} was 1.05 ± 0.42 nmol and the R_{max} was 64.8 ± 6.1%.

Effect of glibenclamide on vasorelaxant responses to dobutamine

In 12 different preparations, dobutamine (8.8 pmol–30 nmol) gave rise to dose-related relaxations of tone (ED_{50} = 294 ± 67 pmol and R_{max} = 109 ± 2%) (Figure 2a). In the presence of glibenclamide the dose-response curve was significantly (P < 0.05) shifted to the right (ED_{50} = 497 ± 115 pmol and the R_{max} = 106 ± 4%).

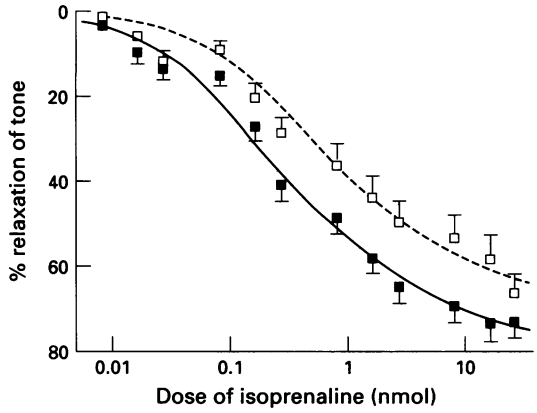

Figure 1 Dose-response curves for the relaxation of established tone in rat isolated perfused superior mesenteric arterial bed by isoprenaline in the absence (■) and presence (□) of 10 μM glibenclamide (both $n=8$). Values are shown as mean ± s.e.mean.

Effect of glibenclamide on vasorelaxant responses to terbutaline

In the 9 preparations to which it was added, terbutaline (10 nmol–22 μmol) gave rise to dose-dependent relaxations of the methoxamine-induced tone ($ED_{50}=157\pm26$ nmol and $R_{max}=100\pm2\%$). Following the addition of glibenclamide the dose-response curve for the relaxant effects of terbutaline was significantly ($P<0.05$) shifted to the right and the ED_{50} was 452 ± 120 nmol and the R_{max} was $99.1\pm2.1\%$ (Figure 2b).

Effect of glibenclamide on vasorelaxant responses to salbutamol

Salbutamol (1.25 nmol–12.6 μmol) caused dose-related relaxations of established tone described by $ED_{50}=142\pm37$ nmol and $R_{max}=96.7\pm4.0\%$ ($n=7-11$). The vasorelaxant effects of salbutamol were unaffected by the addition of glibenclamide ($ED_{50}=237\pm87$ nmol and $R_{max}=86.4\pm4.6\%$) (Figure 2c). However, statistical analysis revealed that at the 1.25 μmol dose, the vasorelaxant response was significantly ($P<0.05$) depressed in the presence of the sulphonylurea ($53.4\pm6.6\%$ v. $77.2\pm3.0\%$).

Effect of glibenclamide on vasorelaxant responses to dibutyryl cyclic AMP

In the 9 preparations to which it was added, dibutyryl cyclic AMP (20 nmol–6.1 μmol) caused dose-dependent relaxations of tone and over the dose-range studied these responses were not affected by the presence of glibenclamide (Figure 3).

Effect of glibenclamide on vasorelaxant responses to verapamil

Verapamil (20 pmol–61 nmol) caused dose-related relaxations of tone ($ED_{50}=1.30\pm0.35$ nmol and $R_{max}=81.7\pm2.9\%$) (Figure 4). Following addition of glibenclamide the vasorelaxant properties of the calcium channel blocker were unaffected ($ED_{50}=1.24\pm0.27$ nmol and the $R_{max}=86.3\pm5.5\%$, all $n=4$).

[^{3}H]-dihydroalprenolol binding assay

Non-specific binding represented $39\pm1\%$ ($n=3$) of total [^{3}H]-DHA binding. A range of concentrations of glibenclamide (1 nM–100 μM) did not displace any [^{3}H]-DHA binding to the rat cerebro-cortical membranes (Figure 5) ($P>0.05$, $n=3$). Under identical experimental conditions, isoprenaline completely displaced all specific [^{3}H]-DHA binding with an IC_{50} of 900 nM.

