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Poster presentation

Regulation of spontaneous activity in rabbit corpus cavernosum myocytes by nitric oxide

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The aim of the present study is two fold. Firstly, to determine if the nitric oxide (NO)/cGMP pathway can modulate spontaneous transient inward and outward currents (STICs and STOCs), in rabbit corpus cavernosum smooth muscle. Secondly, to see if nitric oxide can effect intracellular calcium oscillations observed in these cells. Male New Zealand white rabbits were humanely killed with an injection of pentobarbitone (I.V.), and the corpus cavernosum was dissected from the penis and cut into 1 mm^3 pieces. From this, single smooth muscle cells were isolated in a medium containing collagenase as described previously [1]. The cells were then studied at 37°C using the perforated patch technique. When cells were held under voltage clamp at -60 mV, using caesium containing pipettes, over 70% fired STICs. These events were shown to be carried by a Ca^{2+}-activated Cl^- conductance, and were sensitive to inhibition of IP_3-mediated Ca^{2+}-release [1]. Modulation by the NO/cGMP pathway was investigated by applying nitrosocysteine, 3-(5-hydroxymethyl-2-furyl)-1-benzyl indazole (YC-1), and 8-bromo cGMP, all three of which abolished STIC activity. In addition, the protein kinase G 1 (GK1) specific activator SP-8-Br-PET-cGMPS (25 and 100 μM), significantly reduced STICs. This provides one possible mechanism whereby the NO/cGMP pathway could inhibit tone in corpus cavernosum smooth muscle.

When cells where voltage clamped at -30 mV using K^+ containing pipettes, both STICs and STOCs were observed. The STOCs were abolished by iberiotoxin (300 nM) or by penitrem A (100 nM), indicating that they were mediated by large conductance Ca^{2+}-activated K^+ currents (BK currents). On addition of diethylamine nitric oxide (DEA-NO, 30 μM, nominal), STICs were inhibited but STOC activity increased almost 4-fold. 8-bromo cGMP (1 mM) also increased the STOC activity 3-fold. In contrast, SP-8-Br-PET-cGMPS (25 μM) had little effect on STOCs. Similarly, RP-8-Br-PET-cGMPS (1 μM), a specific inhibitor of GK1, failed to block the effect of 8-bromo cGMP. These results suggest that although cGMP may be involved in the activation of STOCs by NO, GK1 was not involved. This provides another possible mechanism for tone inhibition.

Intracellular calcium oscillations were also investigated using fast confocal microscopy in fluo-4 loaded cells. Preliminary results show that these events are inhibited by DEA-NO (30 μM). Taken together, these results show that the NO/cGMP pathway inhibits STICs, and underlying calcium oscillations, whilst activating STOCs.

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References

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