Abstract  To compare the inhibitory effects of a new group of smooth muscle relaxants, the potassium channel openers cromakalim and pinacidil, with those of oxybutynin on detrusor muscle stimulation in animals. Detrusor strips of guinea pigs (n=16) and rabbits (n=20) were mounted in organ bath for recording of isometric tension. \(\alpha\),\(\beta\)-methylene ATP (10\(^{-7}\), 10\(^{-6}\), 10\(^{-5}\) M), carbachol (10\(^{-6}\), 10\(^{-5}\), 3\(\times\)10\(^{-5}\), 5\(\times\)10\(^{-5}\) M) and transmural electrical-field stimulation (TES) were applied and concentration-response curves in the absence or presence of cromakalim (10\(^{-6}\), 10\(^{-5}\) M), pinacidil (10\(^{-5}\), 5\(\times\)10\(^{-5}\) M) and oxybutynin (10\(^{-5}\), 5\(\times\)10\(^{-5}\) M) were generated. All curves were displaced to the right in a concentration-dependent manner. The order of potency of inhibition was as follows: \(\alpha\),\(\beta\)-methylene ATP (pinacidil>oxybutynin>cromakalim in guinea pigs; pinacidil>cromakalim>oxybutynin in rabbits); TES (pinacidil>cromakalim>oxybutynin in guinea pigs; cromakalim>oxybutynin>pinacidil in rabbits); carbachol (oxybutynin>pinacidil>cromakalim in guinea pigs; oxybutynin>cromakalim>pinacidil in rabbits). Cromakalim and pinacidil mainly inhibited purinergic-induced (\(\alpha\),\(\beta\)-methylene ATP and TES) detrusor contractions.

Keywords  Cromakalim · Detrusor muscle · Pinacidil · Potassium channel openers · Oxybutynin

Introduction
Detrusor instability (DI) is a common urological problem that severely affects the quality of life of many women and men. As yet, the pathophysiology of the condition is incompletely understood and therefore the results of available treatment including drug therapy are usually disappointing [2, 17]. Anticholinergic agents particularly oxybutynin hydrochloride (OH) are usually considered the drugs of first choice because they reduce bladder contractions and associated symptoms in most patients [2, 23]. In many animal models and the isolated human bladder, however, these drugs only partially antagonize the response of the whole bladder smooth muscle to nerve stimulation and of bladder strips to field stimulation although they completely inhibit the response to exogenous cholinergic stimulation [6, 10, 18, 20, 22]. The most widely accepted explanation for this phenomenon is that a significant portion of neurotransmission involved in bladder contraction is non-adrenergic, non-cholinergic [5]. A purinergic system releasing adenosine 5’-triphosphate (ATP) that acts on a subtype of purinoceptors called P2x seems to be the most likely mechanism [6, 10, 18, 20]. Despite these experimental findings, the clinical importance of “atropine resistance” has never been established because of the marked differences between the activation mechanisms of detrusor muscle in humans and animals and because in the normal human bladder, the non-adrenergic, non-cholinergic component is small and sometimes difficult to demonstrate [23].

In the last two decades, a newly developed group of smooth muscle relaxants, the potassium channel openers (PCO), has stimulated interest for their potential therapeutic role in DI [1, 2]. PCO decrease membrane excitability by acting on the ATP-sensitive potassium channel in the cell membrane to increase potassium efflux resulting in membrane hyperpolarization and reduction of opening probability of ion channels involved in depolarization [3, 7, 8]. The best known and most clinically tested members of the group are pinacidil and cromakalim...
[1, 2]. Studies on isolated human detrusor muscle and on bladder tissue from several animal species have shown that both drugs reduce not only spontaneous contractions but also contractions induced by electrical stimulation and carbachol [7, 8, 9, 15, 16, 17]. However, none of those studies has compared the effects of PCO with those of OH, which at present is the mainstay of pharmacological treatment of DI [2, 23].

The use of guinea pig and rabbit as animal models to study normal bladder function and experimentally induced bladder dysfunction is well-established because of their unique properties [14]. The aim of our study, therefore, was to

1) investigate the effects of pinacidil and cromakalim on the contractile response of the detrusor muscle in guinea pigs and rabbits and
2) compare such effects with those of OH.

Materials and methods

In vitro experiments were performed on detrusor muscle strips prepared from the urinary bladder of female guinea pigs (350–400 gm, 3–6 week old, n=16) and female New Zealand white rabbits (2–3 kg, 3–4 month old, n=20). The study was approved by the Research Ethical Committee of the Faculty and conformed to the Code of Practice for the use and care of laboratory animals for research.

