Human, pig and guinea-pig bladder smooth muscle cells generate similar inward currents in response to purinoceptor activation

¹Ryuji Inoue & ²Alison F. Brading

University Department of Pharmacology, South Parks Road, Oxford OX1 3QT

The contribution of purinergic neurotransmission to bladder excitation in pigs and man is small. Exogenously-applied adenosine-trisphosphate (ATP) however, elicits large inward currents in dispersed bladder smooth muscle cells in both species. The essential properties of the ATP-induced current in human and pig detrusor are similar and the current intensity is comparable to those in the guinea-pig, which has a powerful purinergic excitatory innervation. This suggests that other features of the tissue such as the closeness of the innervation and the degree of cell-to-cell coupling may be important in determining the effectiveness of purinergic transmission.

Keywords: Smooth muscle; ATP; urinary bladder; purinoceptors; purinergic transmission

Introduction The urinary bladder of small mammals receives a dual excitatory innervation from cholinergic and non-adrenergic, non-cholinergic (NANC) nerves. Purinergic transmission is probably responsible for the latter, as demonstrated by various functional (Burnstock *et al.*, 1978) and electrophysiological studies (Fujii, 1988). The relative contribution of cholinergic and purinergic transmission varies markedly amongst species, the purinergic contribution being 70–80% at high frequencies in guinea-pigs (Inoue & Brading, 1990) and 15% in pigs (Sibley, 1984). An atropine-resistant tetrodotoxin-insensitive contribution has not been found in normal human bladders from cadaver donors (Sibley, 1984), although a small NANC component has been seen occasionally in some strips from patients with bladder disorders (Sjögren *et al.*, 1982; Hoyle *et al.*, 1989; Luheshi & Zar, 1990; Ruggieri *et al.*, 1990). The differences in the purinergic innervation could be caused by differences in the postsynaptic purinoceptors, or by differences in the organization and innervation of the bladder. In this short paper, in order to distinguish between these possibilities, we describe the effects of postsynaptic purinoceptor activation on membrane currents in single cells from the detrusor of guinea-pigs, pigs and man, and conclude that any small inter-species differences present cannot account for the different degrees of purinergic transmission in the whole animal.

Methods Urinary bladders of Landrace pigs were obtained from a slaughter house and human bladders from patients undergoing cystectomy for bladder cancer at the John Radcliffe Hospital, Oxford. In the latter case, only tumour-free areas of the bladder wall were used, and the results in Table 1 are from four bladders. After preincubation in Ca-free solution, small pieces of detrusor were digested in Ca-free solution supplemented with 0.2% collagenase (Sigma type I and Boehringer) and 0.2% pronase (Serva, type E) for 30–45 min at 36°C with continuous stirring. The resultant cell suspension was diluted 1:1 in modified Krebs solution as previously described (Inoue & Brading, 1990).

The physiological saline solution contained (mm): Na⁺ 140, K⁺ 6, Ca²⁺ 2, Mg²⁺ 1.2, Cl[−] 152.4, glucose 10 and HEPES 10, titrated to pH 7.35–7.40 with Tris base. Ca-free solution was prepared by omitting Ca and Mg from the solution. The

internal pipette solution contained (mm): Ca⁺ 140, Mg²⁺ 2, Cl[−] 144, HEPES 10 and EGTA 1 or 10, titrated to pH 7.2 with Tris base. The recording system was the same as previously described (Inoue & Brading, 1990). For analysis, data were digitized through an A/D converter (DT2801A, Data Translation) under the control of an IBM compatible computer using a programme written in Quick Basic 6.0. For illustrations, an intelligent digital plotter (HP7475A, Hewlett Packard) was used. ATP was made up just before use and applied by use of a concentration-jump apparatus (Inoue & Brading, 1990).

Results Single cells obtained from human or porcine detrusor appear bigger than those from the guinea-pig and have a larger surface area as estimated from the input capacitance (Table 1). The majority of cells contracted immediately on exposure to ATP. When cells were clamped at −60 mV, rapid application of ATP elicited a fast activating, rapidly desensitizing inward current with a very short latency (less than 100 ms) in a concentration-dependent manner in all cells studied (Figure 1). The properties of the ATP-induced inward current are summarized for each species in Table 1. The maximum intensity of the ATP-induced inward current did not differ significantly between the three species. However, half-maximal activation of the current required somewhat higher concentrations for pig and human detrusor than for

Table 1 Characteristics of ATP-induced inward currents recorded in porcine, human and guinea-pig detrusors

	Pig	Man	Guinea-pig
Input capacitance (pF)	80 ± 10 (n = 10)	106 ± 27 (n = 8)	49 ± 14*
Maximum current intensity (μA/μF)	6.7 ± 4.2 (n = 6)	21.1 ± 18.7 (n = 6)	19.6 ± 23.4
50% activation (μM)	3–10 (n = 3)	10–30 (n = 5)	2.3*
Reversal potential (mV)	+1.4 ± 2.3 (n = 3)	+3.2 ± 1.8 (n = 4)	+1.2 ± 1.9*

The maximum current intensity was calculated by dividing the maximum current amplitude recorded at −60 mV by the input capacitance; 50% activation and reversal potentials were estimated from a series of recordings from the same cell by changing the concentration of ATP or the holding potential, respectively.

