Br. J. Pharmacol. (1992), **105**, 129–134

One way cross tolerance between cromakalim and salbutamol in the uterus of the rat *in vivo*

[1]Sandra J. Downing & Michael Hollingsworth

Smooth Muscle Research Group, Department of Physiological Sciences, University of Manchester, Oxford Road, Manchester M13 9PT

1 Cross tolerance between the potassium (K^+) channel opener, cromakalim and the β_2-adrenoceptor agonist, salbutamol, was investigated in the uterus of the non-pregnant rat *in vivo*. Uterine sensitivity to salbutamol was similar in both vehicle-treated and cromakalim-tolerant rats. In salbutamol-tolerant rats, uterine responses to cromakalim were markedly decreased compared with saline-infused rats, such that maximum inhibition of uterine contractions was less than 40%.

2 Propranolol treatment and salbutamol tolerance each produced similar reductions in sensitivity of the uterus to salbutamol of approximately 10 fold. The same dose of propranolol did not influence uterine sensitivity to cromakalim, which suggests that the relaxant action of cromakalim is not due to a direct or indirect activation of uterine β_2-adrenoceptors.

3 Salbutamol produced a marked (11.7 fold) increase in uterine adenosine 3':5'-cyclic monophosphate (cyclic AMP) concentrations measured *ex vivo*, which was completely inhibited by propranolol pretreatment, but was unaffected by glibenclamide pretreatment. Cromakalim did not increase uterine cyclic AMP concentrations, suggesting that stimulation of adenylate cyclase is not significant in the uterine relaxant action of cromakalim.

4 The lack of propranolol antagonism of cromakalim and of cromakalim-induced changes in uterine cyclic AMP concentrations suggests that the cross tolerance observed between salbutamol and cromakalim may be at the level of K^+-channels.

Keywords: Cromakalim; salbutamol; propranolol; cyclic AMP; uterus; K^+-channels

Introduction

It has been suggested that cromakalim, a relaxant of smooth muscle, acts by opening plasma membrane K^+-channels (Cook, 1988; Hamilton & Weston, 1989; Winquist *et al.*, 1989; Newgreen *et al.*, 1990). Glibenclamide, a selective blocker of ATP-dependent K^+-channels, produces competitive-like antagonism of cromakalim in a number of smooth muscles including uterus (Buckingham *et al.*, 1989; Cavero *et al.*, 1989; Eltze, 1989; Wilson, 1989; Winquist *et al.*, 1989; Piper *et al.*, 1990). *In vivo*, a single bolus dose of cromakalim produces prolonged inhibition of uterine contractions (Downing *et al.*, 1989); however, repeated bolus injections of cromakalim do not maintain inhibition of uterine activity. Development of tolerance during repeated administration of cromakalim results in a 25 fold desensitization of the uterus to the drug. The mechanism by which tolerance develops is not known.

β_2-Adrenoceptor agonists such as salbutamol are also relaxants of smooth muscle and act by increasing membrane adenylate cyclase activity leading to increased intracellular concentrations of adenosine 3':5'-cyclic monophosphate (cyclic AMP) (Johansson & Andersson, 1980; 1981; Harden, 1983). It has been suggested that β_2-adrenoceptor agonists also open K^+-channels inducing membrane hyperpolarization and this contributes to their mechanism of smooth muscle relaxation (Diamond & Marshall, 1969; Kroeger & Marshall, 1973; Yamaguchi *et al.*, 1988; Sadoshima *et al.*, 1988; Kume *et al.*, 1989; Longmore *et al.*, 1991). Uterine tolerance to salbutamol has also been observed *in vivo* (Abel & Hollingsworth, 1986). Development of tolerance to β_2-adrenoceptor agonists has been shown to be due to internalization and loss of membrane receptors and uncoupling of the membrane-receptor complex from the adenylate cyclase complex with consequent loss of production of second messenger cyclic AMP (Harden, 1983).

Despite the differences in mechanisms of action of the two relaxants, preliminary studies indicated that cross tolerance between cromakalim and salbutamol occurs in the uterus. The aim of this study was to characterize the phenomenon of cross tolerance between cromakalim and salbutamol in the uterus of the rat *in vivo*. It is possible that an indirect stimulation of uterine cyclic AMP concentrations by cromakalim may occur *in vivo* upon reflex release of adrenaline into the circulation after cromakalim-induced hypotension. The possible involvement of β_2-adrenoceptor stimulation and cyclic AMP production in cromakalim-induced uterine relaxation and cross tolerance with salbutamol was investigated by use of the β_2-adrenoceptor antagonist, propranolol, and by measuring uterine cyclic AMP concentrations *ex vivo* after cromakalim treatment. Preliminary findings have been presented to the British Pharmacological Society (Downing & Hollingsworth, 1991).

Methods

Female rats, 200–250 g, were purchased from Charles River, U.K.

