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Non-adrenergic non-cholinergic neurotransmission at the hamster lower urinary tract

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It is well known that excitatory neurotransmission in the urinary bladder arises predominantly through activation of parasympathetic nerves, and it is only partially cholinergic. In many rodents such as guinea-pig [1], rabbit [2] and rat [3] the neurogenic response of the detrusor smooth muscle evoked by electrical field stimulation is composed by a cholinergic and a larger purinergic component. In agree-ment, atropine alone or atropine plus the P2 receptor an-tagonist suramin, were able to reduce the frequency-evoked contractions of hamster detrusor preparations by about 20% and 90%, respectively [4]. The residual contraction was blocked by indomethacin, whereas tetrodotoxin completely inhibited the frequency-dependent contractions, confirming the neurogenic origin of purinergic neurotransmission (Fig. 1). Furthermore, in hamster detrusor preparations, exogenous ATP and the stable ATP analogue, α,β-methylene ATP evoked rapid and transient contractile responses that were impaired by suramin.

If ATP is the main excitatory neurotransmitter at the bladder level from several species, nitric oxide is the principal inhibitory neurotransmitter involved in the non-adrenergic non-cholinergic (NANC) relaxant response of the urethra, bladder neck and trigone from various mammals including man [5], pig [6], rabbit [7,8], rat [9], sheep [10], cat [11] and dog [12]. In dog and pig urethra the neurogenic relaxation appears to be produced by an unknown neurotransmitter in addition to nitric oxide [12-14]. In hamster proximal urethra too, NANC inhibitory responses to electrical field stimulation are mainly due to nitric oxide [15,16], since are reduced, though not completely blocked, by the nitric oxide synthase-inhibitor L-NG-nitroarginine methyl ester (L-NAME) (Fig. 2A, 2B). The inhibitory responses to electrical field stimulation and to exogenous ATP were also impaired by the non-selective P2X/P2Y receptor antagonist suramin and the more P2Y-selective antagonist Reactive Blue 2, but not by the more P2X-selective antagonist pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS), furthermore were completely inhibited by tetrodotoxin, suggesting that in the hamster proximal urethra ATP may be released as a cotransmitter together with nitric oxide [17]. Our recent pharmacological studies (data unpublished) might supp ort the hypothesis that ATP acts as an inhibitory neurotransmitter on P2Y receptors located on the smooth muscle layer of the hamster proximal urethra. The slow, sustained and not desensitilsing relaxant response to ATP (Fig. 2C) seemed to be evoked by activation of P2Y receptors, based on responses obtained with the P2Y agonists, 2-methylthio ADP and 2-methylthio ATP, and more importantly, based on the involvement of the G-proteins. In fact, The G-protein blocking agent guanosine 5'–O-(2-thiodiphosphate) significantly impaired ATP relaxant responses.

In the hamster urethra, exogenous vasoactive intestinal polypeptide was able to induce concentration-related relaxant responses, however [Lys^1, $Pro^{2,5}$, $Arg^{3,4}$, Tyr^6]-VIP, a reported competitive vasoactive intestinal polypeptide antagonist and α-chymotrypsin were unable to impair either frequency-induced inhibitory responses or relaxant responses to exogenous vasoactive intestinal polypeptide [17], suggesting that it is unlike that this compound acts as an inhibitory neurotransmitter at the hamster urethra level, as already observed in pig [18,19].

Prostaglandins, particularly PGE_2 and $PGF_{2\alpha}$ are potent spasmogens of detrusor smooth muscle of urinary bladder [20]. In hamster urethra PGE_2 caused concentration-related relaxations whereas $PGF_{2\alpha}$ evoked concentration-related contractions [15]. Indomethacin reduced significantly the relaxations induced by exogenous ATP in urethral strips. This result suggests that, as in the urinary bladder [21], prostaglandins play an important role in urethral neurotransmission processes and that activation of P2Y-purinoceptor by ATP can evoke synthesis and release of prostaglandins with relaxant effects.

