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THE EFFECT OF ACIDOSIS IN THE INTRA AND EXTRACELLULAR COMPARTMENTS ON NERVE MEDIATED CONTRACTION IN CORPUS CAVERNOSUM

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Priapism is defined as a pathological condition where penile erection persists beyond, or is unrelated to sexual stimulation. In patients with ischaemic priapism, corporal blood aspirates are acidotic. One hypothesis for ischaemic priapism is reduced contractile function of cavernosal smooth muscle which may be secondary to this alteration in pH. This study aims to determine the effect of acidosis on nerve-mediated contraction in cavernosal smooth muscle and whether intracellular or extracellular pH changes mediate contractile alterations.

Male Duncan-Hartley guinea pigs were humanely killed by cervical dislocation. Isometric contractions were recorded from strips of guinea pig corpus cavernosum in response to electrical field stimulation (EFS at 60 Hz, sensitive to 1 μM tetrodotoxin). Strips were superfused at 37°C with a $HCO3^-/CO_2$ buffered solution (pH 7.39). Acidosis in both the intra and extracellular compartments was generated by increasing the CO_2 content of the superfusate gas mixture from 5% to 10% (pH 6.99). [$HCO3^-$] was reduced in the superfusate in order to create an acidotic extracellular environment (pH 6.97). Intracellular acidosis was mimicked by increasing the CO_2 percentage and superfusate [$HCO3^-$] in proportion (pH 7.44). Total [Ca] was altered appropriately to maintain Ca^{2+} activity. Data are mean ± s.d. Statistical differences ($p<0.05$) between data sets were examined with Student's t tests.

Acidosis induced in both compartments had no significant effect on the EFS response (after 60 mins. 103±12%, 120 mins. 100±11% of control, n=6). Furthermore, tension remained unchanged after 60 mins. of return to normal pH (100±11% of control).

A reduction in extracellular pH alone significantly depressed tension (after 60 mins. 73±12%, 120 mins. 68±15% of control, n=6). After reperfusion with control superfusate for 60 minutes tension partially recovered to 82±11% of control.

By contrast, alteration of intracellular pH significantly augmented tension after 60 min (110±4% of control, n=6). This increase was not sustained at 120 mins. (103±6% of control, n=6). Tension remained unchanged after 60 minutes of reperfusion (90±8% of control)

Corporal blood aspirates from patients with ischaemic priapism show evidence of hypoxia, glucopenia and acidosis. This study would suggest that the detrimental effect of extracellular acidosis on nerve-mediated contraction of cavernosal smooth muscle appears to be ameliorated by the effect of a reduction in intracellular pH. The mechanisms by which intracellular acidosis exerts this protective effect and whether this is sustained in the presence of the other components of ischaemia needs to be assessed.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

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Contractile Activation of Corpus Cavernosal Tissue

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Erectile dysfunction (ED) is a common condition in men and the incidence is rising with life expectancy. There are several pharmacological agents to treat ED, but these are only partially effective. The aims are to compare the contractile activation process between normal and diseased human corporal smooth muscle and to develop an in vitro small-animal model, using guinea-pigs.

Human samples were obtained with ethical approval and informed consent, from patients undergoing penile surgery. Guinea-pigs were humanely killed and the penis removed. Corporal smooth muscle strips were superfused with Tyrode's solution (5%CO_2, 24mM $NaHCO_3$, pH7.4) and attached to an isometric force transducer. Inclusion of the septal membrane was avoided in guinea pigs. Contractions were elicited either by nerve-mediated, electrical field stimulation (EFS, 3s tetanic train, 0.1 ms pulses, 1-80Hz: abolished by 1 μM tetrodotoxin) or by application of phenylephrine. Carbachol and the M3-selective muscarinic antagonist 4-DAMP (4-Diphenylacetoxy-N-methylpiperidine methiodide) were added to pre-contracted preparations. Contractions are expressed as mN/mm^2 and muscarinic relaxation as percentage of previously-induced contraction. Data are mean ± S.D., differences between means ($p<0.05$) were examined with a Mann-Whitney test.

