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K^+ conductance of mechanically dissociated rat sensory neurones in long term cultures in a defined medium

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Introduction

In order to obtain an *in vitro* cell culture model for the study of neuronal development and regeneration in a completely defined culture medium we studied the membrane conductance properties of long term cultured perinatal rat sensory neurones in such a medium. Here we report in particular about K^+ conductance.

Methods

The sensory neurones were obtained by trituration of dorsal root ganglia (DRG) from embryonic (18-21-day embryos) or neonatal rats. After dissociation the cells were cultured at 37°C for at least 5 weeks on poly-D-lysine-coated cover slips in a chemically defined, i.e. no serum containing, medium according to Romijn et al. (1984), supplemented with NGF. Neurones were identified with the use of a monoclonal antibody, detecting all three subunits of neurofilament.

The electrical membrane properties of the smaller size neurones (somata < 25 μm) were studied with the patch-clamp technique in the whole-cell configuration at a temperature of 20-24°C. The standard extracellular solution (ECS) contained (mM): NaCl 140-150, KCl 5, $CaCl_2$ 1, $MgCl_2$ 1, HEPES-NaOH 10, pH 7.2. The standard intracellular solution (ICS) in the patch pipette contained (mM): NaCl 10, KCl140, $CaCl_2$ 1, EGTA 10, $MgCl_2$ 2, HEPES-KOH 10, pH 7.2.

Results and discussion

Neurones surviving the isolation and initial culture procedures as round cells developed processes within 1-2 days and tended to change from a multipolar to a bi- or unipolar shape over a period of two weeks. Other, non-neural cells, probably satellite or Schwann cells, developed in interaction with the neurones over a period of three weeks.

The mean membrane potential of neurones pooled for all culture ages considered (range 0-35 days) was -50 mV (SD=13 mV, n=32). Usually, neurones produced 0 mV overshooting action potentials of 3-4 ms duration upon constant current pulses at all culture ages (32 cells). Often the membrane potential had to be set at -75 mV by a holding current to overcome inactivation at more depolarized membrane potentials.

Current records upon depolarizing voltage-clamp steps from -80 mV to > -50 mV (40 cells) showed the existence of both an initial inward current of 5-10 ms and a delayed outward current at all culture ages, though the relative sizes and the kinetics were variable. When the inward current was relatively small, or when it was blocked by predepolarization or by 5-10 mM external $CoCl_2$ (3 cells; 11-, 12- and 18-day cultures), the delayed current, which was transient in nature, remained. An outward current at > -50 mV, at the given compositions of ECS and ICS, implies that this current at least carries K^+ ions. Selective K^+ conduction was established from measured reversal potentials, which were within 15 mV of the calculated Nernst potential for K^+ (3 cells; 1-, 3- and 12-day cultures).

In some cases the current decline after peak activation was biphasic (examples found in a 2- and a 12-day culture) with an initial rapid phase (300 ms) and a subsequent much slower phase (in the order of seconds), consistent with the existence of a fast and a slow K^+ conductance (Kostyk et al. 1981). In other cases (examples found in a 1-, a 4- and a 25-day culture) only slow inactivation was present or dominant. Inactivation by predepolarization occurred between -80 and 0 mV (one 3-day and two 25-day cultured neurones). So far, no inward rectifying K^+ conductance has been found.

The present results show that mechanically-dissociated rat sensory neurones maintain excitability properties including K^+ conductance expression during long term culturing in a chemically defined culture medium. This provides additional possibilities for *in vitro* studying neuronal function, differentiation and regeneration under defined conditions. Future experiments will be directed towards a better identification of the various neuronal types and membrane conductances in these cultures in order to compare the results with *in vivo* DRG neurone properties and with *in vitro* measurements on dissociated and cultured sensory neurones of others (see Bossu et al.

1985; Kostyuk et al. 1981).

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