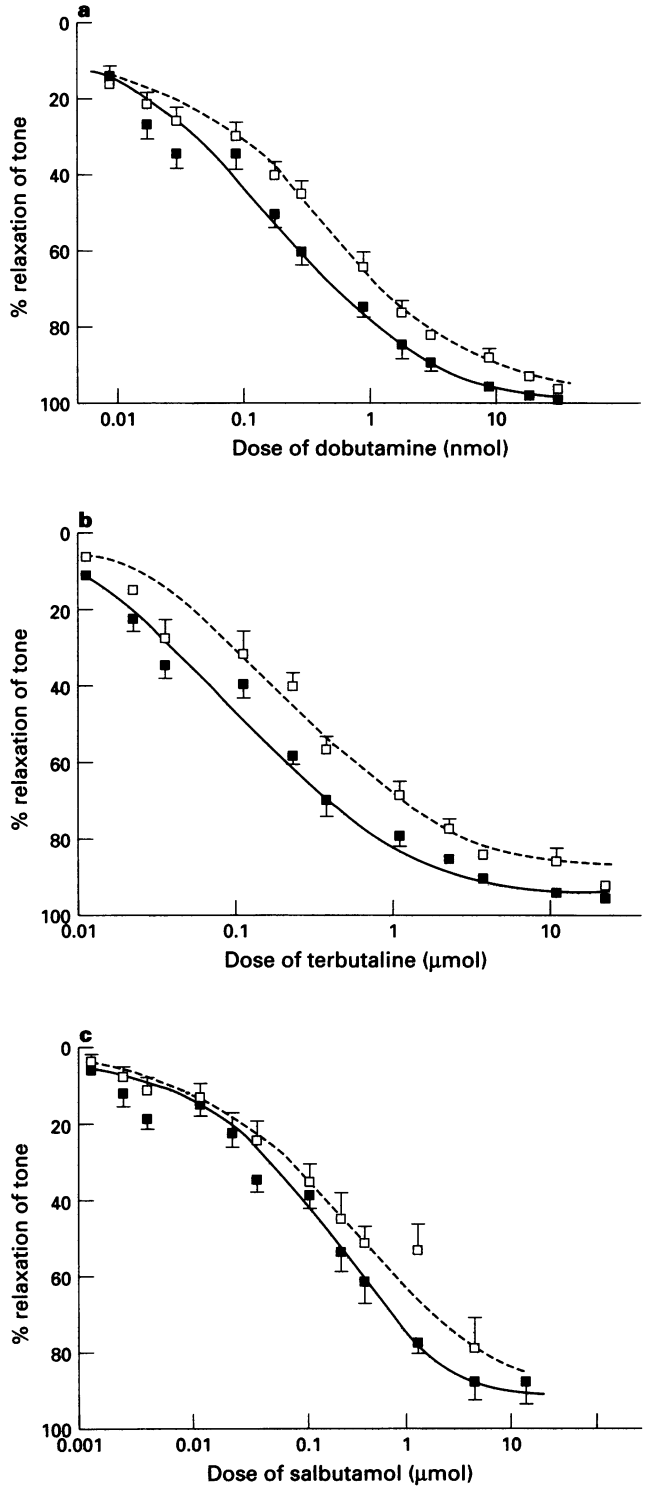

Figure 2 Dose-response curves for the relaxation of methoxamine-induced tone in rat isolated perfused superior mesenteric arterial bed by (a) dobutamine ($n=12$), (b) terbutaline ($n=9$) and (c) salbutamol ($n=7-11$) in the absence (■) and presence (□) of 10 μM glibenclamide. Values are shown as mean ± s.e.mean.

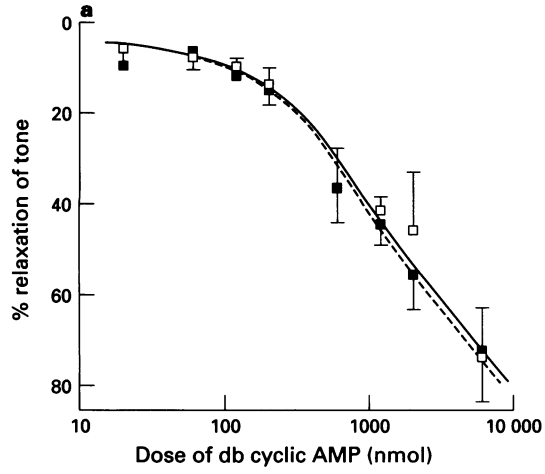

Figure 3 Dose-response curves for the relaxation of methoxamine-induced tone in rat isolated perfused superior mesenteric arterial bed by dibutyryl cyclic AMP in the absence (■) and presence (□) of 10 μM glibenclamide (both $n=9$). Values are shown as mean ± s.e.mean.

