

THE EFFECT OF THE METABOLIC INHIBITOR, ANTIMYCIN A₃, ON THE PHARMACOLOGICAL RESPONSIVENESS OF THE RAT DETRUSOR MUSCLE IN VITRO

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The non-adrenergic, non-cholinergic (NANC) mediated contractile responses observed in mammalian detrusor muscle preparations in vitro following transmural and direct (field) stimulation (1), have been examined in superfused preparations of the rat detrusor muscle during inhibition of the mitochondrial ATP-energising electron transport system (ETS) (2).

Glass suction electrodes have been utilised to specifically stimulate the fine postganglionic parasympathetic nerve fibres innervating the detrusor (trigone-free) muscle preparations to avoid non-specific stimulation of the musculature and intramural neuronal components as in previous studies using 'transmural' stimulation techniques (1). Preparations were stimulated indirectly (neural) with pulse trains (5.0V/0.5ms at 100 Hz/550ms) via glass suction electrodes and then directly (5.0V/1.5s) with Pt-field electrodes 40s later. This pattern of indirect/direct stimulation was repeated every 90s. The detrusor muscle preparations were secured with fine steel pins to the Sylgard 184 resin base floor of a Res-Del perfusion bath and the contractions measured via a Grass FT03 force transducer. Preparations superfused with aerated (95% O₂ / 5% CO₂) RS-I saline (3) at 4.0cm³ min⁻¹ and maintained at 35.0 ± 0.3°C exhibited little (<15%) change in contractile responsiveness over a 24 hr period of in vitro experimentation.

During the superfusion of RS-I containing 10⁻⁶ M antimycin A₃, two distinct phases occurred in the contractile responses to electrical and pharmacological induced stimulation. In the first 2 hrs (phase I) there was a significant reduction (ca.50-80%) in the indirect and direct evoked contractures. In the presence of 10⁻⁶ M atropine there was an absence of 'atropine-resistant' contractions at the end of phase I. Over the next 3 hr (phase II) a 70% restoration of the direct evoked contractile responses occurred and remained as such for a further 3 hrs. Interestingly, the indirect evoked responses showed a similar recovery but then declined to 10% of the initial amplitude within 90 min. During phase II there was a noticeable increase in spontaneously occurring contractions which were unaffected by atropine (10⁻⁵ M) or physostigmine (10⁻⁶ M) but reduced in activity in the presence of adenosine (10⁻⁵ M). Topical applications of ATP (10⁻⁶ M) and ACh (10⁻⁸ M) evoked relatively constant contractile responses over the experimental period.

The effects of antimycin A₃, a specific inhibitor of the mitochondrial ETS and ATP synthesis, have indicated that in the rat detrusor muscle the NANC neuronal components are far more sensitive, in comparison to the cholinergic components, to inhibition of mitochondrial ATP synthesis as might be anticipated if the rat detrusor muscle contained 'purinergic' innervation (1).

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2. Rees, D. (1974b) The effect of metabolic inhibitors on the cockroach nerve-muscle synapse, *Journal of Experimental Biology*, 61; 331-343.
3. Rees, D. (1985) The effect of phosphate buffered RS-I saline on the contractility of the isolated perfused heart preparation. *Proceedings of the Physiological Society of New Zealand*, 5; 18p.