ELSEVIER

European Journal of Pharmacology 287 (1995) 313–316

ejp

Short communication

Relaxant effect of levcromakalim in isolated human small subcutaneous arteries

Birgitta C.P. Hüsken [a,*], Margreet B. Vroom [b], Erik-Jan F. Timmenga [c], Martin Pfaffendorf [a], Pieter A. Van Zwieten [a]

[a] *Department of Pharmacotherapy, University of Amsterdam, Academic Medical Centre, Meibergdreef 15, NL-1105 AZ Amsterdam, Netherlands*
[b] *Department of Anaesthesiology, University of Amsterdam, Academic Medical Centre, Meibergdreef 15, NL-1105 AZ Amsterdam, Netherlands*
[c] *Department of Plastic Reconstructive Surgery, University of Amsterdam, Academic Medical Centre, Meibergdreef 15, NL-1105 AZ Amsterdam, Netherlands*

Received 27 July 1995; revised 23 October 1995; accepted 24 October 1995

Abstract

The effect of levcromakalim, an ATP-sensitive K^+ channel opener, on isolated subcutaneous arteries from mammary tissues obtained from female patients undergoing reconstructive breast surgery was investigated. The small arteries were preserved in the University of Wisconsin (UW) solution. The contractile responses to K^+ and 9,11-dideoxy-11α,9α-epoxy-methano-prostaglandin $F_{2\alpha}$ (U46619) and the relaxant responses to levcromakalim and to the endothelium-dependent vasodilator, methacholine, in these arteries remained fully intact after preservation in UW solution for at least 5 days. The pD_2 value and maximal relaxation obtained from the concentration-response curve of levcromakalim ($n = 7$) were 5.78 ± 0.23 and $81 \pm 6\%$, respectively. The vasodilator effect of levcromakalim was significantly antagonised by the ATP-sensitive K^+ channel blocker, glibenclamide (1 and 3 μM). In conclusion, isolated human arteries contain ATP-sensitive K^+ channels, which can be modulated by K^+ channel openers and blockers. Subcutaneous small arteries, as used in our experiments, appear to be very suitable for pharmacological experiments.

Keywords: Levcromakalim; Glibenclamide; Artery, subcutaneous; (Human)

1. Introduction

ATP-sensitive K^+ (K_{ATP}) channels have been identified in heart (Noma, 1983), pancreatic β-cells (Cook and Hales, 1984) and in skeletal and vascular smooth muscle (Standen et al., 1989). These channels are blocked by antidiabetic sulfonureas such as glibenclamide and are activated by a class of pharmacological agents known as K^+ channel openers which belong to a new category of vasodilator agents. Well-known examples of K_{ATP} channel openers are compounds such as cromakalim, pinacidil, nicorandil, aprikalim, diazoxide, minoxidil sulphate and their analogs (Quast and Cook, 1989).

The hyperpolarization and subsequent vasodilation induced by these K_{ATP} channel openers have been investigated thoroughly in different vascular beds and in different animal species, both in vitro and in vivo (for review see Challinor-Rogers and McPherson, 1994). In humans, cromakalim antagonised the changes in total peripheral resistance induced by noradrenaline and angiotensin II (Van Nguyen et al., 1991). In normotensive patients, cromakalim produced a reflex increase in heart rate without any detectable decrease in blood pressure (Singer et al., 1989; Donnelly et al., 1990). In hypertensive patients, the blood pressure decreased significantly after treatment with cromakalim (Singer et al., 1989). In hypertensive patients treated with atenolol, cromakalim caused a further reduction in blood pressure (Donnelly et al., 1990).

However, the vasodilator effects of K_{ATP} channel openers in isolated human arteries have been little examined.

In the present study we investigated the small subcutaneous arteries from mammary tissues obtained

* Corresponding author. Tel.: +31.20.5664811; fax: +31.20.6968704.

SSDI 0014-2999(95)00714-8

from female patients undergoing reconstructive breast surgery. The aim of the present study was to examine the influence of glibenclamide on the vasodilator action in these arteries of levcromakalim, the active (−)-enantiomer of cromakalim, which acts selectively by opening of ATP-sensitive K^+ channels.

2. Materials and methods

2.1. Isolated human subcutaneous artery preparations

The study was approved by the Ethical Committee of the Academic Medical Centre, and all patients gave informed consent before entering the study.

Small subcutaneous arteries were obtained from 7 healthy female patients undergoing reconstructive breast surgery under general anaesthesia (age range: 16–56 years, mean ± S.E.M.: 26 ± 5 years). None of the patients was receiving chronic drug therapy. The breast tissue was immediately placed in a physiological salt solution (PSS) of the following composition (mM): NaCl (136); KCl (2.5); $MgCl_2$ (0.5); $CaCl_2$ (1.8); NaH_2PO_4 (0.42); $NaHCO_3$ (11.9) and glucose (5.5). After removal of the skin, segments of subcutaneous arteries were dissected and preserved for maximally 5 days in the University of Wisconsin (UW) solution at 4°C. The composition of the UW solution has been described elsewhere in detail (Southard et al., 1990).