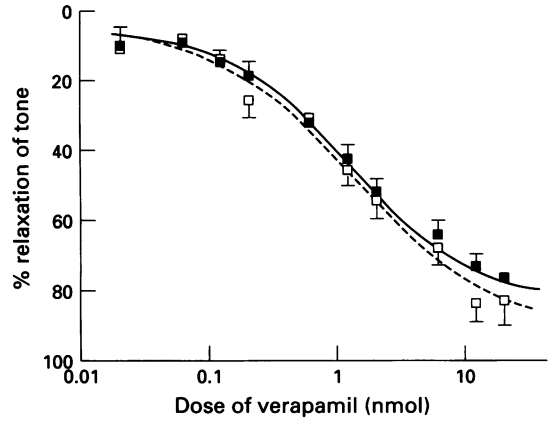

Figure 4 Dose-response curves for the relaxation of methoxamine-induced tone in rat isolated perfused superior mesenteric arterial bed by verapamil in the absence (■) and presence (□) of 10 μM glibenclamide (both $n=4$). Values are shown as mean ± s.e.mean.

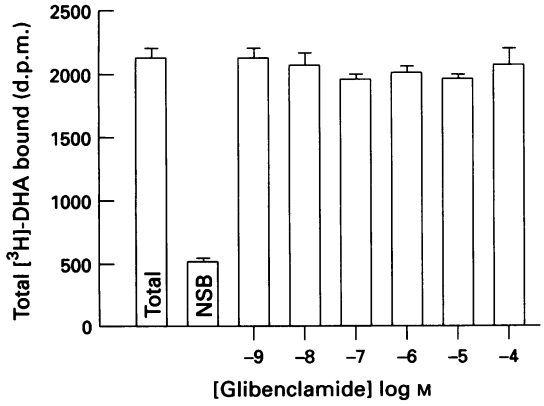

Figure 5 Failure of glibenclamide to compete with [^{3}H]-dihydroalprenolol ([^{3}H]-DHA) binding to a rat brain total particulate fraction. The figure shows data (mean ± s.e.mean) from a single experiment conducted in triplicate. The data represent [^{3}H]-DHA bound per assay (each assay tube contained approximately 250 μg protein) expressed as disintegrations per minute (d.p.m.). Total = binding in the absence of any competing agent and NSB (non-specific binding) = binding in the present of 10 μM propranolol. Glibenclamide did not significantly affect [^{3}H]-DHA binding in two additional experiments.

Discussion

The results of the present study clearly point to an interaction between β-adrenoceptor activation and K_{ATP}-channels. This interaction contributes towards the vasodilator actions of β-adrenoceptor agonists, but would appear to play a supporting rather than a central role in these responses.

In the present investigation the vasodilator potency of isoprenaline, a non-selective β-adrenoceptor agonist, was significantly reduced by addition of glibenclamide which selectively blocks vascular K_{ATP}-channels (Standen *et al.*, 1989). This observation raised the possibility that β-adrenoceptor activation is coupled to the opening of these potassium channels and accords with reports that glibenclamide reduces responses to isoprenaline in both the hamster cheek pouch (Jackson, 1993) and rat aortic rings (Hüsken *et al.*, 1993). Furthermore, a recent study on the rat basilar artery *in vivo* has demonstrated that noradrenaline acts via β_1-adrenoceptors to cause glibenclamide-sensitive vasodilatation (Kitazono *et al.*, 1993). The coupling of β-adrenoceptors to potassium currents has also been reported in non-vascular tissues including pulmonary smooth muscle, where the responses are linked to calcium-activated potassium channels (Cook *et al.*, 1993; Chiu *et al.*, 1993), and cardiac tissue, where the receptors are coupled to K_{ATP}-channels (Schackow & Ten Eick, 1994). Evidence is therefore accumulating to indicate the involvement of K_{ATP}-channels in β-adrenoceptor-mediated responses.

In the present investigation vasorelaxant responses to the selective β_1-adrenoceptor agonist, dobutamine, and the β_2-adrenoceptor agonist, terbutaline, were also influenced by the addition of glibenclamide. These observations would therefore seem to imply that both β_1 and β_2-adrenoceptors are coupled to the activation of K_{ATP}-channels. This finding contrasts with the observation that only β_1 and not β_2-adrenoceptor-mediated vasodilator responses are coupled to K_{ATP}-channels in the canine coronary vasculature (Narishige *et al.*, 1994). However, in the guinea-pig trachealis preparations, both β_1 and β_2-adrenoceptors are similarly coupled to hyperpolarization (Cook *et al.*, 1993), while in canine saphenous vein smooth muscle only β_2 and not β_1-adrenoceptors appear coupled to K_{ATP}-channel activation (Nakashima & Vanhoutte, 1995).