In conclusion, ATP and/or related purines may have a role as excitatory cotransmitters together with acetylcholine at the bladder level, whereas it may be considered an inhibitory cotransmitter together with nitric oxide at the hamster urethra level. The synthesis of endogenous prostaglandins with contractile and/or relaxant effects, secondarily to neural activity, could be important in maintaining the tone in the smooth muscle, as shown in the rabbit proximal urethra [22].

Fig. 1

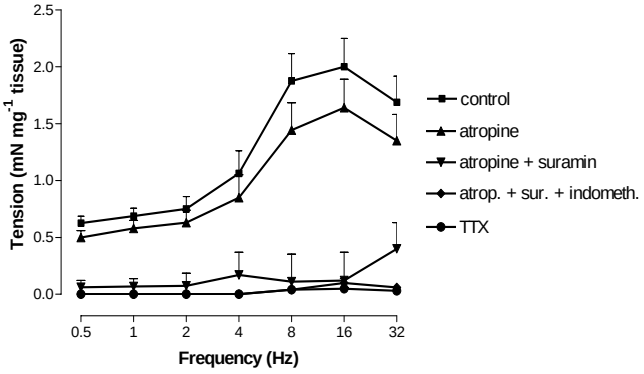

Fig. 2A

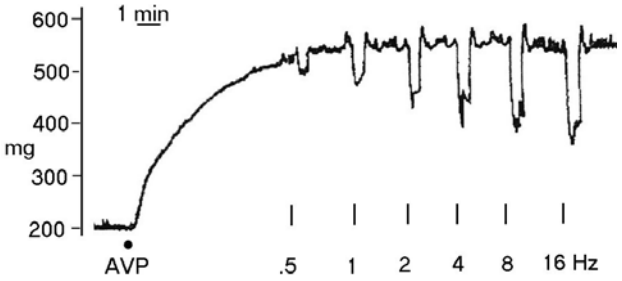

Fig. 2B

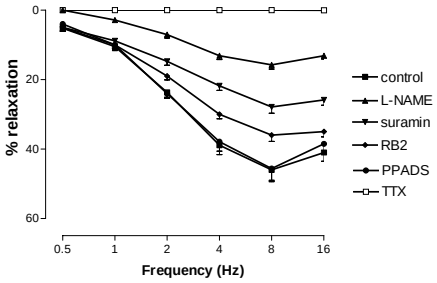

Fig. 2C

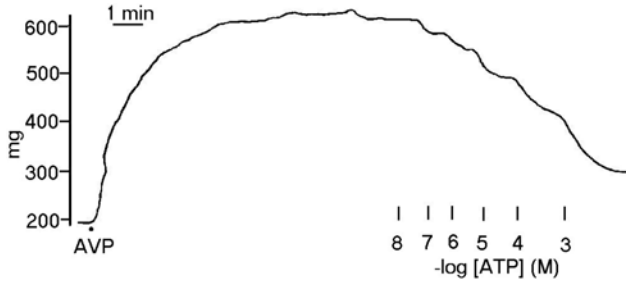

Figure 1: *Frequency-response curves to EFS (70 V, 0.3 ms, 0.5 - 32 Hz) in longitudinal smooth muscle preparations of hamster urinary bladder, before (control) and after incubation with atropine (10^{-6} M), atropine (10^{-6} M) plus suramin (10^{-4} M), atropine (10^{-6} M) plus suramin (10^{-4} M) plus indomethacin (10^{-6} M), tetrodotoxin (TTX: 10^{-6} M). Points show the mean ±s.c. mean of 7 experiments, unless occluded by symbol.*

Figure 2: *Responses of strips of hamster proximal urethra precontracted with arginine vasopressin (AVP, 10^{-8} M) in response to electrical field stimulation (80 V, 0.3 ms, 1 - 16 Hz).* ***A****) Original tracing of frequency-induced relaxation.* ***B****) Frequency-response curves before (control) and after incubation with L-N^{G}-nitroarginine methyl ester (L-NAME: 10^{-4} M), suramin (10^{-4} M), reactive blue 2 (RB2: $2x10^{-4}$ M), pyridoxalphosphate-6-azophenil-2',4'-disulphonic acid (PPADS: 10^{-4} M) and tetrodotoxin (TTX: 10^{-6} M). Propranolol (10^{-6} M), phentolamine*

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