Surgical techniques

Rats were anaesthetized with tribromoethanol (240 mg kg^{-1}, i.p.), bilaterally ovariectomized and the right jugular vein cannulated. A small latex balloon was placed in one uterine horn. Morphine sulphate (5 mg kg^{-1}, s.c.) was given postoperatively to all rats. Animals were allowed 24 h for recovery, then continuous recording of pressure cycles produced by uterine contractions was started in conscious rats (Downing & Sherwood, 1985). Contractions were quantified as the integral of the area under the pressure curve. The rats were allocated to one of the following groups.

In vivo *recordings*

(I) *Cromakalim/salbutamol cross tolerance* Uterine responses to bolus i.v. doses of cromakalim (0.05–0.25 mg kg^{-1}) or salbutamol (20–400 μg kg^{-1}) were recorded for 60 min and expressed as percentage inhibition of the integral of uterine

[1] Author for correspondence.

contractions recorded for the 60 min immediately before each bolus dose. Three hours elapsed between each bolus dose to allow the drug to clear. Three hours after the last bolus dose, the rats were given an i.v. infusion of saline ($0.15\,ml\,h^{-1}$) or salbutamol ($120\,\mu g\,kg^{-1}\,h^{-1}$) for 24 h or 3 i.v. injections of vehicle (14% ethanol/saline, $1\,ml\,kg^{-1}$) or cromakalim ($1\,mg\,kg^{-1}$) 8 h apart. After tolerance induction/vehicle treatment, uterine responses to cromakalim or salbutamol were determined as before, such that the following combinations were examined: (a) Uterine sensitivity to cromakalim before and after salbutamol tolerance (13 rats)/vehicle infusion (10 rats). (b) Uterine sensitivity to salbutamol before and after cromakalim tolerance (10 rats)/vehicle injections (10 rats). Pre- and post-tolerance log ID_{50} values were estimated for each rat by probit analysis. Log ID_{50} values were compared statistically by analysis of variance followed by Student's t test.

(II) *Influence of propranolol on uterine sensitivity to cromakalim and salbutamol* Uterine responses to bolus i.v. doses of cromakalim or salbutamol were determined as described before. Rats were then given an i.v. infusion of saline ($0.15\,ml\,h^{-1}$) for 2 h, salbutamol ($120\,\mu g\,kg^{-1}\,h^{-1}$) for 24 h or propranolol ($400\,\mu g\,kg^{-1}\,h^{-1}$) for 2 h. After infusion, uterine responses to cromakalim or salbutamol were determined as before such that the following combinations were examined: (a) Uterine sensitivity to salbutamol before and after induction of salbutamol tolerance (12 rats). (b) Uterine sensitivity to salbutamol before and after propranolol (9 rats)/saline (10 rats) infusion. (c) Uterine sensitivity to cromakalim before and after propranolol (10 rats)/saline (10 rats) infusion.

Pre- and post-infusion log ID_{50} values were estimated for each rat and compared statistically by analysis of variance followed by Student's t test.

Biochemical studies

Rats were anaesthetized with tribromoethanol and a cannula placed in the right jugular vein. Anaesthesia was maintained throughout the experiments. Rats were then allocated to one of the following groups:

(III) *Time course of cromakalim-induced changes in cyclic AMP concentrations* Rats were given bolus i.v. doses of cromakalim ($0.25\,mg\,kg^{-1}$) or vehicle (14% ethanol/saline, $1\,ml\,kg^{-1}$). Rats were killed at 1, 2, 5, 10, 20, 40 or 60 min after bolus dose ($n = 4$–8 rats per time point), the uterus removed as rapidly as possible and frozen in liquid nitrogen. The uterus was homogenized in 5% w/v trichloroacetic acid, then the homogenate centrifuged at 1200 g and the pellet used for protein determination by the Lowry-Folin method (Lowry *et al.*, 1951). The supernatant was shaken with 4×4 volumes of water-saturated diethyl ether to remove trichloroacetic acid, the ether layer being discarded. Aliquots of the extract (25 and 50 μl) were assayed for cyclic AMP with tritiated cyclic AMP radioimmunoassay kits (Amersham (H^3) cAMP assay system).

(IV) *Influence of propranolol on cromakalim or salbutamol-induced changes in cyclic AMP concentrations* Rats were anaesthetized and jugular veins cannulated as described before. The rats were then given i.v. bolus doses of propranolol ($400\,\mu g\,kg^{-1}$) or saline ($1\,ml\,kg^{-1}$) followed 5 min later by cromakalim ($0.25\,mg\,kg^{-1}$), salbutamol ($100\,\mu g\,kg^{-1}$) or saline ($1\,ml\,kg^{-1}$) ($n = 4$–6 rats). Rats were killed at 2 min after the second bolus dose, the uterus removed, frozen, homogenized and extracted and assayed for cyclic AMP as described before.

