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K_{ATP} channels mediate the β_2-adrenoceptor agonist-induced relaxation of rat detrusor muscle

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Abstract

We propose that ATP-sensitive K^+ (K_{ATP}) channels are normally inactive but involved in β_2-adrenoceptor stimulated relaxation of the rat bladder. Spontaneous detrusor muscle contractions were unaffected by glibenclamide (K_{ATP} channel blocker) but were reduced when pinacidil (K_{ATP} channel opener) concentrations exceeded 10^{-5} M. Inhibition by β_2-adrenoceptor agonist clenbuterol (10^{-6} M) of 1 Hz electrical field stimulated contractions was abolished by glibenclamide (10^{-6} M). Glibenclamide (10^{-6} M) decreased forskolin-induced relaxation (10^{-9}–10^{-4} M) in bladder muscle stimulated with 1 Hz electrical field. In the presence glibenclamide (10^{-6} M) or myristoylated protein kinase A inhibitor (2×10^{-6} M), clenbuterol (10^{-9}–10^{-5} M) failed to inhibit bladder contraction in response to 1 Hz electrical field stimulation. Therefore, K_{ATP} channel opening and the subsequent hyperpolarization of cell membranes in response to β_2-adrenoceptor activation is mediated by raised cyclic-AMP levels and activation of protein kinase A. This counteracts ATP-stimulated depolarization in bladder muscle, thereby reducing cell contraction.

Keywords: Urinary incontinence; β_2-Adrenoceptor; ATP-sensitive K^+ channel; Detrusor muscle; Clenbuterol

1. Introduction

ATP-sensitive K^+ (K_{ATP}) channels, which are closed by intracellular ATP, have been identified in various tissues including pancreatic β-cells, neurons, cardiac, skeletal and vascular smooth muscle (Quayle et al., 1997). More recently, K_{ATP} channels have been identified also in the smooth muscle of the urinary bladder (Bonev and Nelson, 1993a; Matsuda et al., 1995; Gopalkrishnan et al., 1999).

K_{ATP} channel activity in various smooth muscle types has been shown to be important in regulating membrane potential and basal tone (Quayle et al., 1997). In vascular smooth muscle, K_{ATP} channels play a central role in the modulation of contractility by vasoactive substances (Nelson and Quayle, 1995). Similarly, K_{ATP} channel function is important in the urinary bladder where channel activation results in inhibition of the contractile response to low frequency electrical field stimulation, but not that due to carbachol stimulation (Chun et al., 1996).

Cromakalim and pinacidil, both K_{ATP} channel openers, have been shown to hyperpolarize urinary bladder smooth muscle (Foster et al., 1989a; Fujii et al., 1990; Bonev and Nelson, 1993a; Gopalkrishnan et al., 1999). Additionally, K_{ATP} channel openers relax normal and hyper-reflexic bladder smooth muscle (Nurse et al., 1991; Martin et al., 1997), having greater effect on bladder muscle stimulated with low frequency electrical field stimulation (Sudoh et al., 1997). Taken together, these findings suggest that K_{ATP} channels may be important in the control of bladder tone and have a role in the control of the hyper-reflexic bladder (Chun et al., 1996; Heppner et al., 1996; Li et al., 1996).

In rat detrusor muscle, stimulation of β_2-adrenoceptors has been shown to reduce the contractile response of the detrusor muscle only in response to purinergic but not cholinergic stimulation (Hudman et al., 2000). In vascular smooth muscle, K_{ATP} channels are the targets for a number of vasoactive substances (Nelson and Quayle, 1995).

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In particular, stimulation of β-adrenoceptors results in a glibenclamide-sensitive vasodilation in a number of vascular beds (Quayle et al., 1997). This vasodilation may be mediated by either β_1- or β_2-adrenoceptors, alone or together, depending on the vascular bed (Nakashima and Van-Houtte, 1994, 1995; Randall and McCulloch, 1995; Kontos and Wei, 1996; Chang, 1997).

Traditionally, relaxation of smooth muscle by β_2-adrenoceptor agonists has been thought to be via stimulation of adenylyl cyclase and the formation of adenosine 3′,5′-cyclic monophosphate (cyclic AMP) (Scheid et al., 1979). In vascular smooth muscle, there is increasing evidence that hyperpolarization caused by the opening of K_{ATP} channels mediates partially the vasodilatation induced by β_2-adrenoceptor agonists (Quayle and Standen, 1994). In addition, a link between vasodilatation of the rat diaphragmatic micro-circulation, which is mediated by elevation of cyclic AMP levels and K_{ATP} channel activation in vascular smooth muscle, has been demonstrated (Chang, 1997). Similarly, in coronary tissue, activation of K_{ATP} channels through a pathway which involves cyclic AMP and protein kinase A has recently been identified (Wellman et al., 1998). Forskolin-stimulated relaxation of vascular smooth muscle was significantly attenuated by the K_{ATP} channel blocker glibenclamide, again suggesting that a mechanism linking receptor and K_{ATP} channel activation involves the elevation of cyclic AMP levels and protein kinase A activity (Chang, 1997).