The data with a * are taken from Inoue & Brading (1990) (n = 21).

¹ Present address: Department of Pharmacology, Kyushu University, Fukuoka 812, Japan.

² Author for correspondence.

Figure 1 ATP activates large inward currents in pig (a) and human (b) detrusor. The currents recorded at three different concentrations of ATP are superimposed and labelled. ATP was applied by a concentration-jump method and was present throughout each record. Note the rapid activation and inactivation of the inward current, and the difference in the current scale on the two panels.

guinea-pig, although the limited number of trials with the large mammals precludes exact evaluation. The reversal potentials were not significantly different, suggesting similar ionic selectivity of the channels in the three species. In addition, the current was greatly reduced by removal of extracellular Na and was virtually abolished in the absence of Ca and Na (not shown). Insensitivity to a high concentration of intracellular Ca buffer (10 mM EGTA) was also observed among all species and α,β -methylene ATP blocked the response to a following application of ATP in both guinea-pig and pig.

Discussion The present results strongly suggest that the smooth muscle cells of the detrusor of the three species studied all possess a high density of ATP receptors, activation of which can generate large inward currents. The currents have remarkably similar properties, and the small differences recorded in their sensitivity to ATP cannot account for the

weakness of the purinergic innervation in the pig, and it certainly cannot account for the frequent lack of demonstrable purinergic innervation in human bladder strips.

One explanation could be that ATP is not released from intrinsic nerves in man, whilst varying amounts are released in other species. However, ATP is normally thought to be co-released with acetylcholine from peripheral cholinergic nerves, and all three species have a relatively dense and uniform distribution of cholinesterase-positive intrinsic fibres. There is little firm evidence to suggest the existence of separate purinergic nerves. It is thus perplexing that with a large population of ATP-activated channels and a parasympathetic innervation, a purinergic component is either small or absent in human bladder in response to nerve stimulation.

The explanation may lie in differences in the detailed anatomy of the innervation, combined with the rapid desensitization of the ATP-activated channels and poor electrical coupling between the smooth muscle cells in the detrusor. A relatively high concentration of ATP is needed to activate the channels, and if the varicosities releasing transmitter vary in their closeness to the smooth muscle, ATP will undergo diffusional dilution and hydrolysis before reaching the receptors. A slow rise in the concentration of ATP at the receptors may also desensitize them before significant activation occurs. If only a small proportion of cells is innervated, poor electrical coupling would also restrict the spread of excitation. An increase in the cell-to-cell coupling as has been suggested in detrusor instability (Foster *et al.*, 1989) might account for the appearance of a purinergic component of excitatory innervation in this condition (Sjögren *et al.*, 1982).

In conclusion, the variations in the purinergic component of excitatory innervation seen in bladder smooth muscles of different species, cannot be accounted for by differences in the properties or density of the postsynaptic purinoceptors.

This work is supported by Bristol-Myers Squibb Co.

References

- BURNSTOCK, G., COCKS, T., CROWE, R. & KASAKOV, L. (1978). Purinergic innervation of the guinea-pig urinary bladder. *Br. J. Pharmacol.*, **63**, 125–138.
- FOSTER, C.D., SPEAKMAN, M.J., FUJII, K. & BRADING, A.F. (1989). The effects of cromakalim on the detrusor muscle of human and pig urinary bladder. *Br. J. Urol.*, **63**, 284–294.
- FUJII, K. (1988). Evidence for adenosine-trisphosphate as an excitatory transmitter in guinea-pig, rabbit and pig urinary bladder. *J. Physiol.*, **404**, 39–52.
- HOYLE, C.H.V., CHAPPLE, C. & BURNSTOCK, G. (1989). Isolated human bladder: evidence for an adenine dinucleotide acting on P_2 -purinoceptors and for purinergic transmission. *Eur. J. Pharmacol.*, **174**, 115–119.
- INOUE, R. & BRADING, A.F. (1990). The properties of the ATP-induced depolarization and current in single isolated cells from the guinea-pig urinary bladder. *Br. J. Pharmacol.*, **100**, 619–625.
- LUHESHI, G.N. & ZAR, M.A. (1990). Presence of non-cholinergic motor transmission in human isolated bladder. *J. Pharm. Pharmacol.*, **42**, 223–224.
- RUGGIERI, M.R., WHITMORE, K.E. & LEVIN, R.M. (1990). Bladder purinergic receptors. *J. Urol.*, **144**, 176–181.
- SIBLEY, G.N.A. (1984). A comparison of spontaneous and nerve-mediated activity in bladder muscle from man, pig and rabbit. *J. Physiol.*, **354**, 431–443.
- SJÖGREN, C., ANDERSSON, K-E., HUSTED, S., MATTIASSEN, A. & MOLLER-MADSEN, B. (1982). Atropine resistance of transmurally stimulated isolated human bladder muscle. *J. Urol.*, **128**, 1368–1371.

(Received April 16, 1991)

Accepted April 25, 1991)