Temperature sensitivity of dopaminergic neurons in the Substantia Nigra pars compacta

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Certain neurons in the CNS display a high temperature sensitivity (Q_{10}> 2.0) with respect to their firing frequency, e.g. in the hypothalamus, where such neurons are known to play a role in thermoregulation. There are reports of other brain regions expressing similar sensitivity, however, the cellular mechanism and pathophysiological significance of temperature sensitivity in extrahypothalamic neurons remain unclear. We hypothesise that this is due in part to the expression of temperature-sensitive ion channels in cell membranes. This hypothesis is supported by the recent discovery of a family of channels known as TRP (Transient Receptor Potential) channels (Minke & Cook, 2002). One member of the family (TRPV3), which is expressed both in the CNS and at the periphery, is sensitive to temperature changes in the physiological range (around 37°) (Xu *et al.*, 2002). In addition, this cation channel is relatively selective for calcium ions (Xu *et al.*, 2002), which suggests it plays a role not only in the control of neuronal excitability but also of intracellular Ca^{2+} homeostasis

The Substantia Nigra pars compacta (SNc) is a component of the basal ganglia important in motor control. Degeneration of this structure, associated with intracellular Ca^{2+} overload, leads to Parkinson's disease (Hirsch *et al.*, 1997). We have recently found that SNc neurons are temperature sensitive (Lipski *et al.*). The aim of the present study was to further characterise this sensitivity using a combination of whole-cell patch clamp recording and calcium imaging techniques and to explore what role, if any, TRP channels play in the temperature sensitivity of SNc neurons.

Transverse midbrain slices (250 μm) containing the SNc were obtained from young, anaesthetised Wistar rats and kept in aCSF bubbled with 95%O_2/5%CO_2. SNc neurons were visualised with IR-DIC (E600FN microscope, Nikon) and identified using a combination of morphological and electrophysiological criteria. Cells were patched with glass pipettes (2.5-5 MΩ) filled with a solution containing (in mM): 145 K-gluconate, 10 HEPES, 0.75 EGTA, 2 Mg_2ATP, 0.3 Na_3GTP, 2 $MgCl_2$, 0.1 $CaCl_2$, and held at −60 mV under voltage clamp. In some experiments, the Ca^{2+} indicator fura-2 (0.25 mM) was loaded into the cell by diffusion from the pipette solution. The level of free intracellular Ca^{2+} was monitored using the ratiometric technique (340/380 nm). Slices were maintained at 34°C except when temperature ramps were performed.

Transient cooling (by 2, 5 or 10°C) or heating (2 or 5°C) of the slice resulted in an outward (cooling) or inward (heating) current and corresponding changes in cell membrane resistance. The responses were fully reversible on return to control temperature (34°C). Temperature ramps with variable slopes demonstrated slow current kinetics. There was no sign of current desensitisation when steady-state temperature was reached. Cooling of slices by 5°C in the presence of ruthenium red (100 μM; a blocker of TRPV3 channel) produced a small reduction (22%; paired t-test P<0.005, n=5) of cooling-induced outward current. Ca^{2+} imaging experiments revealed temperature dependence of intracellular Ca^{2+} concentration consistent with the hypothesis that Ca^{2+} permeable channels are active at high temperatures and closed during cooling.

These experiments demonstrate a distinct pattern of electrophysiological and Ca^{2+} signal responses evoked by temperature changes in SNc neurons. Further studies are needed to confirm the involvement of TRP channels in temperature sensitivity and control of Ca^{2+} homeostasis in these neurons.

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