The aim of this study was to investigate the possible involvement of K_{ATP} channels in the β_2-adrenoceptor agonist stimulated relaxation of detrusor muscle. This was investigated by examining the effect of modulators of K_{ATP} channel activity on the relaxation response to the β_2-adrenoceptor agonist, clenbuterol. Glibenclamide (10^{-6} M) and pinacidil (10^{-6} M) were used as specific pharmacological tools to close or open K_{ATP} channels, respectively. These agents have been shown in bladder smooth muscle to be effective at closing or opening the channels (Levin et al., 1999; Gopalkrishnan et al., 1999). Secondly, to determine whether K_{ATP} channels in detrusor muscle are modulated in response to changing cyclic AMP levels, forskolin was used to stimulate adenylyl cyclase activity and elevate cyclic AMP levels (Chang, 1997; Wellman et al., 1998). In addition, the ability of an inhibitor of protein kinase A (myristoylated protein kinase A inhibitor (14-22) amide) to block the clenbuterol-stimulated relaxation of detrusor muscle was investigated.

2. Methods

Bladder strips ($4 \times 1 \times 0.5$ mm) from male and female Wistar rats (250–400 g) were mounted in a perfusion organ bath of 0.2 ml volume (Brading and Sibley, 1983) and perfused with Kreb's solution (NaCl 119 mM, KCl 4.4 mM, $NaHCO_3$ 20 mM, NaH_2PO_4 1.2 mM, $MgCl_2$ 1.2 mM, $CaCl_2$ 2.5 mM, and Glucose 11 mM, made up in distilled water (pH 7.2)) as described previously (Hudman et al., 2000).

2.1. Electrical field stimulation

Contraction of the muscle strips was stimulated using electrical field stimulation with recessed platinum electrodes in the wall of the organ bath connected to a stimulator (Digitimer). Stimulation occurred at a frequency of 1 Hz, 50 V with a pulse width of 0.5 ms in 10 s trains at 3 min intervals, unless stated otherwise. At this setting, the contractile response has been shown to be abolished by 1.6×10^{-6} M tetrodotoxin, indicating its neurogenic origin (Hudman et al., 2000). Tetrodotoxin block of electrical field stimulated contraction was confirmed in this study as described by Hudman et al. (2000) (not shown).

The effect of different treatments on the contractile response of detrusor muscle strips to electrical field stimulation at 1 Hz, 50 V, and 0.5 ms pulse width was compared against control contractions normalized to 100% for each experiment. The mean tension from all experiments in response to 1 Hz electrical field stimulation was 4.3 ± 1.4 mN ($n = 30$).

2.2. Drug modulation of K_{ATP} channel activity

To assess the contribution of K_{ATP} channels to the clenbuterol-induced inhibition of the atropine-resistant contractile response of detrusor muscle to electrical field stimulation (Brading and Williams, 1990), the effect of a K_{ATP} channel antagonist, glibenclamide and opener, pinacidil, was investigated in cumulative addition experiments ($n = 5$, in each). The contractile response of strips stimulated at 1 Hz electrical field stimulation was recorded as a control and again after 15 min incubation with dimethylsulfoxide (DMSO) (Sigma, UK) diluted 1000 times in Kreb's solution to control for addition of vehicle. In all the experiments, DMSO concentration never exceeded 0.1% and any effect was considered negligible. Drugs were added to the bath, and after 15 min incubation for each, detrusor muscle strips were then stimulated with 1 Hz electrical field stimulation. Addition of atropine (Sigma, UK) to a concentration of 10^{-6} M was followed by clenbuterol (10^{-6} M). Repeat cumulative addition experiments ($n = 5$) involved the addition of either glibenclamide (Tocris Cookson, UK) (10^{-6} M) followed by clenbuterol (10^{-6} M) or pinacidil (ICN Pharmaceuticals, UK) (10^{-6} M) followed by clenbuterol (10^{-6} M) to atropine pre-treated strips as detailed previously. All additions were made cumulatively to the bathing Kreb's solution.

In addition, bladder strips ($n = 5$) were stimulated with 1 Hz electrical field stimulation as a control and then cumulative additions of pinacidil (10^{-9}–10^{-4} M) were added to the bladder strips and incubated for 15 min before being re-stimulated.