Maximum force of nerve-mediated contractions for guinea-pig tissue, Tmax, was 1.43±0.96 mN/mm^2 and frequency of half-maximal contraction, f1/2, was 39±2 Hz (n=15). For normal human tissue, Tmax was 0.52±0.46 mN/mm^2 and f1/2 42±15 Hz (n=5). For tissue from ED patients, responses to EFS were a mixture of contractions and relaxations: contractions alone (n=9), relaxation alone (n=6) and relaxations at low frequencies with contractions at higher frequencies (n=13). In the latter, relaxation Tmax was 0.10±0.2 mN/mm^2 (f1/2 3±2 Hz), and contraction Tmax was 0.91±1.2 mN/mm^2 (f1/2, 28±10Hz, $p<0.05$ compared to normal human tissue). Phenylephrine induced dose-dependant contractions with pEC_{50} values: guinea-pig, 5.80±0.24 (n=10); normal human, 5.87±0.06 (n=4); human ED, 5.52±0.66 (n=17, $p<0.05$ compared to normal human). Carbachol relaxed both guinea-pig and human tissue, pre-contracted with 1.5 μM phenylephrine, in a dose-dependant manner, pIC_{50}=6.54±0.86 (n=8), 6.41±0.41 (n=9), respectively. In guinea-pigs, 4-DAMP (3-30 nM) decreased pIC_{50} values.

Guinea-pig is a useful animal model for human corporal smooth muscle as they display similar nerve-mediated and agonist-induced contractile responses. Tissue from ED patients exhibits increased contractile sensitivity to EFS, despite large superimposed relaxations. However, this is not due to the increased sensitivity of α-receptors as the phenylephrine pEC_{50} was reduced in the ED group. Carbachol relaxed muscle strips pre-contracted with phenylephrine, at least in part via M3 receptors. The validation of an animal model, as applied to human tissue, will enable better characterisation of the pathophysiology of ED.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

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Modulation of intracellular Ca^{2+} by P1-receptors in isolated guinea-pig detursor myocytes

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Normal detrusor smooth muscle contractions are elicited by a rise of intracellular [Ca^{2+}] via activation of second messenger signalling pathways. These signalling cascades release Ca^{2+} from intracellular stores to activate contractile proteins. Contraction can be modulated by molecules such as cAMP, where a rise in cAMP levels has shown to reduces contractile force (Wheeler et al. 1995). Adenosine-specific P1-receptors are linked to adenylate cyclase activity and could have a role in contractile modulation.

Male Dunkin-Hartley guinea-pigs were humanely killed by cervical dislocation. Bladder tissue was dissociated with a solution of digestive enzymes according to previously determined protocols (Wu et al. 2002). Isolated cells were loaded with a fluorescent indicator; fura-2 and cell suspensions were placed onto a microscope stage and superfused at 37°C in HCO_3^- /CO_2 Tyrode's solution (pH7.35). Ca^{2+}-transients were stimulated with 3μM carbachol (acetylcholine analogue). Effect of the adenylate cyclase inhibitor, MDL-12330A, was investigated on Ca^{2+}-transients in the presence of adenosine. All compounds were from Sigma UK and, with the exception of adenosine, were made as stocks in DMSO and diluted in Tyrode's solution to working concentrations. Data are expressed as mean±S.D., (n) is number of experiments, Student's t test was used to test for significance between data sets (*$p<0.05$).

Previous results (Ikeda et al. 2004) indicated Ca^{2+}-transients may be modulated by the A2b-subtype of P1-receptors. 1μM DPCPX (A1-specific antagonist), 1μM 8-(3-chlorostyryl)caffeine (A2a-specific antagonist) and 1μM MRS-1191 (A3-specific antagonist) with 1mM adenosine reduced transients by 34±31% (n=6), similar to reduction with adenosine alone, 30±26% (n=15), confirming A2b-receptors are involved in adenosine-mediated reductions. MDL-12330A reduced Ca^{2+}-transients dose-dependently (0.03-3μM), with a maximal reduction of 47±22% (n=8). 1μM MDL-12330A reduced Ca^{2+}-transients by 30±31% (n=5) and 1mM adenosine with MDL-12330A were reduced by 39±31% (n=4), therefore possibly affecting similar pathways. Adenosine or MDL-12330A alone did not significantly reduce transients elicited by 80mM KCl-Tyrode's [adenosine (n=3), MDL (n=6)] or 20mM caffeine [adenosine (n=8), MDL (n=6)]. cAMP can modulate Ca^{2+}-transients elicited by muscarinic-receptor activation. Results here show A2b-receptors can also modulate Ca^{2+}-transients, possibly through modulation of adenylate cyclase activity.

Wheeler MA, Martin TV, Weiss RM, (1995). J.Urol. 153, 2044-2049

Wu C, Sui G, Fry CH (2002). J.Physiol. 538, 357-369

Ikeda Y, Wu C, Fry CH (2004). J.Physiol. 577P, PC32.

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