(V) *Influence of glibenclamide on cromakalim or salbutamol-induced changes in cyclic AMP concentrations* Rats were anaesthetized and jugular veins cannulated as described before. The rats were then given an i.v. infusion of glibenclamide ($20\,mg\,kg^{-1}$ in 0.02 N sodium hydroxide in 4% glucose) followed 15 min later by bolus i.v. doses of cromakalim ($0.25\,mg\,kg^{-1}$), salbutamol ($100\,\mu g\,kg^{-1}$) or saline ($1\,ml\,kg^{-1}$) ($n = 4$–6 rats). At 2 min after bolus dose, the animals were killed and the uterus removed, frozen, homogenized and extracted and assayed for cyclic AMP as described previously.

Cyclic AMP radioimmunoassay

Cyclic AMP concentrations in extracts of uterine tissue were assayed over the range 0.25–16 pM. Coefficient of variation within assay was 4.1% ($n = 4$) and for several assays over 6 months was 4.5% ($n = 12$). Recovery of cyclic AMP from uterine tissue during extraction was 62.5 ± 2.5%. Values of cyclic AMP concentrations are uncorrected for procedural losses.

Drugs and analysis of results

Cromakalim was obtained from SmithKline Beecham, salbutamol from Glaxo and glibenclamide from Hoechst. Propranolol was purchased from Sigma Chemical Co., (Poole, U.K.), tribromoethanol from Fluka Chemicals (Glossop, U.K.) and morphine sulphate from Evans (Dunstable, U.K.). Values are quoted as means ± s.e.mean. The significance of difference between means was calculated by analysis of variance followed by Student's t test.

Results

(I) *Cromakalim/salbutamol cross tolerance*

Uterine sensitivity to salbutamol increased in both vehicle-treated and cromakalim-tolerant rats and to similar extents, 4.6 fold and 5.5 fold respectively (pre-vehicle log ID_{50}, 1.94 ± 0.16, post-vehicle log ID_{50}, 1.28 ± 0.10, $P < 0.01$, pre-cromakalim log ID_{50}, 2.18 ± 0.18, post-cromakalim log ID_{50}, $1.44 \pm 0.14\,\mu g\,kg^{-1}$, $P < 0.01$) (Figure 1). Cromakalim tolerance, therefore, had no effect on uterine sensitivity to salbutamol. Uterine sensitivity to cromakalim showed an 8.7 fold increase in saline-infused rats (pre-saline log ID_{50}, -0.76 ± 0.10, post-saline log ID_{50}, $-1.70 \pm 0.09\,mg\,kg^{-1}$, $P < 0.001$). However, uterine responses to cromakalim were markedly decreased in salbutamol-tolerant rats and maximum inhibition of uterine contractions was less than 40% (Figure 2).

(II) *Influence of propranolol on uterine sensitivity to cromakalim or salbutamol* Salbutamol infusion resulted in a decrease in uterine sensitivity to salbutamol of 14.1 fold (pre-tolerance log ID_{50}, 1.69 ± 0.19, post-tolerance log ID_{50}, $2.84 \pm 0.21\,\mu g\,kg^{-1}$, $P < 0.001$) (Figure 3). Propranolol infusion resulted in a similar reduction in uterine sensitivity to salbutamol of 11.2 fold (pre-saline log ID_{50}, 1.73 ± 0.14, post-saline log ID_{50}, 1.69 ± 0.19; pre-propranolol log ID_{50}, 1.87 ± 0.11, post-propranolol log ID_{50}, $2.92 \pm 0.10\,\mu g\,kg^{-1}$, $P < 0.001$). Propranolol did not influence uterine responses to cromakalim. Uterine sensitivity to cromakalim increased in both saline- and propranolol-infused rats (pre-saline log ID_{50}, -0.69 ± 0.10, post-saline log ID_{50}, -1.12 ± 0.16; pre-propranolol log ID_{50}, -0.81 ± 0.09, post-propranolol log ID_{50}, $-1.15 \pm 0.10\,mg\,kg^{-1}$). Uterine sensitivity to cromakalim after propranolol infusion was not significantly different from that observed after saline infusion (Figure 4).

(III) *Time course of cromakalim-induced changes in cyclic AMP concentrations*

Concentrations of cyclic AMP in uterine tissue at various times after cromakalim bolus are given in Table 1. The changes in uterine cyclic AMP concentrations after cromakalim treatment were not significantly different from those after vehicle treatment throughout the 60 min period.

