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Pharmacologic responses of the mouse urinary bladder

Research Article

A. Erdem Canda[1*], Christopher R. Chapple[2], Russ Chess-Williams[1]

[1] *Department of Biomedical Science, University of Sheffield, S102JF Sheffield, United Kingdom*

[2] *The Royal Hallamshire Hospital, Department of Urology, S102JF Sheffield, United Kingdom*

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Abstract: The aim of the study was to determine pathways involved in contraction and relaxation of the mouse urinary bladder. Mouse bladder strips were set up in gassed Krebs-bicarbonate solution and responses to various drugs and electrical field stimulation were obtained. Isoprenaline (b-receptor agonist) caused a 63% inhibition of carbachol precontracted detrusor (EC50=2nM). Carbachol caused contraction (EC50=0.3μM), responses were antagonised more potently by 4-DAMP (M3-antagonist) than methoctramine (M2-antagonist). Electrical field stimulation caused contraction, which was inhibited by atropine (60%) and less by guanethidine and α,β-methylene-ATP. The neurogenic responses were not potentiated by inhibition of nitric oxide synthase. Presence of an intact urothelium significantly depressed responses to carbachol (p=0.02) and addition of indomethacin and L-NNA to remove prostaglandin and nitric oxide production respectively did not prevent the inhibitory effect of the urothelium. In conclusion, b-receptor agonists cause relaxation and muscarinic agonists cause contraction via the M3-receptor. Acetylcholine is the main neurotransmitter causing contraction while nitric oxide has a minor role. The mouse and human urothelium are similar in releasing a factor that inhibits contraction of the detrusor muscle which is unidentified but is not nitric oxide or a prostaglandin. Therefore, the mouse may be used as a model to study the lower urinary tract.

Keywords: *Mouse • Urinary bladder • Urothelium • Contraction • Relaxation*

1. Introduction

A number of animal species have been used to study bladder function; the pharmacological characteristics of the rat and pig bladder appear to be similar to those of the human [1]. In recent years the mouse has become an important model for studying the bladder due to the availability of a wide variety of gene knock-out animals. However, probably due to its size, relatively few studies have examined the receptors and transmitters involved in neurotransmission in this species. In most species the main transmitter appears to be acetylcholine, which acts predominantly via M3 receptors [2] with purinergic mechanisms contributing a minor component to contraction. Nitric oxide relaxes the bladder dome in many species but in the pig [3,4] and human [5] bladder the urothelium also releases an inhibitory factor that is so far unidentified.

To allow a comparison with human bladder responses, the present study examines the transmitters and receptor involved in neurotransmission in the mouse bladder, and also investigates the influence of the urothelium on bladder responses in this species.

2. Material and Methods

Adult male MF1 mice (30 - 35 g) were housed at 22°C and exposed to a 12 h light/12 h dark cycle. Animals had free access to food and water. Mice were killed by cervical dislocation and a lower abdominal incision was made in order to expose the lower urinary tract and the urinary bladders were removed. This research has complied with all relevant national guidelines and institutional policies. Whole urinary bladders were set up in gassed Krebs-bicarbonate solution (composition

* *E-mail: aecanda@yahoo.com*

Table 1. The mean maximum responses and pEC_{50} values of the mouse urinary bladders in response to carbachol, noradrenaline, phenylephrine, histamine and KCl administration.

Agonist	pEC_{50}	Mean maximum response (g)	N
Carbachol	6.4±0.2	0.84±0.06	6
Noradrenaline	7.04±0.6	0.19±0.06	6
Phenylephrine	7.84±6.09	0.25±0.03	6
Histamine	3.12±6.26	0.39±0.08	6
KCl	3.71±0.3	0.51±0.14	5

in mM: NaCl 118.4, $NaHCO_3$ 24.9, KCl 4.7, $CaCl_2$ 1.9, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 11.7) at 37°C under 1g resting tension. Isometric tension was recorded using isometric force transducers (Lectromed UF1, 57 g sensitivity) connected to a Tandon PCA-SL computer *via* an analogue to digital converter (Cambridge Electronic Design). Developed tension was recorded and analysed using "CHART" software (CED). Tissues were equilibrated for 1 h and washed every 15 min before starting the experiments.