2.3. Forskolin induced elevation of cyclic AMP

Bladder strips ($n = 5$) were stimulated at 1 Hz electrical field stimulation to determine the contractile response. Cumulative additions of forskolin (Sigma, UK) (10^{-9}–10^{-5} M) were added to the bath with 15 min incubation between each addition. After incubation with each concentration, the preparations were stimulated again at 1 Hz electrical field stimulation and the contractile response recorded. A sigmoidal standard dose–response curve was fitted to the data where $Y = \text{Bottom} + (\text{Top} - \text{Bottom})/1 + 10^{(\log \text{EC}_{50} - X)\text{hillslope}}$ using Graphpad Prism software.

2.4. Involvement of K_{ATP} channel activity in the response to elevated cyclic AMP

The above forskolin experiments were repeated in the presence of glibenclamide (10^{-6} M) or pinacidil (10^{-6} M) equilibrated for 15 min before cumulative doses of forskolin (10^{-9}–10^{-5} M) were added to the bath. Each concentration was equilibrated for 15 min before being stimulated at 1 Hz.

2.5. Effect of protein kinase A inhibitor or glibenclamide on clenbuterol-induced relaxation

Bladder strips ($4 \times 0.5 \times 1$ mm) were suspended from sutures in a 50 ml static organ bath filled with Kreb's solution at 37°C and aerated with 95% O_2 and 5% CO_2. A static bath was used to reduce the amount of protein kinase A inhibitor required during these experiments. The bottoms of the strips were fixed to a hook suspended in the bath and the top to an isometric force transducer attached to an oscillograph as before. The strips were passed through a Chick biventer stimulating electrode (Harvard) and stimulated at 10 V, 1 Hz, 0.5 ms in 10 s trains at 3 min intervals. Agents were added directly to the organ bath and concentrations reported were the final bath concentrations assuming an even mixing. The preparations were tensioned and equilibrated as before.

A dose–response curve to 1 Hz electrical field stimulation was constructed in the presence of cumulative concentrations of clenbuterol (10^{-9}–10^{-5} M). Either myristoylated protein kinase A inhibitor (14-22 amide) *N*-Myr-Gly-Arg-Thr-Arg-Arg-Asn-Ala-Ile-NH_2 (Calbiochem-novabiochem, UK) (2×10^{-6} M) or glibenclamide (10^{-6} M) was added to the bath and allowed to equilibrate for 15 min and then the dose–response curve for clenbuterol was repeated ($n = 4$).

2.6. Solutions

A 10^{-2} M stock solution of clenbuterol was made up fresh in distilled water before being diluted with Kreb's solution just prior to addition. Stock solutions of 10^{-2} M glibenclamide and pinacidil were made in DMSO. Glibenclamide and pinacidil stock solutions were diluted in Kreb's solution to give an organ bath concentration of 10^{-6} M for both. Myristoylated protein kinase A inhibitor (14-22) amide was dissolved in distilled water and then added directly to the bath to a final bath concentration of 2×10^{-6} M. A stock solution of forskolin was made in DMSO at a concentration of 10^{-2} M that was then diluted in Kreb's solution as required.

2.7. Statistical analysis

Data were expressed as mean ± S.E.M. Statistical analysis of drug effect and the difference between treatment groups were determined using analysis of variance (ANOVA) where Tukeys-honestly significantly different (HSD) test was used for multiple comparison. A P value of < 0.05 was regarded as significant in all cases.

3. Results

3.1. Closure of K_{ATP} channels

To investigate the mechanism for the clenbuterol-induced inhibition of the contractile response of detrusor muscle to electrical field stimulation, the possible relationship between the stimulation of β_2-adrenoceptors and K_{ATP} channel activity was examined in the presence of the K_{ATP} channel blocker glibenclamide. β-Addition of glibenclamide (10^{-6} M) to non-stimulated detrusor muscle had no effect on the spontaneous contractions or the tone of the preparation ($n = 5$) (Fig. 1a).

In control experiments, the contractile response of the detrusor muscle to 1 Hz electrical field stimulation after addition of DMSO was not significantly different to the control response (Fig. 2a). Similarly, the addition of 10^{-6} M atropine had no significant effect on the contractile response of the detrusor muscle to 1 Hz electrical field stimulation (Fig. 2a). The addition of clenbuterol (10^{-6} M) however, significantly inhibited the contractile re-

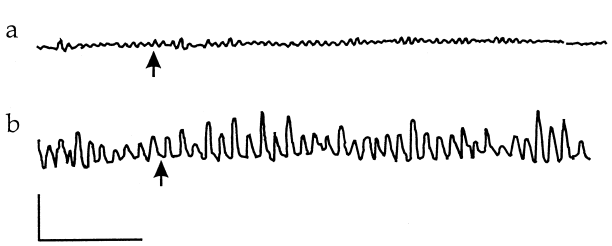

Fig. 1. Oscillograph recording of non-stimulated spontaneous contractile activity (a) before and after the addition of glibenclamide (10^{-6} M) showing that the base line tone and spontaneous activity remain unaltered by glibenclamide. (b) Before and after the addition of pinacidil (10^{-6} M) showing that the base line tone and spontaneous activity remain unaltered. Horizontal bar represents a time scale of 3 min and vertical bar 10 mN of contractile force. The upward pointing arrows indicate the point of addition of glibenclamide or pinacidil.