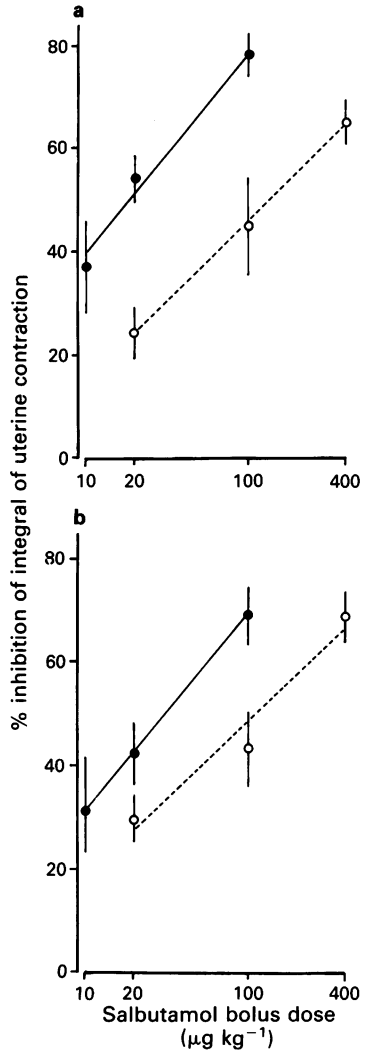

Figure 1 Effect of salbutamol on uterine contractions (a) before (○) and after (●) treatment with 14% ethanol/saline vehicle (3 × 1 ml kg^{-1}, i.v., 8 h apart, 10 rats) and (b) before (○) and after (●) treatment with cromakalim (3 × 1 mg kg^{-1}, i.v., 8 h apart, 10 rats). Ordinate scale: % inhibition of integral of uterine contractions. Abscissa scale: salbutamol bolus dose (μg kg^{-1}) log scale.

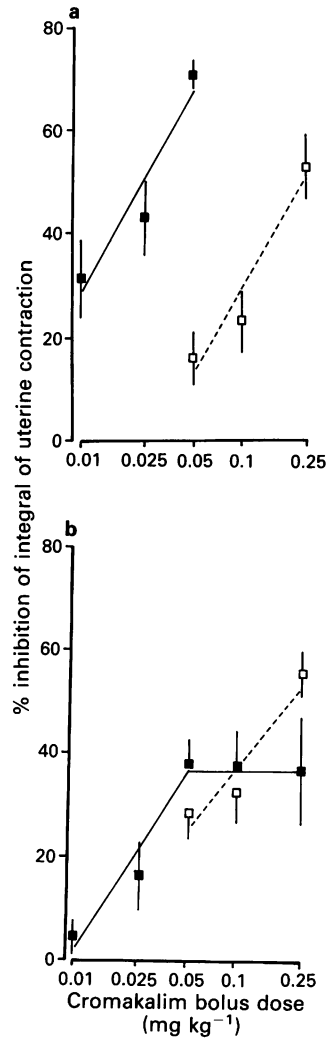

Figure 2 Effect of cromakalim on uterine contractions (a) before (□) and after (■) infusion of saline (0.15 ml h^{-1} i.v. for 24 h, 10 rats) and (b) before (□) and after (■) infusion of salbutamol (120 μg kg^{-1} h^{-1} i.v., for 24 h, 13 rats). Ordinate scale: % inhibition of integral of uterine contractions. Abscissa scale: cromakalim bolus dose, (mg kg^{-1}), log scale.

(IV) *Influence of propranolol on cromakalim or salbutamol-induced changes in cyclic AMP concentrations*

Propranolol plus saline treatment had no effect on uterine cyclic AMP concentrations (Figure 5). Salbutamol produced a marked increase in uterine cyclic AMP concentrations at

Table 1 Time course of changes in uterine cyclic AMP concentrations after cromakalim or vehicle treatment

	Cyclic AMP concentrations (pmol mg^{-1} protein)	
Time (min)	*Vehicle*	*Cromakalim* (0.25 mg kg^{-1})
1	9.61 ± 0.67 (6)	11.32 ± 1.55 (6)
2	8.71 ± 1.09 (8)	11.63 ± 2.60 (8)
5	8.60 ± 1.84 (6)	10.53 ± 3.66 (7)
10	7.53 ± 2.24 (6)	8.92 ± 1.73 (6)
20	9.18 ± 1.29 (5)	7.91 ± 1.07 (4)
40	11.14 ± 3.33 (4)	6.87 ± 1.00 (5)
60	6.95 ± 0.46 (4)	7.47 ± 0.92 (4)

Values are means ± s.e.mean and numbers of rats are given in parentheses. There were no significant differences with treatment.

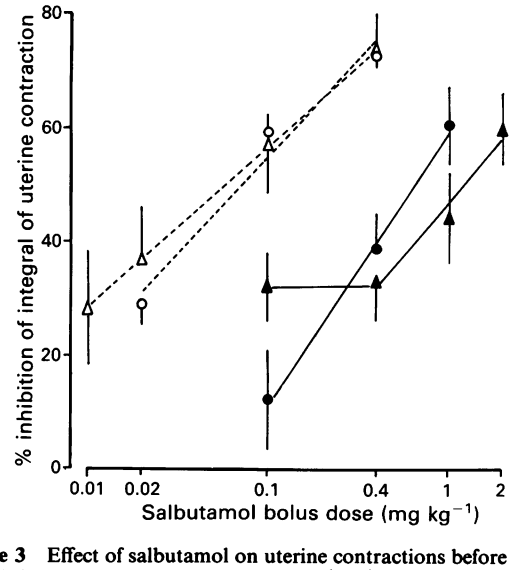

Figure 3 Effect of salbutamol on uterine contractions before (○) and after infusion of salbutamol (120 μg kg^{-1} h^{-1} for 24 h, 12 rats, ●), propranolol (400 μg kg^{-1} h^{-1} for 2 h, 9 rats, ▲) or saline (0.15 ml h^{-1} for 2 h, 10 rats, △). Ordinate scale: % inhibition of integral of uterine contraction. Abscissa scale: salbutamol bolus dose (mg kg^{-1}) log scale.