Cumulative concentration-response curves to carbachol, KCl, noradrenaline, phenyephrine and histamine were obtained. Responses to carbachol were identified in the absence and presence of the muscarinic antagonists 1,1-dimethyl-4-diphenylacetoxypiperidinium iodide (4-DAMP, 10nM) and methoctramine (3μM) and apparent pKB values were calculated from the shifts of carbachol curves. In separate experiments, concentration-relaxation curves to isoprenaline (non-selective β-adrenoceptor agonist) were also obtained in carbachol precontrated bladders. In another series of experiments, urinary bladders were electrically field-stimulated (10Hz, 0.01ms duration, 40V for 5s) at 100s intervals and the effects of the following drugs investigated: Nω-nitro-L-arginine (L-NNA) (10μM), guanethidine (10μM), atropine (1μM), α,β-methylene-ATP (10μM) and tetrodotoxin (1μM).

To investigate the influence of the urothelium on responses, longitudinal strips of paired mouse urinary bladders were isolated and the urothelium (approximately 30% of the tissue mass) was removed under the microscope from one strip of each pair. Both tissues from each pair were then suspended under 1g tension in Krebs-bicarbonate solution gassed with 95% in oxygen at 37°C. Cumulative concentration-response curves to carbachol were constructed before and after administering indomethacin (5μM) and L-NNA (10μM).

2.1. Statistical analysis

For each curve, responses were plotted as a percent of the individual maximal response and the concentration of agonist producing a 50% response of the maximum response (EC_{50} value) was calculated using Prism (GRAPHPAD software, San Diego, CA, U.S.A.). The mean EC50 values are presented as the geometric mean EC_{50} values with 95% confidence limits. Antagonist affinities estimates (apparent pK_B values) were determined from the equation:

$pK_B = \log (CR-1)-\log[B]$

CR is the concentration ratio (ratio of the EC_{50} values) in the absence and presence of the antagonist obtained with a concentration [B] of antagonist [6].

To compare responsiveness between pairs of tissues (± urothelium), contractions to carbachol were expressed as a percentage of the maximum contraction obtained in the absence of an intact urothelium. Mean responses (±s.e.mean) were calculated and used to plot concentration-response curves.

2.2. Drugs and solutions

All drugs were obtained from Sigma, Poole, UK and were prepared fresh in distilled water except indomethacin which was prepared as a stock solution in ethanol and then diluted in distilled water.

3. Results

Carbachol, noradrenaline, phenylephrine, histamine and potassium all induced contraction of the mouse bladder. Noradrenaline and phenylephrine were the most potent but responses to these agonists were relatively small and responses to carbachol were significantly greater (Table 1). In contrast, the β-adrenoceptor agonist isoprenaline relaxed carbachol precontracted strips by 63% with a pEC50 of 8.9.

Responses to carbachol were antagonised by both 4-DAMP and methoctramine with rightward shifts of the carbachol concentration-response curves, but without any change in maximum response (Table 2, Figures 1 and 2). However, 4-DAMP (M3-antagonist) was >100-fold more potent than methoctramine (M2-antagonist) and the affinity estimate was 9.9 compared with only 7.2 for methoctramine (Table 2, Figures 1 and 2).

Electrical field stimulation caused contraction of the mouse bladder strips and these were not potentiated in

Table 2. The mean maximum responses, pEC_{50} and pKB values of the mouse urinary bladders in response to carbachol in the presence of muscarinic antagonists 4-DAMP and methoctramine.

	pEC_{50}		Mean maximum response (g)			
Antagonist	Control	Antagonist	Control	Antagonist	pKB	n
4-DAMP	6.50±0.32	4.78±0.10	0.92±0.18	0.94±0.22	9.94±0.18	6
Methoctramine	6.31±0.09	4.89±0.37	1.44±0.26	1.52±0.48	7.2±0.22	5

Figure 1. Antagonism of detrusor responses to carbachol by 4-DAMP (10nM). Cumulative concentration-response curves to carbachol in the absence and presence of 4-DAMP. Responses are expressed as a percentage of the maximum response obtained for the first curve (pKB= 9.94±0.18).