In each experiment a segment (length: approximately 2 mm) of an artery was dissected of free superficial fat and loose connective tissue in PSS at 4°C. Subsequently, a 40-μm diameter stainless-steel wire was inserted into the arterial lumen and the preparations were mounted in an isometric wire myograph according to Mulvany and Halpern (1977). The vessels were fixed to a micrometer screw and, after insertion of a second wire, to an isometric force transducer. The preparations were equilibrated for 15 min in PSS at 37°C which was oxygenated with carbogen (95% O_2, 5% CO_2), at a pH of 7.4. Subsequently, the ratio between passive wall tension and internal circumference was determined by a normalisation procedure (Mulvany and Halpern, 1977). The internal circumference was adjusted to a value which equaled 90% of the diameter at an intraluminal pressure of 100 mm Hg (13.3 kPa). This value was then divided by π to obtain the normalised internal lumen diameter.

2.2. Experimental protocol

After an equilibration period of 15 min the preparations were subjected for 5 min to a depolarizing PSS (containing 120 mM K^+), thus causing contraction of the vascular smooth muscle. In this high-K^+ solution 117.5 mM NaCl had been replaced by KCl. After a 20-min equilibration interval, 5-min periods of vasoconstriction were provoked by the thromboxane A_2 receptor agonist, 9,11-dideoxy-11α,9α-epoxy-methano-prostaglandin $F_{2\alpha}$ (U46619) (1 μM). On top of this vasoconstriction, methacholine (10 μM) was added to induce endothelium-dependent vasodilation, followed after 20 min of equilibration by exposure to high-K^+ PSS for 5 min.

Twenty minutes after the last K^+-induced contraction, the human arteries were incubated for 1 h with 0, 0.1, 1 and 3 μM glibenclamide, respectively. Subsequently, the preparations were exposed to 1 μM U46619, which produced a nearly maximal response. After the contraction had reached its equilibrium, increasing doses of levcromakalim were added to the bath in order to induce relaxation. Concentration-response curves made in drug-treated preparations were compared to those obtained in control vessels on the basis of pD_2, E_{max} and slope (Hill coefficient) values.

2.3. Drugs

(Acetyl-β) methacholine chloride and U46619 (9,11-dideoxy-11α,9α-epoxy-methano-prostaglandin $F_{2\alpha}$) were obtained from Sigma (St. Louis, MO, USA). Levcromakalim was kindly donated by SmithKline Beecham (Surrey, UK) and glibenclamide was purchased from Hoechst (Frankfurt am Main, Germany). All drugs, except glibenclamide, were dissolved in distilled water before being added to the PSS containing organ bath. Glibenclamide was dissolved in 100% dimethyl sulfoxide (DMSO) and subsequently diluted to a stock solution of 10^{-2} M, this stock solution was diluted with 50% DMSO to the concentrations 10^{-3} M and 10^{-4} M, after which they were added to the organ bath. The vehicle did not affect the results, as demonstrated in separate control experiments.

2.4. Evaluation of data

Using a computer program (GraphPad, Institute for Scientific Information, USA), concentration-response curves based on the relationship

$$E = E_{max} \cdot A^P \cdot \left(A^P + EC_{50}^P\right)^{-1}$$

were fitted to log concentration-response data of individual experiments.

In the equation, E is the response obtained at a given concentration A, E_{max} is the maximally attainable response, EC_{50} is the concentration required to induce the half-maximal effect, and the exponent P describes the slope of the relationship (Hill coefficient).

The pD_2 is defined as the negative logarithm of the EC_{50} value ($-\log [EC_{50}]$).

The maximal effects (E_{max}) of the vasodilator agents

were calculated as the percentage relaxation of the vascular tone induced by 1 μM U46619.

The parameters of the concentration-response curves of at least 6 individual experiments were averaged. The data shown are means ± S.E.M. Statistical significance was established by means of an unpaired, double-sided Student's *t*-test. A *P* value of less than 0.05 was considered significant.

3. Results

The optimal lumen diameter of the isolated human subcutaneous arteries amounted to 428 ± 51 μm (n = 26). Accordingly, the vessels studied should be classified as small arteries.

The experimental protocol is shown in Fig. 1, with the contractile response to 120 mM K^+ PSS and 1 μM U46619. The same figure shows the relaxant actions of the endothelium-dependent vasodilator, methacholine (10 μM), as well as those of levcromakalim. The same responses were obtained after preservation of the arteries in UW solution for up to 5 days.

