The response of isolated tunica dartos muscle to acute and prolonged cold stimulation and noradrenaline

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Human infertility is a problem affecting 10-15% of couples, with approximately equal contribution from both partners (DeKretser and Baker, 1999). Spermatogenesis is a temperature sensitive process that is regulated by the hypothalamo-pituitary gonadal axis of hormones. In most mammals testicular temperature is maintained 2-6°C below the core body temperature (Maloney & Mitchell, 1996). The tunica dartos muscle lining the scrotal skin plays an important role in the regulation of testicular temperature and therefore, male fertility. Contraction of the dartos reduces the surface area of the scrotum and the blood flow to the scrotal skin, preventing heat loss. Dartos relaxation causes excess heat to be removed (Shafik, 1973). Dartos contraction can be induced by sympathetic stimulation, and cold-induced contraction was previously thought to be mediated solely through a spinal reflex involving the sympathetic nerves and noradrenaline. However, cooling of the isolated dartos produces a contractile response indicating that other mechanisms also induce contraction in the dartos (Maloney *et al*., 2005). Therefore, we have studied the contractile physiology of the isolated dartos muscle with regard to cold stimulation and noradrenaline.

Isolated dartos muscle strips (2-3 mm wide) were obtained from the glabrous part of the scrotum of Wistar rats and were connected using 3/0 surgical silk to a force transducer system in an organ bath. The tissue suspended organ chamber contained Krebs-Ringer solution (NaCl 121 mM, KCl 5.4 mM, $MgSO_4 \cdot 7H_2O$ 1.2 mM, $NaHCO_3$ 25 mM, HEPES 5 mM, glucose 11.5 mM, $CaCl_2 \cdot H_2O$ 2.5 mM) with a pH of 7.35. The bath was aerated with carbogen (5% CO_2 and 95% O_2) and its temperature was controlled via a recirculating water bath at 33°C.

The isolated dartos muscle contracted in response to electrical field stimulation, noradrenaline exposure and cooling to 15°C. The cooling response was greater with the overlying skin present, compared to the isolated muscle (115 ± 6.1% compared to 37 ± 7.3% of EFS response, $p = 3.03 \times 10^{-7}$, $n = 8$). The dose response curve to noradrenaline was sigmoidal with an EC_{50} of 10^{-5} M ($n = 6$). Prolonged cooling caused the tension to gradually decrease to baseline after approximately 3 hours, allowing the noradrenaline response at 15°C to be determined in the absence of cooling induced force. The contractile response to noradrenaline at 15°C was 153% of that measured at 33°C. The contractile reactivity to noradrenaline was decreased at higher temperatures, as previously shown by another method (Gibson et al., 2002). Repeated cooling (after rewarming) led to a marked reduction in the contractile response to further cooling (3rd cooling peak tension 28.5 ± 11.7% of 1st peak, $n = 6$). No such reduction in response was observed with repeated electrical field stimulation or noradrenaline.

This study shows that the cooling response in the tunica dartos is enhanced by the presence of the skin, and the cooling response may be in part due to greater sensitivity to basal noradrenaline release at lower temperatures.

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