The animals were sacrificed by cervical dislocation following brief general anesthesia with 70% ether, the abdomen opened and urinary bladder removed. The bladder was opened by a vertical incision from urethra to the dome and the mucosa removed from the bladder detrusor muscle. Longitudinal muscle strips measuring about 10x2x1 mm were cut from the bladder dome and each was immediately mounted in 30 mL Schuller organ bath containing Krebs solution at 37°C continuously bubbled with 95% O2 and 5% CO2 to give a pH of 7.4. The strips were fitted with electrodes for transmural electrical-field stimulation (TES) and attached to HSE-force transducer type 372 (Hugo Sachs Elektronik, March-Hugstetten, Germany). Isometric tension of each muscle strip (the transducer output) was recorded on a Graphite-Wattnabe thermal array recorder (WR 3600 Mark 10) via a plug-sys DC bridge amplifier (Hugo Sachs Elektronik, March-Hugstetten, Germany). Experiments were started following an equilibration period between 30 and 45 min.

Influence of PCO and OH on the responses to α,β-methylene ATP and carbachol

Following the equilibration period, cumulative concentrations of the excitatory drugs α,β-methylene ATP and carbachol were applied to the bath. Either of the three inhibitory drugs, cromakalim, pinacidil and OH, were subsequently applied and the cumulative concentrations of the excitatory substances were repeated in its presence. The concentration-response curves for the two excitatory drugs were generated in the absence and presence of each of the three inhibitory drugs.

Influence of PCO and OH on the responses to TES

The effects of cromakalim, pinacidil and OH on the responses of detrusor muscle strips to TES were also studied. Electrical impulses for field stimulation of intrinsic nerves were delivered by an HSE stimulator (Hugo Sachs Elektronik, March-Hugstetten, Germany). Stimuli were of 50 V intensity and their frequency was varied between 1–10 Hz. These stimulation parameters were chosen because they were previously found to elicit almost a pure nerve-mediated response, without a direct smooth muscle stimulation [18]. The responses were obtained in the absence and presence of each of the three inhibitory drugs.

Drugs and solutions

The composition of Krebs solution was (mM) NaCl 121, KCl 5.9, NaHCO3 15.5, MgCl2 1.2, NaH2PO4 1.2, CaCl2 2.5 and glucose 10. Carbachol and α,β-methylene ATP were obtained from Sigma Chemical Co., St. Louis, Missouri; cromakalim from SmithKline Beecham, Herts, U. K.; pinacidil from Leo, Ballerup, Denmark and OH from Leiras Oy, Turku, Finland. Pinacidil was dissolved in 4% v/v hydrochloric acid and cromakalim in 50% dimethyl sulfoxide at concentrations of 10-5 M and 10-3 M, respectively, as stock solutions. All other drugs were dissolved in distilled water at concentrations of 10-2 to 10-4 M as stock solutions. Stock solutions were kept at 4°C for as long as 7 days before use and were further diluted with Krebs solution to obtain the desired concentrations of drugs. The drug concentrations reported are the final concentrations in the organ bath: α,β-methylene ATP: 10-7, 10-6 and 10-5 M; carbachol: 10-6, 10-5, 3x10-5 and 5x10-5 M; cromakalim: 10-6 and 10-5 M; pinacidil: 10-5 and 5x10-5 M; OH: 10-5 and 5x10-5 M.

Calculation of the effects of PCO and OH on the responses to α, β methylene ATP and carbachol

The changes in basal tone were selected for presentation in this article since changes in total tension showed similar results. Changes in phasic tension were also difficult to interpret especially at high agonist concentrations where excitatory effects on basal tension caused strong contractions that caused a decrease in the force of phasic contractions (Fig. 1). Basal tone was defined as the tone maintained by the tissue between phasic contractions i.e. the tone when phasic contractions are fully relaxed.

For each tissue, all responses were compared to the tissue activity before adding any drugs. The percentage change in the basal tension was calculated according to the following equation:

\[
\text{Tone after agonist administration} - \text{Tone before any drug administration} \times 100.
\]

For example in the tracing of Fig. 1a, the effect of carbachol 5x10-5 M in the absence of pinacidil was calculated as:

\[
\frac{X - C}{C} \times 100.
\]

and in the presence of pinacidil (Fig. 1b) as:

\[
\frac{Xe - Ce}{Ce} \times 100.
\]

A negative value can result when the basal tone generated by carbachol in the presence of pinacidil was lower than the control basal tone.

Calculation of the effects of PCO and OH on the responses to TES

To eliminate the effects of tissue variability, all the results were normalized to the maximum response obtained upon TES i.e. the response to the 10 Hz stimulus in the absence of any drugs (Fig. 2). All responses were calculated as percentage difference from this maximum response according to the following equation:

\[
\frac{\text{Response to stimulus} - \text{response to 10 Hz in absence of drugs}}{\text{response to 10 Hz in absence of drugs}} \times 100.
\]