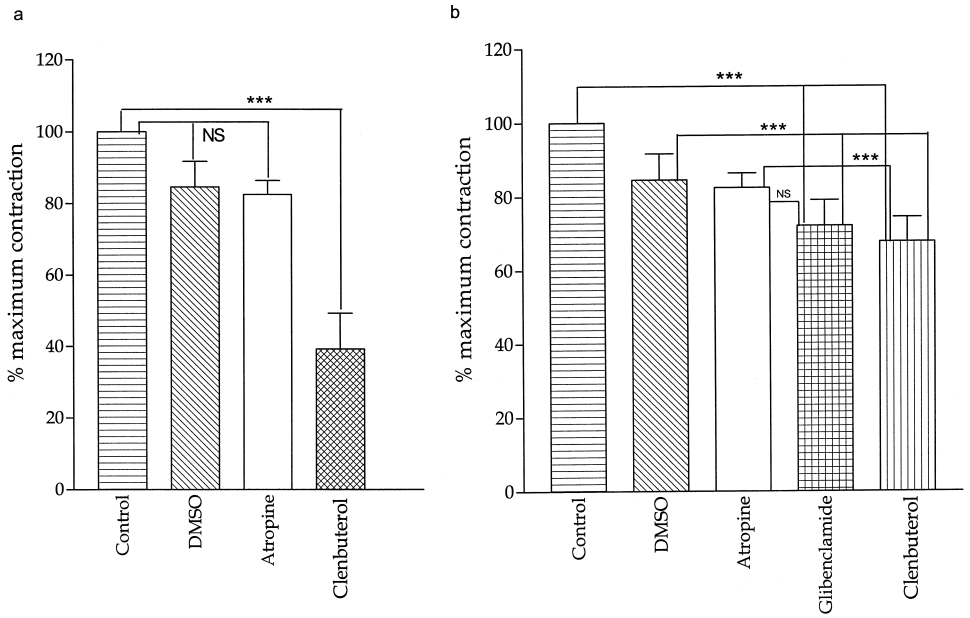

Fig. 2. (a) The effect drugs on the contractile response to 1 Hz electrical field stimulation ($n = 5$). Control contractions to 1 Hz electrical field stimulation were normalized to 100%, and the subsequent contractile response in the presence of different drugs expressed as a percentage of this contraction. Intervals of 15 min incubation were included between all drug additions and were followed by 1 Hz electrical field stimulation. In the control experiments, DMSO (1:1000), atropine (10^{-6} M) and clenbuterol (10^{-6} M) were added sequentially ($n = 5$). b. The effect of the cumulative addition of DMSO (1:1000), atropine (10^{-6} M), glibenclamide (10^{-6} M) and clenbuterol (10^{-6} M) on 1 Hz electrical field stimulated contractile response, expressed as a percentage of the control contractile response ($n = 5$). Bars in all experiments are the mean of 5 detrusor muscle strips with error bars showing S.E.M. $^{***}P < 0.001$ and NS is not significantly different.

sponse to electrical field stimulation when compared to the control, the response after DMSO and the atropine-resistant contractile response ($P < 0.001$, in all cases).

In similar experiments, ($n = 5$) glibenclamide (10^{-6} M) was added to strips treated with DMSO and atropine as previously described. In the presence of glibenclamide the contractile response was significantly inhibited ($P < 0.001$) when compared to the contractile response of the control but was not significantly different when compared to the atropine-resistant contractile response (Fig. 2b). The response following the further addition of clenbuterol was significantly reduced ($P < 0.001$) when compared to the control response but was not significantly different to the effect after addition of glibenclamide only (Fig. 2b). A comparison of the effect of clenbuterol (10^{-6} M) on DMSO, atropine and glibenclamide pre-treated strips with the control experiment strips treated with DMSO, atropine and clenbuterol only (Fig. 2a) was significantly different ($P < 0.001$). Thus, glibenclamide blocked the clenbuterol-induced inhibition of the atropine-resistant contractile response (Fig. 2a and b).

