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The actions of isoprenaline and mirabegron in the isolated whole rat and guinea pig bladder

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ABSTRACT

β_3-adrenoceptor agonists influence overactive bladder in humans and animal models. However, data is emerging that the mode of action of these drugs is complex. The present study explored the actions of the β_3-adrenergic agonist mirabegron and the non-selective agonist isoprenaline on the contractile systems in the rat and guinea pig bladder.

Intravesical pressure was measured in isolated whole bladders from female adult animals. In both species spontaneous contractile activity was observed. The muscarinic agonist arecaidine produced complex responses consisting of an initial transient pressure rise followed by complex phasic activity. Three contractile elements were identified: intrinsic micro-contractile activity, initial transient response and steady state phasic activity. The intrinsic and steady state activity could be further divided into a baseline pressure with superimposed phasic activity. The effects of isoprenaline and mirabegron were investigated on these elements.

In the rat, the micro-contractile activity could be completely inhibited by isoprenaline (full agonist). The arecaidine-induced initial and steady state baseline pressures were partially reduced, while the phasic activity was little affected. In the guinea pig, both the arecaidine-induced baseline pressure and the phasic activity were affected by isoprenaline. Mirabegron didn't produce significant inhibitory effects in any of the contractile elements in either species.

These results show that complex contractile systems operate in the rat and guinea pig bladder that can be modulated by β_1/β_2-adrenoceptor mechanisms. No evidence was obtained for any β_3-dependent regulation of contraction. These data support similar data in humans. Therefore the primary site of therapeutic action of β_3-adrenergic agonists remains unknown.

1. Introduction

Small contractile events have been recorded in the bladder during urine storage in different species including: cat (Sherrington, 1892), rat (Streng et al., 2006), guinea pig (Biallosterski et al., 2011) and human (Bristow and Neal, 1996). Such activity increases in amplitude and frequency as the bladder fills, but it is not associated with urine loss. Consequently, it has been described as non-voiding activity (NVA). This NVA has been demonstrated to be linked to bursts of afferent activity (Iggo, 1955; Iijima et al., 2009). Taken together, these observations have led to the concept that NVA forms the motor component of a motor-sensory system that sends information related to bladder volume to the central nervous system (Gillespie, 2004). Therefore, the mechanisms generating and modulating NVA may be of fundamental importance in bladder physiology and the generation of bladder sensation.

Spontaneous micro-contractions (MC) or autonomous activity has been recorded from isolated strips and were thought to be related to NVA (Gillespie, 2004; Vaughan and Satchell, 1995). The mechanisms underlying this autonomous activity in muscle strips differ from the in vivo NVA, because of the lack of central input. A myogenic origin of activity was suggested (Brading, 1997). More recently, the idea has been introduced that the spontaneous MC are generated and coordinated in the mouse and rat by a specialized system of interstitial cells (Gillespie et al., 2015a; Lagou et al., 2006). A similar situation has been proposed in the guinea pig but in this species an additional role for the intramural ganglia has been postulated (Gillespie, 2004; Gillespie et al., 2006). Therefore, by studying whole isolated bladder autonomous activity, more integrated responses responsible for the generation of bladder sensation could be observed. The rat bladder is devoid of ganglia and so experiments on the whole bladder may reveal information on the interstitial cell network. In the guinea pig, where there are intramural ganglia, experiments in the whole bladder may shed light not only on

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the interstitial cell network but also on the role of the ganglia. For these reasons it is essential to study the two species in parallel.

Recently, interest in the peripheral control of bladder sensation has intensified following the addition of β_3-selective adrenoceptor agonists to pharmacotherapy in overactive bladder (OAB) treatment. Drugs such as mirabegron have been reported to decrease voiding frequency, reduce incontinence episodes and consequently, improve quality of life (Chapple et al., 2014; Sacco et al., 2014). Although these drugs were designed to relax the detrusor via a direct action on detrusor cell β_3-adrenoceptors and so alleviate the symptoms of overactivity (Igawa and Michel, 2013), evidence is now emerging that additional modes of actions should be considered. The plasma concentrations of therapeutic doses of mirabegron are in the order of 30–75 nM (Krauwinkel et al., 2012). In this concentration range, little relaxation can be seen in carbachol-induced contractions in isolated strips of human bladder (Svalo et al., 2013). Parallel experiments have also been done on isolated strips of the rat bladder demonstrating little or no effect of mirabegron (Gillespie et al., 2015b, 2015c). The implication is that mirabegron does not therapeutically act on the smooth muscle. Alternative ideas have been put forward regarding the mode of action of mirabegron, for example, afferent recording from mechanosensitive afferents have been shown to be decreased in the presence of mirabegron, an effect possibly linked to inhibition of MC activity (Aizawa et al., 2012). In an obstructed rat model, it was shown that mirabegron reduced NVA, having little effect on detrusor contractility during voids, suggesting that this motor-sensory system might be a potential therapeutic target for β_3-selective adrenoceptor agonists (Gillespie et al., 2012). In addition, other targets have been suggested that include the urothelium (Kullmann et al., 2011; Masunaga et al., 2010) or pelvic ganglia (Eastham et al., 2015). However, with these additional speculative mechanisms the precise nature of the adrenergic component of the overall physiological system remains to be fully characterized (see Eastham and Gillespie, 2013 for an overview).