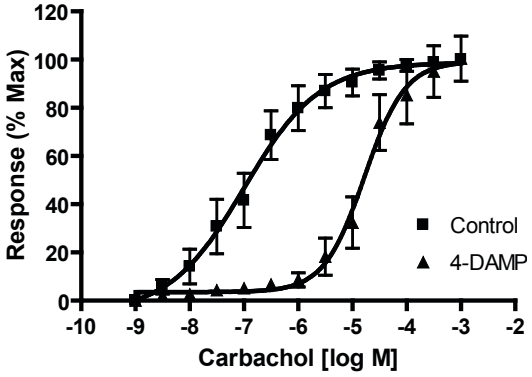
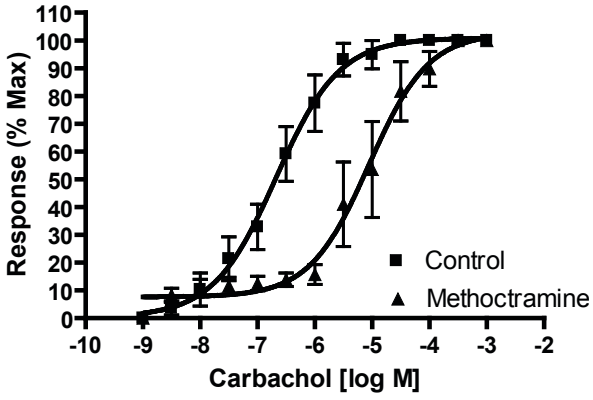


Figure 2. Antagonism of detrusor responses to carbachol by methoctramine (3μM). Cumulative concentration-response curves to carbachol in the absence and presence of methoctramine. Responses are expressed as a percentage of the maximum response obtained for the first curve (pKB= 7.2±0.22).



the presence of the nitric oxide synthase inhibitor L-NNA (responses were 97.8±7.9% of controls). However, the contractile responses to field stimulation were depressed in the presence of inhibitors of cholinergic, adrenergic and purinergic neurotransmission. In the presence of the muscarinic agonist atropine, the adrenergic neurone blocker guanethidine and the purinergic P2X receptor desensitiser α,β-methylene-ATP responses to field stimulation were reduced to 58.6±5.1% (p=0.011), 88.5±2.7% (p=0.018) and 62.2±3.6% (p=0.009), respectively. In combination these drugs reduced responses to field stimulation by 89% and the remaining response was almost completely abolished by the neurotoxin, tetrodotoxin indicating that they were non-neuronal in origin.

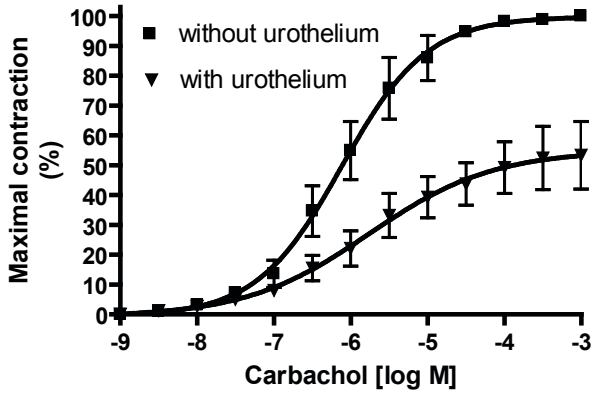
The presence of an intact urothelium significantly depressed responses to carbachol, the maximum responses being reduced from 2.2±0.8g to 1.18±0.6g in the presence of the urothelium (p=0.02). The addition of indomethacin and L-NNA to remove prostaglandin and nitric oxide production respectively did not prevent the inhibitory effect of the urothelium, the inhibition being 39.7±11.2% in the presence of these drugs and 48.6±9.2% in control tissues (Figures 3 and 4).

4. Discussion

Electrical field stimulation of detrusor smooth muscle strips from the mouse bladder induced contractile responses that were sensitive to atropine, guanethidine and α,β-mATP, but were not influenced by the presence of L-NNA. Responses were completely abolished by the neurotoxin tetrodotoxin indicating that responses were neurogenic in origin. Since α,β-mATP and atropine reduced responses by 41% and 38% respectively, these data suggest that in this species Ach and ATP act equally as neurotransmitters while NA and NO exert only minor effects. In most species Ach is the main transmitter in the bladder with a variable contribution from ATP [7]. In the human bladder, Ach appears to be the sole or predominant transmitter in healthy detrusor tissue [8-10] but with ATP playing an enhanced role in the overactive bladder via P2X1 receptors [8,11]. Stronger contractile responses to ATP have also been detected in bladder tissues obtained from patients with overactive bladders compared with normal subjects suggesting the involvement of ATP in detrusor overactivity [12]. In the mouse the ATP contribution is almost equal to that of Ach, suggesting that the mouse bladder most resembles the diseased human bladder.