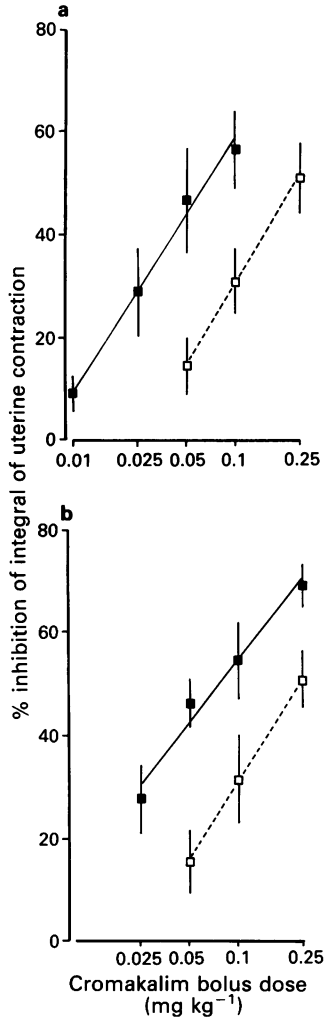

Figure 4 Effect of cromakalim on uterine contractions (a) before (□) and after (■) infusion of saline (0.15 ml h^{-1} i.v. for 2 h, 10 rats) and (b) before (□) and after (■) infusion of propranolol (400 $\mu g\,kg^{-1}\,h^{-1}$ i.v. for 2 h, 10 rats). Ordinate scale: % inhibition of integral of uterine contractions. Abscissa scale: cromakalin bolus dose (mg kg^{-1}) log scale.

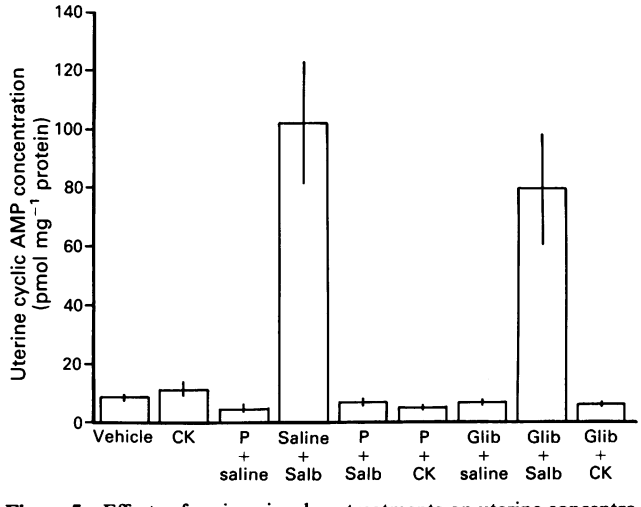

Figure 5 Effects of various i.v. drug treatments on uterine concentrations of cyclic AMP measured *ex vivo*. Ordinate scale: cyclic AMP concentrations (pmol mg^{-1} protein). Abscissa scale: drug treatment. Vehicle = 14% ethanol/saline. CK = cromakalim (0.25 mg kg^{-1}). P = propranolol (400 $\mu g\,kg^{-1}$). Saline = 1 ml kg^{-1}. Salb = salbutamol (100 $\mu g\,kg^{-1}$). Glib = glibenclamide (20 mg kg^{-1}). Values are means of n = 4–8 rats; vertical lines show s.e.mean.

2 min after bolus dose ($P < 0.001$) which was inhibited by prior propranolol treatment. Cyclic AMP concentrations in uterine extracts from propranolol plus cromakalim-treated rats were similar to those from animals treated with vehicle for propranolol.

(V) *Influence of glibenclamide on cromakalim or salbutamol-induced changes in cyclic AMP concentrations*

Glibenclamide plus saline treatment had no effect on uterine cyclic AMP concentrations (Figure 5). Glibenclamide did not significantly reduce salbutamol-induced increase in uterine cyclic AMP concentrations. Uterine cyclic AMP concentrations in glibenclamide plus cromakalim-treated rats were similar to those observed in vehicle-treated rats.