3.2. Opening of K_{ATP} channels

An experiment was conducted to determine whether the effect of clenbuterol could be mimicked by direct opening of K_{ATP} channels with pinacidil. Pinacidil had a small dose-dependent inhibitory effect on the contractile response of the rat detrusor muscle to 1 Hz electrical field stimulation (Fig. 3). The amount of inhibition due to pinacidil was small with 37% and 30% inhibition of

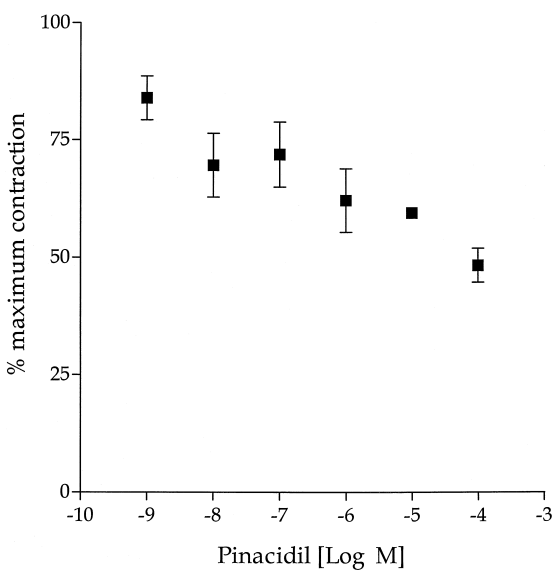

Fig. 3. A dose response curve for pinacidil 10^{-9}–10^{-4} M added to detrusor muscle strips from rats. Bladder strips were stimulated 15 min after addition of each concentration with 1 Hz 0.5 ms electrical field stimulation. The results are expressed as percentage of the maximum control contraction to 1 Hz electrical field stimulation. Error bars show S.E.M. ($n = 5$).

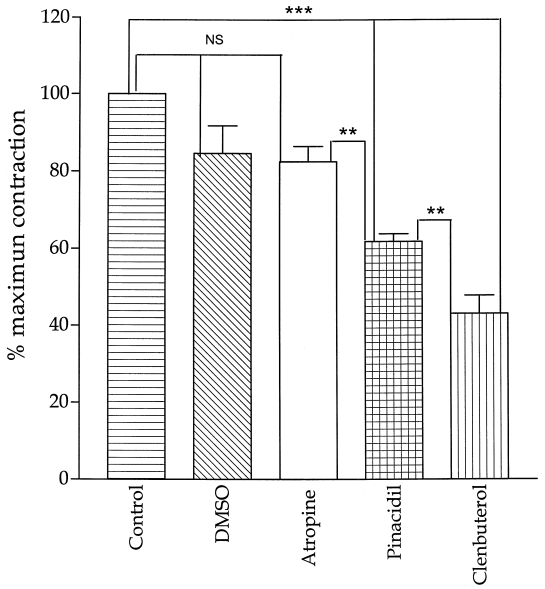

Fig. 4. The effect on 1 Hz electrical field stimulated contractions after the cumulative addition of drugs. The control contractions to 1 Hz electrical field stimulation were normalized to 100%, and the subsequent contractile response in the presence of different drugs expressed as a percentage of this control contraction. Intervals of 15 min incubation were included between all additions followed by 1 Hz electrical field stimulation. DMSO 1:1000 in Kreb's solution; atropine 10^{-6} M, pinacidil 10^{-6} M, and clenbuterol 10^{-6} M. Bars show the mean of five rats with error bars showing S.E.M. $^{**}P < 0.01$, $^{***}P < 0.001$, and NS = not significant.

contraction at 10^{-6} and 10^{-5} M, respectively. However, pinacidil at high concentrations (10^{-5} M and above) either decreased spontaneous contractions or in 50% of preparations abolished them (not shown). The amount of inhibition caused by pinacidil at 10^{-6} M and 10^{-5} M was not significantly different, therefore, further studies with this agent were carried out at a concentration (10^{-6} M) below that, which abolished spontaneous contractions. Although it was not possible to calculate an EC_{50} from our data, the concentration of pinacidil used in our studies was 10-fold higher than the reported EC_{50} in bladder smooth muscle (Gopalkrishnan et al., 1999). Under these conditions, the addition of pinacidil to non-stimulated detrusor muscle had no effect on the spontaneous contractions or the tone of the bladder muscle preparations ($n = 5$) (Fig. 1b). DMSO and atropine again had no significant effect on the contractile response of the detrusor muscle to 1 Hz electrical field stimulation (Fig. 4). The effect of added pinacidil (10^{-6} M) was inhibitory when compared to the contractile response of the control ($P < 0.001$) and when compared to the atropine-resistant component of the contractile response ($P < 0.05$) (Fig. 4). The further addition of clenbuterol (10^{-6} M) was inhibitory to the contractile response of detrusor muscle when compared to the contractile response of the control and when compared to the contractile response of the pinacidil pre-treated strips ($P < 0.001$) (Fig. 4). The effect of clenbuterol on bladder strips that had been pre-treated with pinacidil (Fig. 4) showed no significant difference to those treated with clenbuterol only (Fig. 2a).

