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SHORT COMMUNICATION

PATCH-CLAMP RECORDING OF POTASSIUM CHANNELS FROM GLIAL CELLS IN THE COCKROACH CENTRAL NERVOUS SYSTEM

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Glial cells in the insect nervous system are believed to play a role in regulating the extracellular potassium concentration, both during neuronal activity through spatial buffering (Gardner-Medwin, Coles & Tsacopoulos, 1981), and also by forming a blood–brain barrier (Schofield & Treherne, 1984). The membrane potential of glial cells has been thought to be exclusively dependent on potassium (Kettenmann, Sonnhof & Schachner, 1983), but recent studies on cultured glia have revealed conductance mechanisms for chloride (Coles & Orkand, 1984), sodium (Munson, Westermark & Glaser, 1979; Reiser, Loffler & Hamprecht, 1983) and calcium (MacVicar, 1984); and calcium and potassium channels have also been reported from *in vivo* retinal glial cells (Newman, 1985). This paper presents some patch-clamp recordings from *in situ* glial cells in the cockroach central nervous system.

The ventral abdominal nerve cord from adult, male cockroaches (*Periplaneta americana*) was used throughout. The nerve cord was isolated and desheathed, using fine needles, between the 4th and 5th abdominal ganglia. This procedure removes the neural lamella and causes some damage to the underlying perineurial glial cells. However, all axons retain an intact layer of glial cells (C. A. Leech & L. S. Swales, unpublished observations). The nerve cord was then mounted in the experimental chamber and treated with a 1% solution of collagenase (Sigma type 1-S, in cockroach saline) for 45 min at room temperature. All experiments were performed at room temperature (20–23 °C).

Normal cockroach saline contained (in mmol l^{-1}): NaCl, 157; $CaCl_2$, 2; $MgCl_2$, 2; trehalose, 5; KOH, 3; Hepes, 10 (pH 7·2). After enzyme treatment the cord was transferred to a '210K' saline: K_2SO_4, 105; Hepes, 10; trehalose, 5; $MgSO_4$, 2; EGTA, 2; $CaSO_4$, 1 (brought to pH 7·2 with KOH giving a total [K] of about 220 mmol l^{-1}, free [Ca] = 10^{-7} mol l^{-1}). In some experiments, K_2SO_4 was partly replaced with Na_2SO_4. Patch pipettes were produced from borosilicate glass tube (Clark electromedical, GC120–15) and filled with 210K saline containing 2 mmol l^{-1} $CaSO_4$ (without EGTA). Currents were recorded using a Dagan 8900 amplifier with the output signal filtered at 1 kHz. Signals were recorded on tape (Racal Store 4) for

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subsequent analysis. The usual convention for potentials and current are used. All records were obtained from isolated, inside-out patches (Hamill *et al.* 1981).

Patches generally contained more than one conductance level. Patches with several identical conductance levels and also ones containing different conductance levels were observed (Fig. 1). Some of these different levels will represent various types of channel while others may reflect a number of conductance states for an individual channel. Channel activity was observed with the membrane potential held at different levels. Under these conditions, a wide range of values for channel conductance was obtained (Fig. 2). For 24 channels measured in separate patches, the range was