The concentration-dependent effect of levcromakalim allowed us to make a concentration-response curve from which values for the following parameters were calculated (n = 7): pD_2 value = 5.78 ± 0.23; E_{max} = 81 ± 6%; Hill coefficient = −2.9 ± 0.4.

Glibenclamide (0.1, 1 and 3 μM) influenced neither the basal vascular tone nor the effects induced by 1 μM U46619, which was used for precontraction of the vessels.

The vasodilator effect of levcromakalim was significantly antagonised by glibenclamide (1 and 3 μM), but not by a low concentration of 0.1 μM glibenclamide (Fig. 2). The concentration-response curve of levcromakalim was significantly decreased from a concentration of 10^{-6} M levcromakalim onwards, in the presence of 1 and 3 μM glibenclamide when compared to the concentration-response curve obtained without glibenclamide.

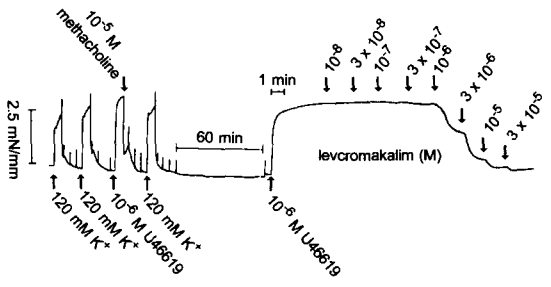

Fig. 1. Recorder tracing of the experimental protocol followed for an isolated human subcutaneous artery with an optimal lumen diameter of 457 μm. After two contractions induced by 120 mM K^+ a contraction was elicited with 1 μM U46619, followed directly by a vasodilator response induced by 10^{-5} M methacholine. Twenty minutes after another contraction elicited by 120 mM K^+ the artery was equilibrated for 60 min. Subsequently, 10^{-6} M U46619 (thromboxane A_2 agonist) was added to induce a precontraction. When maximal contraction had been achieved a concentration-response curve for the vasodilator response to levcromakalim was obtained. The spikes represent the brief periods of wash-out.

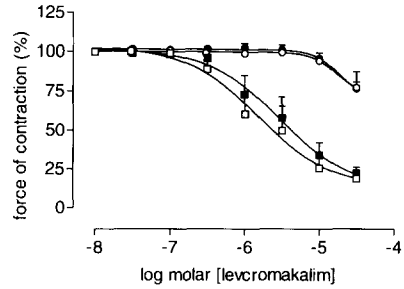

Fig. 2. Concentration-response curves for the effects of 0 (□), 0.1 (■), 1 (○) and 3 (●) μM glibenclamide on the dilator response curves of levcromakalim in isolated human subcutaneous arteries. The preparations were precontracted with 1 μM U46619.

4. Discussion

Aalkjær et al. (1987) investigated artery segments from biopsies of skin and subcutaneous tissue taken under local anaesthesia from the gluteal region of normotensive controls and hypertensive patients. To our knowledge human small subcutaneous artery preparations from the mammary region have been little studied in vitro. To us this preparation, obtained from healthy women not subjected to any chronic drug treatment, seemed attractive, since it consists of small arteries which can be obtained readily without a biopsy. This is a great advantage, it is less inconvenient for the patients and the amount of tissue obtained from breast surgery is much greater than that obtained from a biopsy. This preparation is limited by the fact that only arteries from healthy women can be investigated, but it can be used successfully in vitro for pharmacological studies concerning vasoactive agents. The arteries were taken from the subcutaneous bed, which like all vascular beds has a specialized function but, in addition, takes part in peripheral resistance control.

The introduction of the University of Wisconsin (UW) preservation solution, currently the organ-preservation solution of choice (Ploeg et al., 1992), has opened up possibilities to cold-store whole organs for prolonged periods. The storage of blood vessels for extended periods with preservation of vessel wall viability and endothelial function is of vital importance, both in cardiovascular surgery and in fundamental studies. The vessel wall of UW-preserved arteries in dog arterial grafts remained fully intact for up to 14 days of storage. The endothelial layer, examined by scanning electron microscopy, proved to be mostly intact for up to 14 days of storage in the UW-preserved

arteries (Vischjager et al., 1995). The efficacy of the UW solution for the preservation of blood vessels has also been demonstrated by others, although only for short periods of time (Ekin et al., 1993). In this connection both stimulated vascular smooth muscle and its relaxation, reflecting endothelial function, proved a valuable tool for assessing the functional viability of the artery during the preservation process. In the present study we observed that the contractile response to K^+-PSS and the thromboxane A_2 receptor agonist, U46619, and the relaxant response to levcromakalim and to the endothelium-dependent vasodilator, methacholine, in human small subcutaneous arteries remained fully intact after preservation of the preparations in UW solution for at least 5 days.