Clearly there is some controversy regarding the mode of action of the sympathetic system and adrenergic agonists in the bladder wall. The present study was done to explore this question using isolated rat and guinea pig bladders, to characterize in greater detail the actions of the selective β_3-adrenoceptor agonist mirabegron in the bladder wall and compare this to the actions of isoprenaline, a non-selective β-adrenergic agonist.

2. Materials and methods

2.1. Animals

The protocol was approved by the Animal Ethics Committee of the University of Antwerp (EC 2013-37). Experiments were done in accordance with the European Communities Council Directive (89/609/EEC) on the protection of animals used for experimental and other scientific purposes. Female Sprague-Dawley rats ($n = 11$) and Dunkin Hartley guinea pigs ($n = 9$) weighing 200–250 g were used. The animals were kept under standard laboratory conditions with a cycle of 12 h light and 12 h darkness and free access to food pellets and tap water. The animals were killed using CO_2 and cervical dislocation.

2.2. Isolated whole bladder preparation

The procedures for whole bladder isolation have been described before (Drake et al., 2003). The urinary bladder and urethra were removed and placed in Krebs solution: (mM) NaCl 114, KCl 4.7, CaCl2 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11.7, ascorbic acid 1.1 gassed with 95% O_2 and 5% CO_2 at 37 °C (pH = 7.4). A BD Insyte-WTM intravenous bladder catheter (rat: 22 gauge; ⌀ 0.5 mm and guinea pig: 16 gauge; ⌀ 1.7 mm) was inserted transurethrally and secured at the bladder neck using a thread ligature (Silk 4–0). Residual urine was removed using Krebs solution. The bladder was transferred to a heated organ bath (volume 20 ml, 37 °C) gassed with Krebs solution. The catheter was connected to a pressure transducer (Emka-technologies, France) and a NE-1000 syringe pump (New Era Pump Systems, Farmingdale, New York, USA) via three-way stopcocks. All data were monitored with time using WINDAQ DI-710 data acquisition (Dataq Instruments, Inc., Akron, OH, USA).

2.3. Study protocols

The bladder was filled until spontaneous micro-contractile (intrinsic) activity was observed: 400–600 μl in rats and 1000–1200 μl in guinea pigs. These volumes are normal voiding volumes for rats and guinea pigs (Andersson et al., 2011; Smith et al., 2008). Before starting manipulations, the bladder was left to equilibrate for at least 20 min. All drugs were added to the solution bathing the serosal surface of the bladder.

Protocol 1: In these experiments the responses of the isolated bladder to the muscarinic agonist, arecaidine but-2-ynyl ester tosylate (Tocris, UK) were determined. Arecaidine concentrations of 30, 100, 300 and 1000 nM were administered for 300 s in the rat and 500 s in the guinea pig in intervals of 300 s in the rat and 500 s in the guinea pig, with Krebs washes in between (see Fig. 1A and 1D for further details).

Protocol 2: In these experiments an arecaidine concentration of 300 nM was present throughout the whole experiment. After a 300 or 500 s control period of exposure to arecaidine (300 nM), a β-adrenergic agonist: isoprenaline hydrochloride (Sigma Aldrich, Belgium) or mirabegron (Kemprotec Limited, UK) was added cumulatively, at increasing concentrations (0.001, 0.01, 0.1, 1 and 10 μM) without washing off the arecaidine. During this protocol (see Figs. 6A,B and 7A,B), changes of the arecaidine-induced steady state response, which includes the overall bladder pressure and phasic activity, can be observed.

Protocol 3: A last set of experiments were set up to evaluate the effect of the β-adrenergic agonists on the intrinsic activity and both the initial and steady state arecaidine-induced responses of the isolated bladder. In each experiment, an initial control response to arecaidine (300 nM) was elicited for 300 or 500 s. After removing the agonist from the bathing solution, bladder activity returned to baseline (intrinsic) activity for 300 or 500 s and was subsequently exposed to isoprenaline or mirabegron for 300 or 500 s. A second exposure to the same concentration of arecaidine was given for 300 or 500 s and the effect of isoprenaline or mirabegron determined. During the subsequent wash period, isopenaline or mirabegron was added to the bathing solution at a concentration equal to the one given before the second arecaidine administration. This sequence was then repeated for increasing concentrations of isoprenaline or mirabegron (0.01 to 10 μM). Original records using this protocol are illustrated in Figs. 2A–5A.

2.4. Data analysis

Bladder pressure was analyzed using chart software, AD-instruments. The responses are characterized by slow changes in pressure upon which high frequency MC events are visible. In order to isolate different components, the slower component and the high frequency MC element, the records were filtered and subtracted according to the method described by Gillespie et al. (2015c). Briefly, the original record was smoothed (Triangular (Barlett) Window, running smoothing average using 5555 data points for rat data and 33,333 for the guinea pig, sampling rate 1000 Hz) to determine an estimate of the underlying slow pressure change, baseline pressure. Baseline pressure measurements were measured before arecaidine application (intrinsic response), immediately after the arecaidine application (initial response) and in the steady state. The high frequency activity was quantified using the subtracted records (raw data − smoothed data) by determining the integral of the activity (area under the curve (pressure × time)). In the rat, the integral was determined over a 150 s sampling interval from both the intrinsic activity in the absence of agonist and the steady state during arecaidine application. In the guinea pig, the integral of the arecaidine-induced phasic activity was determined over a 250 s sampling interval.