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Modulation of nerve-evoked contractions by β_3-adrenoceptor agonism in human and rat isolated urinary bladder

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ABSTRACT

Activation of β_3-adrenoceptors has been shown to have a direct relaxant effect on urinary bladder smooth muscle from both rats and humans, however there are very few studies investigating the effects of β_3-adrenoceptor agonists on nerve-evoked bladder contractions. Therefore in the current study, the role of β_3-adrenoceptors in modulating efferent neurotransmission was evaluated. The effects of β_3-adrenoceptor agonism on neurogenic contractions induced by electrical field stimulation (EFS) were compared with effects on contractions induced by exogenous acetylcholine (Ach) and αβ-methylene adenosine triphosphate (αβ-meATP) in order to determine the site of action. Isoproterenol inhibited EFS-induced neurogenic contractions of human bladder ($pD_2 = 6.79$; $E_{max} = 65\%$). The effect of isoproterenol was selectively inhibited by the β_3-adrenoceptor antagonist L-748,337 ($pK_B = 7.34$). Contractions induced by exogenous Ach (0.5–1 μM) were inhibited 25% by isoproterenol (3 μM) while contractions to 10 Hz in the same strip were inhibited 67%. The selective β_3-adrenoceptor agonist CL-316,243 inhibited EFS-induced neurogenic contractions of rat bladder ($pD_2 = 7.83$; $E_{max} = 65\%$). The effects of CL-316,243 were inhibited in a concentration dependent manner by L-748,337 ($pA_2 = 6.42$). Contractions induced by exogenous Ach and αβ-meATP were significantly inhibited by CL-316,243, 29% and 40%, respectively. These results demonstrate that the activation of β_3-adrenoceptors inhibits neurogenic contractions of both rat and human urinary bladder. Contractions induced by exogenously applied parasympathetic neurotransmitters are also inhibited by β_3-agonism however the effect is clearly less than on neurogenic contractions (particularly in human), suggesting that in addition to a direct effect on smooth muscle, activation of prejunctional β_3-adrenoceptors may inhibit neurotransmitter release.

1. Introduction

Overactive bladder (OAB) is a syndrome characterized by urinary urgency, with or without incontinence, usually with frequency and nocturia [1]. Population-based estimates suggest that OAB affects 12–17% of adults in Europe and the United States, and greatly affects the quality of life of those afflicted [2–4]. Until recently, antimuscarinics were the only compound class approved for treating OAB. However, the first compound targeting a new mechanism has recently been approved in the US, Europe and Japan. Mirabegron is a β_3-adrenoceptor agonist that has been shown to be an effective treatment of OAB symptoms [5]. Several pharmaceutical companies are now developing other promising β_3-adrenoceptor agonists for OAB treatment.

The activity of the urinary bladder during voiding and urine storage is governed by a complex neural control system. During bladder emptying, parasympathetic nerves are stimulated leading to the release of acetylcholine (Ach) and adenosine triphosphate (ATP) that act on post-junctional muscarinic and purinergic receptors, respectively, to produce contraction of the bladder smooth muscle. During bladder filling, sympathetic nerve activity to bladder smooth muscle results in β-adrenoceptor-mediated relaxation [6]. The subtype of β-adrenoceptor that mediates relaxation of the smooth muscle has been well studied and varies among mammalian species. In humans, β_3-adrenoceptor activation has been shown to produce relaxation of basal bladder tension and bladder that has been pre-contracted with carbachol or KCl [7–9]. In the rat,

Abbreviations: Ach, acetylcholine; αβ-meATP, αβ-methylene adenosine (5′)-triphosphate; EFS, electrical field stimulation; OAB, overactive bladder.

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the same has been found, although there is some evidence to suggest that stimulation of β_2-adrenoceptors might also play a role [10–12]. There is also evidence to suggest that there is crosstalk between the parasympathetic and sympathetic systems as stimulation of postjunctional M2 receptors has been shown to inhibit β_3-adrenoceptor induced relaxation [13,14].

While the β-adrenoceptor subtypes involved in direct smooth muscle relaxation have been clearly identified, very little has been done to investigate the identity, function and particularly the location of β-adrenoceptors that affect neurogenic contractions of the bladder. If, in addition to being located postjunctionally on bladder smooth muscle, β_3-adrenoceptors can affect neuronal release of Ach by acting prejunctionally, it could have implications on how these compounds behave in the clinic and potentially interact with antimuscarinics. Therefore in the current study, the ability of β-adrenoceptor agonists to affect contractions mediated by parasympathetic nerves (induced by electrical field stimulation – EFS) through β_3-adrenoceptor agonism in isolated urinary bladder strips from humans and rats was tested. The magnitude of the inhibitory effects of β_3-adrenoceptor stimulation on neurogenic contractions and those induced by exogenous neurotransmitters were then compared in order to determine if the site of action is prejunctional, postjunctional or a combination of both.

