

Evidence against purinergic motor transmission in guinea-pig urinary bladder

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Much of the bladder motor transmission is atropine-resistant in many species. In guinea-pig detrusors, Ambache & Zar (1970) reported that ATP induced brief, small contractions, not maintained for >10 s despite the continued presence of ATP for 30 s; and, with closely-spaced repetitions, tachyphylaxis to ATP occurred sometimes, without corresponding decline in transmission. The following results provide further evidence against purinergic transmission.

From 11 guinea-pigs mucosa-free strips (10–20 mm × 2–4 mm) were cut sagittally over the apex, avoiding trigone and ganglion-containing bladder-neck, and were suspended at 35°C in Krebs (Rang, 1964) ± atropine (2.9 μM) ± hexamethonium (280 μM). Tetrodotoxin-susceptible twitches were elicited by transmural stimulation (1–32 pulses; 0.1 ms; 10 Hz). Disodium ATP (Sigma; pH 3.4 at 1%) was neutralized with solid NaHCO₃. To avoid tachyphylaxis, ATP-contacts were reduced to 15 s and spaced >10 min apart.

Smooth muscles are usually sensitive to their motor neurotransmitters. But Figure 1 (typical of 11 preparations) illustrates the remarkable insensitivity of atropinized detrusors to ATP, and ATP's inability to mimic the growth of the atropine-resistant motor transmission, even in such massive dosage as 15 mM (0.8%). Thus, the ATP-receptors do not possess the characteristics displayed by the transmission and expected of the post-synaptic receptors for the unknown motor transmitter.

This insensitivity to ATP is difficult to reconcile with purinergic transmission in guinea-pig bladder and supports Ambache & Zar's (1970) conclusion, subsequently denied by Burnstock, Dumsday & Smythe (1972). However, their finding that most rat bladders failed to respond to ATP, even at 330 μM, in fact suggests non-purinergic transmission in the rat, too.

The adenosine-uptake inhibitors dipyridamole (0.02–2 μM), hexobendine (1.5 μM) and dilazep (1.5 μM) failed to potentiate ATP or neurogenic responses.

Prolonged exposures to large doses of ATP, 1.8 mM, reduced histamine and acetylcholine responses, indicating muscle depression.

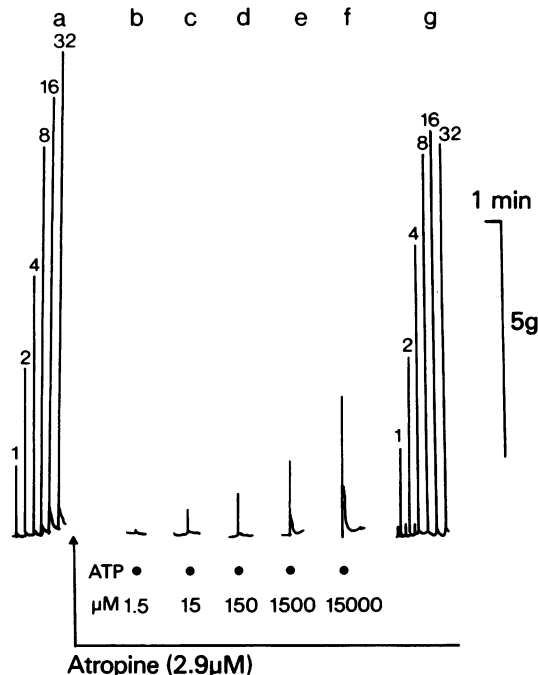


Figure 1 Guinea-pig detrusor, isometric record; atropine (2.9 μM), present after (a). Relative insensitivity to ATP and inability to match the growth of atropine-resistant neurogenic responses as the ATP-concentration is increased in tenfold steps up to 10,000 times threshold.

Panels (a) and (g) show, before and after atropine, the growth of twitches elicited at 1 min intervals by transmural stimulation with 1–32 pulses of 0.1 ms (10 Hz; constant voltage). In panels b–f, responses to neutralized ATP (in atropine); to avoid tachyphylaxis, the administration of ATP was spaced at intervals of at least 10 min and each dose was given for only 15 s: at (b), 1.5 μM (0.8×10^{-6} g/ml); (c) 15 μM; (d), 150 μM; (e), 1500 μM and (f), 15,000 μM. The maximum ATP-response is only 35% of the largest twitch in panel (g). Note the disparity between the growth of the neurogenic responses with each doubling of the number of pulses and that of the ATP-responses with each tenfold increase in concentration.

References

- AMBACHE, N. & ZAR, M. ABOO. (1970). Non-cholinergic transmission by post-ganglionic motor neurones in the mammalian bladder. *J. Physiol. (Lond.)*, **210**, 761–783.
- BURNSTOCK, G., DUMSDAY, B. & SMYTHE, A. (1972). Atropine resistant excitation of the urinary bladder: the possibility of transmission via nerves releasing a purine nucleotide. *Br. J. Pharmac.*, **44**, 451–461.
- RANG, H.P. (1964). Stimulant actions of volatile anaesthetics on smooth muscle. *Br. J. Pharmac.*, **22**, 356–365.