Discussion

In vehicle-treated rats, an increase in uterine sensitivity to salbutamol and cromakalim was observed. For salbutamol, the increase in uterine sensitivity of approximately 5 fold, is apparent by 48 h after surgical balloon implantation, whereas for cromakalim, uterine sensitivity appears to increase progressively from 30 h after surgery. The apparent change in sensitivity may, in part, reflect a decline in uterine prostaglandin production, which would have been stimulated during balloon insertion and would have stimulated uterine contractions. It may be that 24 h recovery between surgery and uterine sensitivity testing is insufficient. However, integrals of uterine contractions recorded at 48 h after surgery (65.2 ± 3.3 units per h) are slightly greater than those recorded at 24 h after surgery (53.9 ± 2.7, n = 23 rats). Therefore, the change in uterine sensitivity to the drugs is not due solely to the *in vivo* recording technique. The increase in uterine sensitivity to salbutamol may also be, in part, a result of ovariectomy. Oestrogen has been shown to reduce β_2-adrenoceptor-mediated cyclic AMP production in rabbit uterus (Riemer *et al.*, 1988). Uterine sensitivity to β_2-adrenoceptor agonists may increase progressively during the period of declining oestrogen influence. In contrast to salbutamol, uterine sensitivity to cromakalim is increased by oestradiol treatment (Downing & Hollingsworth, unpublished), and therefore, declining oestrogen influence after ovariectomy is unlikely to be a cause of the marked increase in sensitivity to cromakalim with time. The mechanism of the increase in uterine sensitivity to cromakalim is not known. No increase in sensitivity is observed *in vitro* at 4 h after initial cromakalim concentration-effect testing, and therefore this phenomenon is unlikely to be cromakalim-induced (Piper *et al.*, 1991). In addition, no increase in cardiovascular sensitivity to cromakalim has been observed (Downing & Hollingsworth, 1989) suggesting that this phenomenon is specific to the uterus.

Cross tolerance between cromakalim and salbutamol was observed in the uterus of the rat, but in one direction only. Uterine sensitivity to salbutamol was unaffected by development of tolerance to cromakalim. However, uterine responses to cromakalim were markedly depressed by development of tolerance to salbutamol.

The possibility that cromakalim relaxes uterine smooth muscle indirectly *in vivo* by reflex release of adrenaline resulting from cromakalim-induced hypotension, thus activating uterine β_2-adrenoceptors, was tested by use of propranolol. Propranolol produces blockade of uterine β_2-adrenoceptors and antagonizes adrenaline released in response to cromakalim-induced fall in blood pressure. At doses which were effective at reducing salbutamol-induced inhibition of uterine contractions and stimulation of uterine cyclic AMP concentrations, propranolol had no effect on uterine sensitivity to cromakalim. Furthermore, a large bolus dose of cromakalim (0.25 mg kg^{-1}, i.v.) did not increase

uterine cyclic AMP concentrations. Although in most isolated tissues cromakalim does not change cyclic AMP concentrations (Newgreen *et al.*, 1990), cromakalim has been shown to increase cyclic AMP concentrations in taenia coli (McHarg *et al.*, 1989). It may be that significant losses of cyclic AMP occurred due to the action of endogenous phosphodiesterases before the tissue was frozen; however, marked increases in uterine cyclic AMP concentrations in response to salbutamol were observed with the same methodology, suggesting that cromakalim-induced changes in cyclic AMP would have been observed, had they occurred. It therefore appears unlikely that cromakalim acts directly or indirectly on β_2-adrenoceptors in the uterus.

The lack of antagonism of cromakalim by propranolol and the absence of changes in uterine cyclic AMP concentrations after cromakalim bolus suggest that the desensitization to cromakalim observed in salbutamol-tolerant rats is not due to loss of β_2-adrenoceptors or uncoupling of adenylate cyclase. Potassium channel opening may be involved in the mechanism of action of β_2-adrenoceptor agonists (Diamond & Marshall, 1969; Kroeger & Marshall, 1973; Izumi & Kishikawa, 1982; Yamaguchi *et al.*, 1988). Kume *et al.* (1989) have demonstrated that isoprenaline and cyclic AMP activate calcium-dependent K^+-channels in rabbit isolated tracheal myocytes. Cromakalim has also been reported to activate calcium-dependent K^+-channels in arterial vascular smooth muscle (Kusano *et al.*, 1987; Gelband *et al.*, 1989; Post *et al.*, 1989). The activity of calcium-activated K^+-channels is modulated by a number of regulatory factors, including ATP (Groschner *et al.*, 1991), cyclic AMP-dependent phosphorylation (Sadoshima *et al.*, 1988; Kume *et al.*, 1989) at a site closely associated with the channel (Ewald *et al.*, 1985), and guanosine 5'-monophosphate (Williams *et al.*, 1988). These factors may in turn be modulated by salbutamol and/or cromakalim and may also represent a site at which tolerance develops. The K^+-channel itself may also become tolerant to salbutamol or cromakalim. Although glibenclamide does not antagonize salbutamol as an inhibitor of uterine contractions (Downing & Hollingsworth, 1990), nor influence salbutamol-induced changes in cyclic AMP concentrations, ATP-dependent K^+-channel opening may be a minor component of β_2-adrenoceptor agonist action (Longmore *et al.*, 1991). Blockade or desensitization of potassium channels by cromakalim may be overridden by the cyclic AMP-mediated inhibition of uterine contractions and thus uterine sensitivity to salbutamol would not be reduced in cromakalim-tolerant rats. Desensitization of potassium channels in addition to loss of β_2-receptors may occur during development of tolerance to salbutamol and may result in cross tolerance with cromakalim.