3.3. Forskolin induced elevation of cyclic AMP

To investigate whether the cyclic AMP signaling pathway was involved in K_{ATP} channel activation forskolin was applied to stimulate directly adenylyl cyclase activity. Forskolin inhibited the contractile response of detrusor muscle to 1 Hz electrical field stimulation in a dose-dependent manner with an EC_{50} of $1.1 \pm 0.2 \times 10^{-7}$ M (Fig. 5). The sigmoid dose–response equation gave a goodness of fit for the line through the mean data of $r^2 = 0.98$

3.4. Effect of glibenclamide on forskolin induced relaxation

Glibenclamide (10^{-6} M) blocked forskolin-induced relaxation at concentrations of forskolin less than 10^{-7} M (Fig. 5). However, in the presence of glibenclamide, the dose–response curve for forskolin-induced relaxation was shifted to the right. The EC_{50} for forskolin-induced relaxation in the presence of glibenclamide was $5.8 \pm 1.2 \times 10^{-7}$ M and the sigmoid dose–response curve of the mean data had a goodness of fit of $r^2 = 0.99$ (Fig. 5). The difference in EC_{50} values in the absence and presence of glibenclamide, using an unpaired Student's *t*-test for both curves, was statistically significant ($P < 0.02$).

3.5. Effect of pinacidil on forskolin-induced relaxation

The possible synergistic action of pinacidil on forskolin-induced relaxation was investigated. Pinacidil

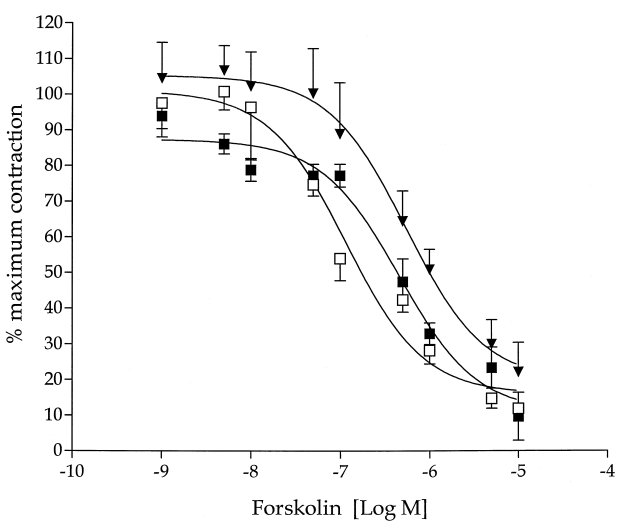

Fig. 5. Dose–response curve for forskolin-induced relaxation of detrusor muscle stimulated with 1 Hz electrical field stimulation in the absence (□) or presence of a 15 min pre-incubation with either 10^{-6} M glibenclamide (▼) or 10^{-6} M pinacidil (■). Results are expressed as a percentage of the control contraction to 1 Hz electrical field stimulation in the absence of forskolin. Error bars show S.E.M. ($n = 5$).

(10^{-6} M) did not significantly affect the dose-dependent relaxation induced by forskolin on the detrusor muscle response to 1 Hz electrical field stimulation (Fig. 5). The EC_{50} ($4.4 \pm 1.5 \times 10^{-7}$ M) for the forskolin–dose–response curve in the presence of pinacidil was not significantly different from that determined in the presence of forskolin alone.

3.6. Effect of protein kinase A inhibitor and glibenclamide on clenbuterol-induced relaxation of detrusor muscle

To investigate the involvement of protein kinase A in the mechanism of clenbuterol-induced relaxation of detrusor muscle, the effect of protein kinase A inhibition was investigated. The addition of 2×10^{-6} M protein kinase A inhibitor to the bladder strips had no significant effect on the tone or spontaneous contractions of the preparation (not shown). Following the addition of the protein kinase A inhibitor, the contractile response of the bladder muscle to electrical field stimulation at 1 Hz (10 V, 0.5 ms) was essentially unchanged ($102.4 \pm 1.6\%$) when compared to untreated control strips. Subsequent addition of clenbuterol (10^{-9}–10^{-5} M) produced no significant additional change in response to 1 Hz electrical field stimulation (Fig. 6). However, the contraction measured in the presence of the protein kinase A inhibitor and clenbuterol together was significantly different from the contractile response to 1 Hz electrical field stimulation in the presence of clenbuterol alone ($P < 0.01$).