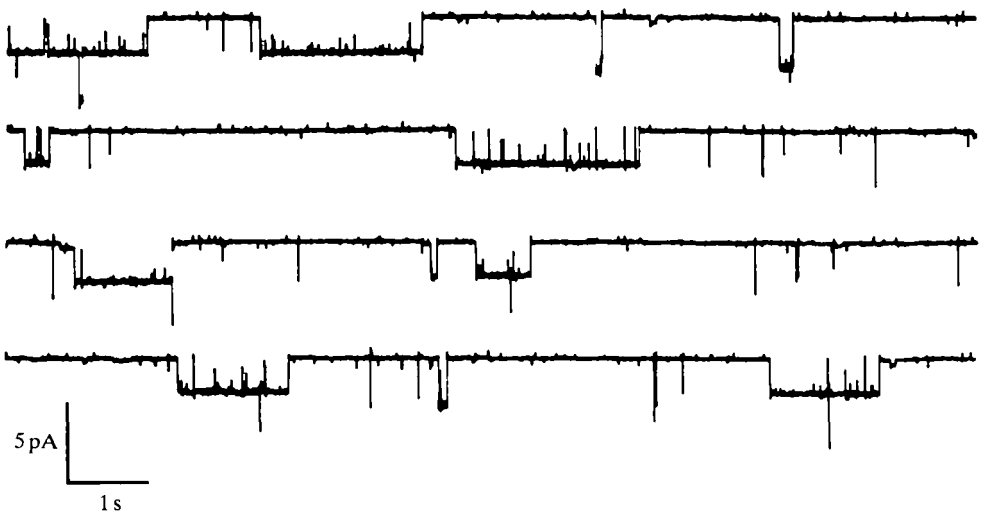

Fig. 1. Continuous records obtained from a patch containing multiple conductance levels at a membrane potential of −60 mV. Records low pass filtered at 200 Hz.

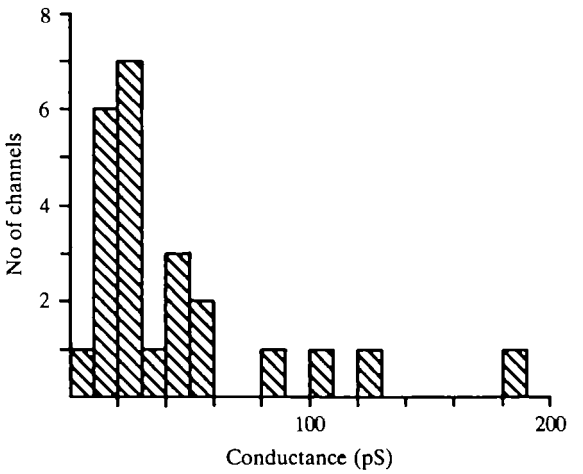

Fig. 2. Histogram of channel conductances. Currents were measured for at least four membrane potentials. Data are shown for 24 channels from separate patches.

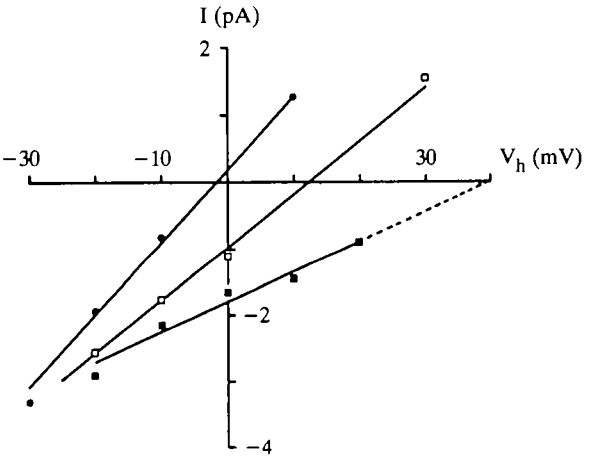

Fig. 3. Effect of potassium concentration. [K] at the cytoplasmic face of the membrane was changed by substitution with Na. The reversal potential changed by about 40 mV for decade change in [K]. Channel conductance was 109 pS in 210K (●), 78 pS in 110K (□) and 45 pS in 20K (■).

8–184 pS. A similar wide range of conductance values was reported by Kettenmann, Orkand & Lux (1984) for potassium channels in cultured mouse oligodendrocytes. No analysis of the voltage dependence of channel kinetics has yet been performed.

To investigate the ionic nature of these channels, the potassium concentration at the cytoplasmic face of the patch was changed using a 'drainpipe' system (Yellen, 1982). Fig. 3 shows current–voltage relationships for a channel in a patch where the potassium concentration was changed in this way. The reversal potential of the currents shifted in the direction predicted from the Nernst equation, but not by as much as expected for a pure potassium electrode. Similar results were obtained from three patches containing channels with conductances in the range 13–109 pS (in 210K saline).

No attempts were made during these experiments to determine whether the activation of the channels was calcium dependent. The calcium concentration of the saline bathing the cytoplasmic face of the membrane was about 10^{-7} mol l^{-1}, which is in the range of concentrations reported to activate calcium-dependent potassium channels in other systems.

Recordings of single channel currents from *in situ* glial cells in the cockroach central nervous system are presented. These channels show a wide range of conductances and their reversal potential changes when the potassium concentration at the cytoplasmic face of the patch is changed.

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