An alternative explanation for cross tolerance between salbutamol and cromakalim in one direction only is that cromakalim tolerance may be due to loss of cromakalim binding sites only. Such a mechanism would be unlikely to influence uterine sensitivity to salbutamol. Cromakalim may not induce tolerance of putative second messenger systems or of K^+-channels. Development of tolerance to salbutamol, however, may include loss of salbutamol binding sites, uncoupling of second messenger systems and desensitization of K^+-channels. In this case, uterine sensitivity to cromakalim would be reduced in salbutamol-tolerant rats.

In conclusion, cross tolerance between salbutamol and cromakalim has been observed in one direction; uterine sensitivity to cromakalim was reduced in salbutamol-tolerant rats, but sensitivity to salbutamol was unchanged in cromakalim-tolerant rats. Propranolol treatment did not influence uterine sensitivity to cromakalim, nor were significant changes in uterine cyclic AMP concentrations observed after cromakalim treatment. Cross desensitization between cromakalim and salbutamol may be due to tolerance at the level of uterine K^+-channels.

We thank Action Research for financial support, SmithKline Beecham for cromakalim and Glaxo for salbutamol.

References

ABEL, M.H. & HOLLINGSWORTH, M. (1986). The effects of long term infusion of salbutamol, diltiazem and nifedipine on uterine contractions in the ovariectomized, post-partum rat. *Br. J. Pharmacol.*, **88**, 577–584.

BUCKINGHAM, R.E., HAMILTON, T.C., HOWLETT, D.R., MOOTOO, S. & WILSON, C. (1989). Inhibition by glibenclamide of the vasorelaxant action of cromakalim in the rat. *Br. J. Pharmacol.*, **97**, 57–64.

CAVERO, I., MONDOT, S. & MESTRE, M. (1989). Vasorelaxant effects of cromakalim in rats mediated by glibenclamide-sensitive potassium channels. *J. Pharmacol. Exp. Ther.*, **248**, 1261–1268.

COOK, N.S. (1988). The pharmacology of potassium channels and their therapeutic potential. *Trends Pharmacol. Sci.*, **9**, 21–28.

DIAMOND, J. & MARSHALL, J.M. (1969). A comparison of the effects of various smooth muscle relaxants on the electrical and mechanical activity of rat uterus. *J. Pharmacol. Exp. Ther.*, **168**, 21–30.

DOWNING, S.J. & HOLLINGSWORTH, M. (1989). Cardiovascular tolerance to cromakalim in the rat *in vivo*. *Br. J. Pharmacol.*, **98**, 886P.

DOWNING, S.J. & HOLLINGSWORTH, M. (1990). Antagonism of relaxin by glibenclamide in the uterus of the rat *in vivo*. *Br. J. Pharmacol.*, **99**, 10P.

DOWNING, S.J. & HOLLINGSWORTH, M. (1991). Cross tolerance between salbutamol and cromakalim in the uterus of the rat *in vivo*. *Br. J. Pharmacol.*, **104**, 164P.

DOWNING, S.J., MILLER, M. & HOLLINGSWORTH, M. (1989). Tolerance to cromakalim in the rat uterus *in vivo*. *Br. J. Pharmacol.*, **96**, 732–738.

DOWNING, S.J. & SHERWOOD, O.D. (1985). The physiological role of relaxin in the pregnant rat: the influence of relaxin on uterine contractile activity. *Endocrinology*, **116**, 1206–1214.

ELTZE, M. (1989). Glibenclamide is a competitive antagonist of cromakalim, pinacidil and RP49356 in guinea pig pulmonary artery. *Eur. J. Pharmacol.*, **165**, 231–239.

EWALD, D.A., WILLIAMS, A. & LEVITAN, I.B. (1985). Modulation of single Ca^{2+}-dependent K^+-channel activity by protein phosphorylation. *Nature*, **315**, 503–506.

GELBAND, C.H., LODGE, N.J. & VAN BREEMAN, C. (1989). A Ca^{2+}-activated K^+-channel from rabbit aorta: modulation by cromakalim. *Eur. J. Pharmacol.*, **167**, 201–210.

GROSCHNER, K., SILBERBERG, S.D., GELBAND, C.H. & VAN BREEMAN, C. (1991). Ca^{2+}-activated K^+-channels in airway smooth muscle are inhibited by cytoplasmic adenosine triphosphate. *Pflügers Arch.*, **417**, 517–522.

HAMILTON, T.C. & WESTON, A.H. (1989). Cromakalim, nicorandil and pinacidil: novel drugs which open potassium channels in smooth muscle. *Gen. Pharmacol.*, **20**, 1–9.

HARDEN, T.K. (1983). Agonist-induced desensitization of the β-adrenergic receptor-linked adenylate cyclase. *Pharmacol. Rev.*, **35**, 5–32.