In the present investigation both a selective β_1- and a β_2-adrenoceptor agonist evoked glibenclamide-sensitive responses but why the β_2-adrenoceptor agonist salbutamol was only weakly influenced is at present unclear. However, this observation parallels the finding in guinea-pig trachealis smooth muscle that isoprenaline, salbutamol, procaterol (a β_2-selective agonist) but not salmeterol (also β_2-selective) activate potassium channels, and the authors ascribe this apparent heterogeneity to salmeterol having a low intrinsic efficacy at β_2-adrenoceptors (Cook *et al.*, 1993). Hence, the participation of K_{ATP}-channels in vasodilator responses may also be agonist- rather than subtype-specific.

The findings from the present investigation point to the involvement of K_{ATP}-channels in β-adrenoceptor-mediated vasorelaxation. By contrast, in human bronchial tissue the relaxant effects of isoprenaline are unaffected by the blockade of K_{ATP}-channels. However, they are sensitive to charybdotoxin, implicating the involvement of large conductance calcium-activated potassium channels (Miura *et al.*, 1992). Hence there may be species and tissue differences in the way in which β-adrenoceptors are coupled to potassium conductances.

An alternative explanation to the above interaction between glibenclamide and β-adrenoceptor agonists could reflect sulphonylurea-binding at adrenoceptors (Cherksey & Altsulzer, 1984). However, in the present investigation glibenclamide, up to 100 μM, did not influence the binding of [^{3}H]-dihydroalprenolol to rat β-adrenoceptors. A non-selective inhibition of vasodilatation by glibenclamide may also be ruled out as vasorelaxant responses to verapamil, a calcium antagonist which acts independently of K_{ATP}-channels, were unaffected by glibenclamide. However, given the consistent reductions in α-

adrenergic tone experienced on addition of glibenclamide it cannot be ruled out that this agent may act as an α-adrenoceptor antagonist in this preparation (Cherksey & Altsulzer, 1984).

How are β-adrenoceptors linked to the activation of K_{ATP}-channels? It is well established that β-adrenoceptors are coupled, via a G-protein, to the activation of adenylate cyclase and a rise in intracellular cyclic AMP. There is also evidence to indicate that protein kinase A links receptor activation to channel opening (Ribalet *et al.*, 1989). In this respect the catalytic subunit of protein kinase A is known to activate glibenclamide-sensitive potassium currents in rabbit mesenteric arterial vascular smooth muscle (Qualye *et al.*, 1994). However, in the present investigation, and in the hamster cheek pouch (Jackson, 1993), K_{ATP}-channel blockade does not influence the vasodilator responses to dibutyryl cyclic AMP, the cell permeable analogue of cyclic AMP. This implies that cyclic AMP is not involved in the activation of K_{ATP}-channels and accords with the observation that relaxant responses due to the adenylate cyclase activator, forskolin, are similarly independent of K_{ATP}-channel activation (Jackson, 1993; Bouchard *et al.*, 1994). Similarly, in cat ventricular myocytes the coupling of β-adrenoceptors and K_{ATP}-channels is unaffected by protein kinase A inhibition (Schackow & Ten Eick, 1994). The precise mechanisms underlying the coupling between receptor and channel activation are not clear from the present investigation, although it is clear that it is independent of cyclic AMP. The activation of K_{ATP}-channels is known to be linked to membranous G-proteins (Brown & Birnbaumer, 1988) and patch clamp studies on guinea-pig ventricular myocytes have shown that both adenosine and acetylcholine may open these channels in a G-protein-dependent manner (Terzic *et al.*, 1994). It is therefore possible that β-adrenoceptor coupling to K_{ATP}-channels is mediated via G-proteins.

The interaction between β-adrenoceptors and K_{ATP}-channels may perhaps account for a previous report of an interaction between these systems. Specifically, salbutamol induces one-way cross tolerance to the potassium channel opener cromakalim in the rat uterus (Downing & Hollingsworth, 1992), with the likely site of interaction being the K_{ATP}-channel.

The findings of the present investigation clearly link a significant element of β-adrenoceptor-mediated vasodilator responses to the activation of K_{ATP}-channels. These findings not only add to the accumulating evidence that β-adrenoceptors are linked to systems other than adenylate cyclase but also to the range of mechanisms by which β-adrenoceptor agonists cause vasodilatation in the cardiovascular system. The observation that β-adrenoceptors are coupled to the activation of K_{ATP}-channels increases the number of endogenous mediators which may interact with these channels and contribute towards vascular regulation in this manner. These findings accordingly increase our knowledge of the physiological significance of K_{ATP}-channels.

This work was funded by a project grant from the British Heart Foundation (PG94060).

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(Received December 14, 1994
Revised March 9, 1995
Accepted March 10, 1995)