In our experiments glibenclamide influenced neither the basal vascular tone nor the contractions induced by 1 μM U46619 in the human isolated subcutaneous artery. These findings may be explained by the fact that K_{ATP} channels in vascular smooth muscle are closed under physiological conditions, when sufficient intracellular [ATP] is available (Standen et al., 1989).

As expected, we found, in human isolated small subcutaneous artery preparations, a potent vasodilator effect of levcromakalim, the (−) enantiomer of cromakalim. The vasodilator potency of levcromakalim in the human small subcutaneous artery preparations was similar to that observed in an earlier in vitro study with rat small mesenteric arteries (Hüsken et al., 1995). This vasodilator effect was antagonised by glibenclamide (1 and 3 μM), suggesting the involvement of the K_{ATP} channels in the vascular responses to levcromakalim.

The results of the present study demonstrated clearly that isolated human arteries obtained from surgical material can be used successfully in vitro for pharmacological studies concerning vasoactive agents.

In conclusion, the results indicated that activation of ATP-sensitive K^+ channels in arterial smooth muscle appears to be an effective mechanism to dilate isolated human small subcutaneous arteries, which can be modulated by K_{ATP}-channel openers and blockers.

Acknowledgements

We thank Dr. J.B. De Boer, Dr. C.M.A.M. Van der Horst and Dr. M.J.P.F. Ritt of the Department of Plastic Reconstructive Surgery, Academic Medical Centre, Amsterdam, for their help with the supply of breast tissue.

References

Aalkjær, C., A.M. Heagerty, K.K. Petersen, J.D. Swales and M.J. Mulvany, 1987, Evidence for increased media thickness, increased neuronal amine uptake, and depressed excitation-contraction coupling in isolated resistance vessels from essential hypertensives, Circ. Res. 61, 181.

Challinor-Rogers, J.L. and G.A. McPherson, 1994, Potassium channel openers and other regulators of KATP channels, Clin. Exp. Pharmacol. Physiol. 21, 583.

Cook, D.L. and C.N. Hales, 1984, Intracellular ATP directly blocks K^+ channels in pancreatic β-cells, Nature 311, 271.

Donnelly, R., H.L. Elliott, P.A. Meredith and J.L. Reid, 1990, Clinical studies with the potassium channel activator cromakalim in normotensive and hypertensive subjects, J. Cardiovasc. Pharmacol. 16, 790.

Ekin, S.T., P.J. Pearson, P.R.B. Evora and H.V. Schaff, 1993, One-hour exposure to University of Winconsin solution does not impair endothelium-dependent relaxation or damage vascular smooth muscle of epicardial coronary arteries, J. Heart Lung Transplant. 12, 624.

Hüsken, B.C.P., M. Pfaffendorf and P.A. Van Zwieten, 1995, Influence of the hypertensive state on the effect of potassium channel modulators in mesenteric arteries, Br. J. Pharmacol. 114 (Suppl.), 35P.

Mulvany, M.J. and W. Halpern, 1977, Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats, Circ. Res. 41, 19.

Noma, A., 1983, ATP-regulated K^+ channels in cardiac muscle, Nature 305, 147.

Ploeg, R.J., J.H. Van Bockel, P.T.H. Langendijk, M. Groenewegen, F.J. Van der Woude, G.G. Persijn, J. Thorogood and J. Hermans, 1992, Effect of preservation solution on results of cadaveric kidney transplantation, Lancet 340, 129.

Quast, U. and N.S. Cook, 1989, Moving together: K^+ channel openers and ATP-sensitive K^+ channels, Trends. Pharmacol. Sci. 10, 431.

Singer, D.R., N.D. Markandu, M.A. Miller, A.L. Sugden and G.A. MacGregor, 1989, Potassium channel stimulation in normal subjects and in patients with essential hypertension: an acute study with cromakalim (BRL 34915), J. Hypertens. Suppl. 7, S294.

Southard, J.H., T.M. Van Gulik, M.S. Ametani, P.K. Vreugdenhil, S.L. Lindell, B.H. Pienaar and F.O. Belzer, 1990, Important components of the UW solution, Transplantation 49, 251.

Standen, N.B., J.M. Quayle, N.W. Davies, J.E. Brayden, Y. Huang and M.T. Nelson, 1989, Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle, Science 245, 177.

Van Nguyen, P., D.L. Holliwell, A. Davis, T.C.G. Tasker and F.H.H. Leenen, 1991, Effects of the potassium channel activator, cromakalim, on arterial and cardiac responses to norepinephrine, angiotensin II, and isoproterenol in normotensive men, J. Cardiovasc. Pharmacol. 18, 797.

Vischjager, M., T.M. Van Gulik, M. Pfaffendorf, P.A. Van Zwieten, J. Van Marle, J.G. Kromhout, P.J. Klopper and M.J.H.M. Jacobs, 1995, Morphology and function of dog arterial grafts preserved in UW-solution, Br. J. Clin. Pharmacol. (in press).