IZUMI, H. & KISHIKAWA, T. (1982). Effects of ritodrine, a β_2-adrenoceptor agonist, on smooth muscle cells of the myometrium of pregnant rats. *Br. J. Pharmacol.*, **76**, 463–471.

JOHANSSON, S.R.M. & ANDERSSON, R.G.G. (1980). Effects of β-adrenergic agonists on rat uterine motility and cAMP levels *in vivo*. *Acta Pharmacol. Toxicol.*, **47**, 5–10.

JOHANSSON, S.R.M. & ANDERSSON, R.G.G. (1981). Mechanisms of β-adrenergic desensitization in rat myometrium. *Acta Pharmacol. Toxicol.*, **49**, 241–247.

KROEGER, E.A. & MARSHALL, J.M. (1973). Beta-adrenergic effects on rat myometrium: mechanisms of membrane hyperpolarization. *Am. J. Physiol.*, **225**, 1339–1345.

KUME, H., TAKAI, A., TOKUNO, H. & TOMITA, T. (1989). Regulation of Ca^{2+}-dependent K^+-channel activity in tracheal myocytes by phosphorylation. *Nature*, **341**, 152–154.

KUSANO, K., BARROS, F., KATZ, G., GARCIA, M., LACZOROWSKI, G. & REUBEN, J.P. (1987). Modulation of K channel activity in aortic smooth muscle by BRL 34915 and a scorpion toxin. *Biophys. J.*, **51**, 54a.

LONGMORE, J., MILLER, M., TRESIZE, D.J. & WESTON, A.H. (1991). Further studies on the mechanism of action of isoprenaline in bovine tracheal smooth muscle. *Br. J. Pharmacol.*, **102**, 182P.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.

McHARG, A.D., SOUTHERTON, J.S., WEIR, S.W. & WESTON, A.H. (1989). A comparison of the effects of cromakalim, noradrenaline and isoprenaline in guinea pig isolated taenia coli. *Br. J. Pharmacol.*, **96**, 27P.

NEWGREEN, D.T., BRAY, K.M., McHARG, A.D., WESTON, A.H., DUTY, S., BROWN, B.S., KAY, P.B., EDWARDS, G., LONGMORE, J. & SOUTHERTON, J.S. (1990). The action of diazoxide and minoxidil sulphate on rat blood vessels: a comparison with cromakalim. *Br. J. Pharmacol.*, **100**, 605–613.

PIPER, I., DOWNING, S.J. & HOLLINGSWORTH, M. (1991). Uterine cross tolerance between cromakalim and RP49356; common mechanism of action? *Fund. Clin. Pharmacol.*, **5**, 409.

PIPER, I., MINSHALL, E., DOWNING, S.J., HOLLINGSWORTH, M. & SADRAEI, H. (1990). Effects of several potassium channel openers and glibenclamide on the uterus of the rat. *Br. J. Pharmacol.*, **101**, 901–907.

POST, J.M., SMITH, J.M. & JONES, A.W. (1989). BRL 34915 (cromakalim) stimulation of ^{42}K efflux from rabbit arteries is modulated by calcium. *J. Pharmacol. Exp. Ther.*, **250**, 591–597.

RIEMER, R.K., WU, Y.Y., BOTTARI, S.P. JACOBS, M.M., GOLDFIEN, A. & ROBERTS, J.M. (1988). Estrogen reduces β_2-adrenoceptor-mediated cAMP production and the concentration of the guanyl nucleotide regulatory protein, G_s, in rabbit myometrium. *Mol. Pharmacol.*, **33**, 389–395.

SADOSHIMA, J.-I., AKAIKA, N., KANAIDE, H. & NAKAMURA, M. (1988). Cyclic AMP modulates Ca-activated K channel in cultured smooth muscle cells of rat aortas. *Am. J. Physiol.*, **255**, H754–H759.

WILLIAMS, D.L., KATZ, G.M., ROY-CONTANCIN, L. & REUBEN, J.P. (1988). Guanosine 5'-monophosphate modulates gating of high conductance Ca^{2+}-activated K^+-channels in vascular smooth muscle cells. *Proc. Natl. Acad. Sci., U.S.A.*, **85**, 9360–9364.

WILSON, C. (1989). Inhibition by sulphonylureas of vasorelaxation induced by K^+-channel activators *in vitro*. *J. Auton. Pharmacol.*, **9**, 71–78.

WINQUIST, R.J., HEANEY, L.A., WALLACE, A.A., BASKIN, E.P., STEIN, R.G., GARCIA, M.L. & KACZOROWSKI, G.J. (1989). Glyburide blocks the relaxant response to BRL 34915 (cromakalim), minoxidil sulfate and diazoxide in vascular smooth muscle. *J. Pharmacol. Exp. Ther.*, **248**, 149–156.

YAMAGUCHI, H., HONEYMAN, T.W. & FAY, F.S. (1988). β-Adrenergic actions on membrane electrical properties of dissociated smooth muscle cells. *Am. J. Physiol.*, **254**, C423–C431.

(Received August 16, 1991
Revised September 18, 1991
Accepted October 1, 1991)