Pre-incubation with glibenclamide (10^{-6} M) alone had no effect on the contractile response of detrusor muscle to 1 Hz electrical field stimulation (not shown). When incubated for 15 min prior to addition of cumulative doses of clenbuterol glibenclamide blocked the clenbuterol induced relaxation of the detrusor muscle (Fig. 6).

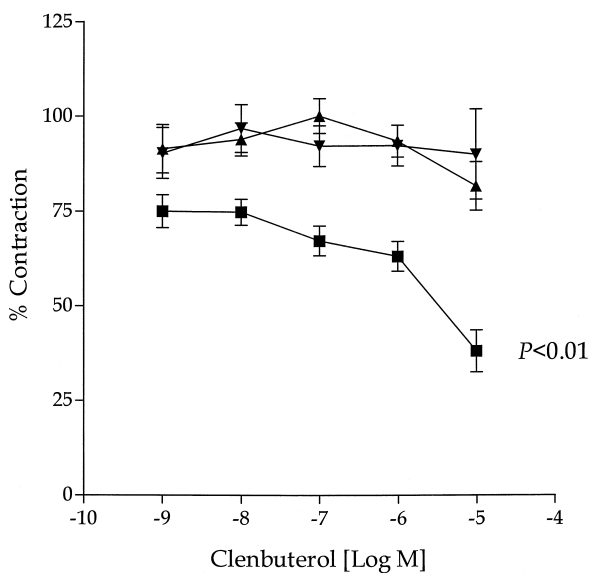

Fig. 6. Dose–response curve for the clenbuterol (10^{-9}–10^{-5} M)-induced relaxation of detrusor muscle stimulated with 1 Hz electrical field stimulation in the absence (■) or presence of 2×10^{-6} M PKA inhibitor (▲) or 10^{-6} M glibenclamide (▼). Error bars show S.E.M. ($n = 4$).

4. Discussion

The recent identification of K_{ATP} channels in the urinary bladder of the guinea pig (Bonev and Nelson, 1993a; Masuda et al., 1995; Gopalkrishnan et al., 1999) is consistent with observations that K_{ATP} openers and blockers have a physiological effect on the detrusor muscle (Foster et al., 1989a,b; Fujii et al., 1990; Nurse et al., 1991; Martin et al., 1997). In this study, the contribution of K_{ATP} channel activity to the β_2-adrenoceptor-induced inhibition of contractile activity in the rat urinary bladder was investigated. Our results revealed two important findings. Firstly, clenbuterol had no significant effect on the contractile response of the bladder to 1 Hz electrical field stimulation when the K_{ATP} channels were blocked. Secondly, opening of K_{ATP} channels was dependent on signaling via cyclic AMP and activation of protein kinase A.

The first part of this study investigated the relationship between K_{ATP} channel activity and the inhibition of contraction produced by the β_2-adrenoceptor agonist, clenbuterol. Clenbuterol has been shown to inhibit the contractile response of the detrusor muscle to 1 Hz electrical field stimulation in a dose-dependent manner (Hudman et al., 2000). This study showed that clenbuterol inhibited the purinergic but not the cholinergic component of the response to electrical field stimulation (Hudman et al., 2000). In addition, clenbuterol was shown to inhibit the contractile response to exogenously applied ATP but not that in response to carbachol. Moreover, the spontaneous contractions seen in rat detrusor muscle were observed to be inhibited by clenbuterol (10^{-6} M) (Hudman et al., 2000).

Addition of the K_{ATP} channel blocker glibenclamide to bladder strips had no effect on the spontaneous contractions or tone of the strips. However, addition of high concentrations of the K_{ATP} opener pinacidil (10^{-5} M and above) had an inhibitory effect on spontaneous contractions. Taken together, these results suggest that in non-stimulated detrusor muscle K_{ATP} channels are predominately closed. Consistent with this conclusion, Teramota et al. (1997) reported that glibenclamide-sensitive K_{ATP} channels in the bladder demonstrated only brief openings in non-stimulated cells, suggesting that K_{ATP} channels may not contribute to maintenance of the membrane potential under resting conditions. Similarly, in vascular smooth muscle cells K_{ATP} channels have also been found to be closed in non-stimulated muscle (Chang, 1997).

By contrast to the absence of effect on non-stimulated bladder muscle, the K_{ATP} channel blocker glibenclamide, at 10^{-6} M, reversed the clenbuterol-induced (10^{-9}–10^{-5} M) inhibition of the contractile response to 1 Hz electrical field stimulation, whereas opening K_{ATP} channels with pinacidil (10^{-6} M) did not. It was inferred that the in-

hibitory action of clenbuterol on detrusor muscle was through the opening of K_{ATP} channels. Although yet to be shown directly in detrusor muscle, it has been shown electrophysiologically in vascular smooth muscle that glibenclamide attenuates the hyperpolarization induced by β-adrenoceptor agonists linking K_{ATP} channel activity to the inhibition of contraction induced by β_2-adrenoceptor stimulation (Nakashima and Van-Houtte, 1994; Wellman et al., 1998).

