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SYNERGY BETWEEN NEUROTRANSMITTER ACTIONS IN TRIGONAL SMOOTH MUSCLE

Hypothesis / aims of study

The trigone is distinct from the dome of the bladder in having different innervation and structure, but its function remains unclear. In part this is due to a poor understanding of the factors that regulate its contractile function, and when in the micturition cycle these are active. Theer are two distinct layers: a deeper one which is a continuation of the detrusor; and a superficial layer that develops with the ureter. The NO-cGMP system has been implicated in relaxing the tissue (1) but there are no studies that describe the relative significance of the muscarinic, adrenergic and purinergic systems in regulating contractility, and any interaction that may exist between these potential excitatory systems. The aim of the study was to investigate the relative importance and any interplay between these excitatory systems.

Study design, materials and methods

Experiments used male guinea-pigs; the bladder with adjacent urethra was removed and the dome resected cranially to the ureteral entrance into the dorsal bladder base. The base was longitudinally opened on the ventral site and the trigone exposed. After removing the mucosa, a thin strip of muscle (0.5 mm diameter) between the ureteral orifices was cut from the superficial part of the trigone. Strips were superfused with a CO_2/HCO_3^- buffered medium (pH 7.4, 37^{o}C) trough and contractile responses evoked either by field stimulation (EFS, 0.1 ms pulses, 1-64 Hz, 3-s trains) or exposure to exogenous agonists. To evaluate the effects of ROK- and PKC-inhibitors, tissues were incubated with 5 μM Y-27632 and 5 μM GF 109203 X, respectively, and then challenged with different agonists. Values are means±SD and significant ($p<0.05$) differences between the data sets were evaluated by Student´s *t*-tests.

Results

The α-agonist phenylephrine (PE) and the muscarinic agonist carbachol generated contractures with respective EC_{50} values of 15.2±1.2 (n=7) and 22.5±1.3 μM (n=10) and maximum responses of 21.4±2.9 and 57.3±25.9 mN.mm^{-2}. Raised KCl had an EC_{50} of 56.5±1.1 mM (n=9) and a maximum of 65.7±27.2 mN.mm^{-2}. EFS generated contractions with half-maximal frequency at 23.0±1.6 Hz (n=9) and a maximum response of 30.2±7.2 mN.mm^{-2}. In separate experiments 1 □M atropine, prazosin and ABMA (to desensitise purinergic receptors) reduced EFS contractions by 68.5±4.9% (n=7), 30.1±10.0% (n=10) and 18.6±6.3% (n =7), respectively. The sum of the individual reductions (117.2%) was significantly greater than 100%, suggesting a synergistic effect between adrenergic, muscarinic and purinergic pathways. To elucidate the synergistic effects, EFS was carried out in the presence of agonists. PE (3 or 10 μM) enhanced contractions by 3.5±0.7 and 6.4±1.5-fold, respectively. A PE concentration >10 μM did not further enhance contractions. Prazosin (0.1 μM) completely abolished the basal contraction and the augmented EFS response (n=7). A low carbachol concentration (0.3 μM) also augmented EFS contractions by 2.4±0.4-fold. To show that the augmenation was not presynaptic but on the muscle, carbachol, ABMA and high-KCl responses were measured in the presence of 10 μM PE. PE augmented contractions induced by carbachol (1 μM; 3.9±1.2-fold control), the non-hydrolysable ATP analogue ABMA (1 μM; 4.3±0.4-fold) and 10-20 mM KCl (5.0±2.0-fold). Calcium-sensitization of contractile proteins by rho-kinase (ROK) and protein kinase C (PKC) play a role in detrusor. We investigated the effect of the ROK-inhibitor Y-27632 and the PKC-inhibitor GF 109203 X (both 5 μM) on contractions evoked by 10 μM PE, 3 μM carbachol and 60 mM KCl (n = 7). Both agents reduced significantly the PE contracture to 54.9±7.8% and 37.3±7.4% control, respectively; with no significant effects on the other contractures. EFS contractions (32 Hz) were also attenuated by GF 109203 X and Y-27632: 43.1 ± 8.9 and 77.8 ± 19.4 % control, respectively.

Interpretation of results

We have demonstrated the predominant muscarinic control of EFS-induced contractions in the superficial trigone. To our knowledge, this is the first study to demonstrate synergistic effects in neurotransmitter activation of trigonal muscle. That PE also potentiates ABMA and carbachol contractures suggests the effect is on the muscle. The effect was not merely additive as the activating concentrations of PE or carbachol produced only minor contractions themselves (1-12% of EFS response). A small rise of intracellular calcium ($[Ca^{2+}]_i$), by pre-activating muscarinic or adrenergic receptors could result in a subsequent further increase of $[Ca^{2+}]_i$ by EFS or another agonist being on a steeper region of the $[Ca^{2+}]_i$-tension curve. However, an increase of $[Ca^{2+}]_i$ by raising extracellular KCl was effective in augmenting EFS contractions only over a limited range (10-20 mM) and the effect was at most only 2.3-fold over control, unlike the 6-fold increase with PE. These data are therefore consistent with the hypothesis that agonists sensitises the muscle to further agonist application. The selective reduction of the PE contracture by ROK and PKC inhibitors suggests that, for this agonist in particular, this is the sensitising pathway; the second messengers remain to be elucidated.

Concluding message

Trigonal muscle demonstrates muscarinic, adrenergic and purinergic mechanisms for nerve-mediated activation. These different systems act synergistically to generate contraction. For adrenergic systems at least this synergy is modulated via the rho-kinase pathway.

References

1 Scand J Urol Nephrol Suppl. 1995;175:43-53.

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