Five subtypes of muscarinic receptors (M1-5) have been cloned and in urinary bladder smooth muscle it is

Figure 3. Responses to carbachol of detrusor strips with an intact urothelium or with the urothelium removed. Responses are plotted as a percentage of the maximum response of the denuded tissue (p=0.02).

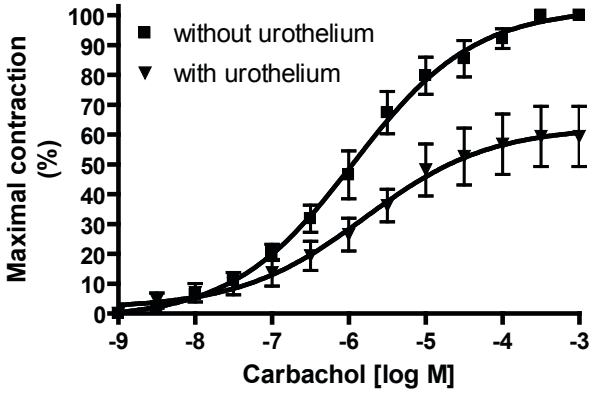


the M2-muscarinic receptor subtype that predominates at the protein level with a smaller population of M3-receptors also being present [13]. Surprisingly it is the minor population of M3-receptors that mediates detrusor muscle contraction *in vitro* in all species so far examined (for review see ref 14) including human [15,16] and mouse bladder [17]. Recent studies using mutant mice lacking M2 [18], M3 [19] or both [20] also suggest that the M3 receptor is the predominant muscarinic receptor subtype mediating detrusor contraction in vivo. The present study further supports the idea that M3 receptors are responsible for detrusor contraction in the mouse, since responses to carbachol were antagonised more potently by the M3-antagonist 4-DAMP than the M2-antagonist methoctramine.

Contractile responses to sympathomimetic agents (noradrenaline and phenylephrine) were also observed in this study. Although the prostate and bladder neck were removed under the microscope, complete removal proved difficult due to the small size of these preparations and therefore some remaining bladder outlet tissue would explain the responses to these sympathetic agents and also the small (11%) but significant effect of the adrenergic neurone blocker on field stimulated responses.

In the urinary bladder, β-adrenoreceptors are predominant over α-receptors postjunctionally [21]. It has been demonstrated that β1, β2, and β3 AR mRNAs are expressed in human detrusor muscle, which is effectively relaxed by β3-AR agonists [22]. In the rat bladder, it was suggested that relaxation of detrusor is mediated by β2 and β3 ARs [23,24]. In our study the nonselective β-adrenoceptor agonist isoprenaline caused 63% inhibition of carbachol precontracted detrusor muscle strips supporting the relaxation effect of β-ARs in the mouse bladder, although the receptor subtype present was not determined.

Figure 4. Responses to carbachol of detrusor strips with an intact urothelium or with the urothelium removed after L-NNA (10μM) and indomethacin (5μM) administration. Responses are plotted as a percentage of the maximum response of the denuded tissue.



L-Arginine-derived nitric oxide (NO) is the major inhibitory non-adrenergic non-cholinergic (NANC) neurotransmitter in the bladder outlet [8,25] and the presence of NO synthase (NOS) has been demonstrated in lower urinary tract of animals and humans [26-28]. However, the influence of NO on bladder detrusor smooth muscle appears minor. NANC-mediated relaxation in the human detrusor is short lasting and only seen occasionally. Fujiwara *et al.* [29] have identified nNOS immunoreactive nerves in the mouse bladder, but neither L-arginine nor the NOS inhibitor N-nitro-L-arginine exerted any effect on bladder contractions induced by electrical field stimulation. In the same study it was found that NO-donors cause accumulation of cGMP in mouse bladder, but do not induce smooth muscle relaxation. In support of this finding, our results suggest that NO does not seem to have much influence on detrusor contractility since neurogenic responses were not potentiated by inhibition of NOS with L-NNA. The mouse is therefore similar to human but the role of NO in the bladder body has yet to be established.

In conclusion, neurotransmission in the mouse bladder is mediated primarily via Ach and ATP with NO and NA having only minor roles. This is similar to that seen in the human bladder except that the large purinergic component suggests that the mouse most resembles the diseased human bladder. As in the human bladder, relaxation can be induced by β-adrenoceptor agonists whilst the urothelium releases a factor to inhibit detrusor contraction. These data suggest that the mouse is a good model for studying human bladder physiology/pharmacology.

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