The second part of this study aimed to identify the mechanism through which clenbuterol activates K_{ATP} channels to produce the inhibition of the contractile response of rat detrusor muscle. Inhibition of the contractile response in smooth muscle by β-adrenoceptor agonists has been linked to activation of adenylyl cyclase and increased formation of cyclic AMP (Scheid et al., 1979). In addition, a variety of endogenous neurohormones have been shown to modulate K_{ATP} channel activity in vascular smooth muscle cells by either a cyclic AMP-dependent mechanism or possibly a direct action of G-proteins (Quayle and Standen, 1994; Nelson and Quayle, 1995; Quayle et al., 1997). In our experiments, the relationship between cyclic AMP and K_{ATP} channel activation was investigated using forskolin to activate adenylyl cyclase and elevate cyclic AMP levels, bypassing the actions of the β_2 adrenoceptor. The dose-dependent inhibition of detrusor muscle contractions in response to electrical field stimulation in the presence of forskolin at low doses ($< 10^{-7}$ M) was reversed by the K_{ATP} channel blocker glibenclamide but the full dose–response curve to forskolin in the presence of glibenclamide was shifted to the right. We conclude that at lower cyclic AMP concentrations, attained in response to low concentrations of forskolin, the relaxation was predominantly via activation of K_{ATP} channels. The rightward shift of the full dose–response curve in the presence of glibenclamide indicated that at higher concentrations of cyclic AMP, relaxation was induced by additional unidentified mechanisms. In the presence of pinacidil, which alone produced a small but non-significant relaxation, the EC_{50} for forskolin induced relaxation was unmodified.

To examine whether protein kinase A mediated the signaling between raised cyclic AMP concentration and K_{ATP} channel activation, the effect of a protein kinase A inhibitor on clenbuterol-induced inhibition of contraction was investigated. In the presence of myristoylated protein kinase A inhibitor (14-22) amide, stimulation of β_2-adrenoceptors with clenbuterol failed to inhibit the electrical field stimulated detrusor muscle contractions indicating that K_{ATP} channels are activated in response to protein kinase A phosphorylation.

The results of this study, together with those of (Hudman et al., 2000), allow the proposal of a mechanism which describes how the contractile response of the detrusor muscle to purinergic stimulation can be inhibited by clenbuterol, while the contractile response to cholinergic stimulation cannot (Hudman et al., 2000). Stimulation with ATP, either directly or through release from nerves, activates purinoceptors (P_{2x}) in the bladder smooth muscle to open a non-specific cation channel, resulting in depolarization of the cell membrane (Fry and Wu, 1998). The membrane depolarization and consequent opening of L-type calcium channels allows an influx of Ca^{2+} ions into the muscle cell leading to contraction (Fry and Wu, 1998). Thus, contraction caused by ATP is dependent on depolarization of the plasma membrane. Conversely, stimulation with acetylcholine causes activation of the muscarinic receptors (M_2 and M_3), production of the second messenger inositol 1,4,5 trisphosphate (IP_3), and release of Ca^{2+} from the sarcoplasmic reticulum (SR) (Fry and Wu, 1998). Thus, contraction caused by acetylcholine is primarily independent of changes in the membrane potential. Only the contractile responses to purinergic stimulation would be susceptible to inhibition by β_2-adrenoceptor activation and the consequent opening of K_{ATP} channels, while cholinergic responses would remain essentially unaffected. It is noteworthy that muscarinic receptor activation inhibits K_{ATP} channel activity in urinary bladder smooth muscle in a protein kinase C-dependent manner (Bonev and Nelson, 1993b). Such inhibition could mask an effect of clenbuterol on K_{ATP} channels in cholinergic synapses.

In conclusion, K_{ATP} channels appear to be functional but inactive in the rat detrusor muscle. The inhibition induced by β_2-adrenoceptor agonists in this tissue appears, however, to be mediated by these K_{ATP} channels. Our results indicate a link between the accumulation of cyclic AMP and activation of protein kinase A in the urinary bladder smooth muscle, which results in the opening of K_{ATP} channels.

Detrusor instability in the human has been linked recently to the appearance of an accessory purinergic excitatory system in the human unstable detrusor muscle (Fry and Wu, 1998). Further elucidation of this signaling pathway may provide a novel strategy for therapeutic treatment of this debilitating condition. The potential for drug therapies involving β_2-adrenoceptor agonists, like clenbuterol, is an important area for further investigation.

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