2. Materials and methods

2.1. Tissue preparation

Human bladder specimens were obtained from twenty patients (69 ± 2 years old, 19 males and 1 female) undergoing cystectomy due to bladder cancer at the Urology Departments of Rangueil Hospital, Toulouse (France) or Foch Hospital, Suresnes (France). For each patient, an anonymous patient sheet containing information on patient's age, sex, body weight, height, anaesthetics used during surgery, nature of drug administered before hospitalization was provided. No attempt was made to control medication before surgery. Institutional approvals for the use and shipment of human tissues were given, and all patients gave written informed consent. The patients were negative for HIV1–2, HTLV1–2, hepatitis B–C and syphilis. Tissues were placed in a cold storage solution (Custodiol®, EUSA Pharma, Limonest, France) and transported to the laboratory immediately after surgery in a container at 4 °C. Upon receipt, tissues were stored at 4 °C until the start of the experiment. After removal of the serosa and mucosa, 8–10 mm long × 2–3 mm wide detrusor smooth muscle strips were prepared. Samples were divided as follows: samples from 14 patients were used for EFS β-adrenoceptor agonist/antagonist experiments, samples from 4 patients for frequency–response curves and samples from 2 patients for both antagonist and EFS/Ach comparisons.

The current study was conducted according to European Council Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes. Fifty two female Wistar rats (body weight 190–260 g) were obtained from Charles River Laboratories (Saint-Germain-sur-l'Arbresle, France). All animals were group-housed in cages at least 4–5 days before the experiments with free access to food and water. Rats were humanely euthanized using CO_2. The whole urinary bladder was isolated, freed from connective and fat tissues, the dome and base removed and the bladder bisected into two halves (strips).

Both human and rat strips were mounted in 5 mL glass organ baths containing a Krebs–Henseleit solution of the following composition (mM): NaCl 114, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11.7, ascorbic acid 1.1 (pH 7.4) and gassed with 95% O_2 and 5% CO_2 at 37 °C. Tissues were allowed to equilibrate under a resting tension of 1 g for 60 min. Strips were then exposed to KCl (80 mM) to measure their viability. Bladder responses were measured using isometric transducers (EMKA Technologies, Paris, France) and recorded using a data acquisition system (PowerLab 16s, ADInstruments, Sydney, Australia).

2.2. Effect of compounds on EFS-induced contractions

After washout and 45 min of re-equilibration, EFS was applied using the following parameters for human strips: maximal current 800 mA, frequency of 10 Hz, square pulses of 0.1 ms, trains of 5 s every 1 min; and for rat strips: maximal current 800 mA, frequency of 15 Hz, square pulse of 0.1 ms, trains of 4 s every 2 min. Ten to fifteen minutes later antagonists (L-748,337, CGP-20712A, ICI-118,551) or vehicles were added and EFS-induced contractions continued for an additional 10–20 min until stable basal contractions were obtained. Then cumulative concentrations to isoproterenol (10 nM to 100 μM), CL-316,243 (1 nM to 100 μM), L-755,507 (1 nM to 10 μM) or vehicle were added in log or semi-log unit increments. At the end of experiments, 1 μM tetrodotoxin (TTX) was added to verify the neurogenic nature of contractions. Responses to EFS were determined by measuring the amplitude of response and expressed as % inhibition from basal EFS contractions (before agonist addition) for all concentration response curves.

In another set of experiments, two frequency response curves were conducted using the same parameters described above and frequencies of 1.25, 2.5, 5, 10, 20 and 40 Hz (plus 0.625 Hz for rat) applied for 6 min at each frequency and with intervals of 10 min between stimulations. Before the second curve was performed isoproterenol (3 μM) or CL-316,243 (1 μM) (or their vehicle) was added to human and rat strips, respectively. Results are expressed as % of the KCl (80 mM) induced maximal contraction.

In the third set of EFS experiments, two reproducible control responses to EFS (10 min) were performed at 30 min intervals. Atropine (1 μM) was then added to the organ bath for 30 min before a third response to EFS. Then, coincubation of atropine (1 μM) and PPADS (30 μM) was added for 30 min, before further EFS responses were evoked. Results are expressed as % of the KCl (80 mM) induced maximal contraction.

2.3. Effects of β-adrenoceptor agonists on contractions evoked by exogenous Ach or αβ-meATP

Human bladder strips were electrically stimulated at 10 Hz for 15 min, then a single concentration of Ach (0.5 or 1 μM) necessary to obtain a similar contraction as that induced by EFS in the same tissue was added. After a washout period of 30 min, strips were once again stimulated by EFS at 10 Hz for 15 min, before the addition of isoproterenol (3 μM) or vehicle for 10–15 min (time required for the stabilization of the response). EFS was then stopped and the same concentration of Ach (determined earlier in the experiment) added.

In experiments with rat bladder, following the 45 min re-equilibration and washout period after the challenge to KCl (80 mM), three reproducible control responses to Ach (500 μM) were obtained at 20 min intervals. Then CL-316,243 (1 μM) (or vehicle) was added, 10 min before the final application of agonist. Differences in amplitude between the third (without agonist) and the fourth contraction (with agonist or vehicle) are expressed as % of KCl (80 mM) maximal contraction. A similar protocol was used to test the effects of CL-316,243 (1 μM) on responses to αβ-meATP (10 μM) except that the washout period was 45 min in order to avoid desensitization of P_2-purinoceptors.