VOLTAGE-GATED CALCIUM AND PROTON-ACTIVATED SODIUM CURRENTS IN CO-CULTURED CORTICAL ASTROCYTES FROM MOUSE (MUS MUSCULUS) IN PRIMARY CULTURE

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Astrocytes play an important role in the regulation of ionic microenvironment, which is essential for normal neuronal functions. The role of astrocytes in calcium homeostasis is slowly beginning to appear (Hertz et al., 1989, J. Neurosci. Res, 22: 209; MacVicar et al., 1989, Soc. Neurosci. Abst, 15: 15; Kraig, 1989, Soc. Neurosci. Abst, 15: 352; Finkbeiner et al., 1989, Soc. Neurosci. Abst, 15: 1162). These functions could be mediated through voltage-sensitive Ca^{2+} channels and kainate/quisqualate type glutamate receptors.

Astrocytes grown in tissue culture are known to express voltage-dependent Ca^{2+}-channels, which are of the L-type and are induced by substances known to increase the intracellular levels of cAMP (MacVicar, 1984, Science, 226: 1345; MacVicar and Tse, 1988, Glia, 1:359; Barres et al 1989, J. Neurosci. 9: 3169). We were interested to see if astrocytes grown in the presence of neurons will express the voltage dependent Ca^{2+} channels. The whole cell patch-clamp technique has been used to characterize the Ca^{2+} currents in mouse co-cultured astrocytes. Astrocytes were prepared as described by Hertz et al., 1985, in Neuromethods, AA Boulton and GB Baker eds., Humana Press Clifton, NJ, vol. 1, pp.,117-167. The Ca^{2+} currents were recorded using patch-clamp electrodes filled with (in mM): 115 CsCl, 5 NaCl, 5 MgATP, 0.1 cAMP, 14 EGTA, and 10 HEPES (pH 7.4). Superfusion solution was composed of (in mM): 137 NaCl, 5.4 KCl, 1 $MgCl_2$, 10 glucose, 10 HEPES (pH 7.4) and 10 $CaCl_2$ or 10 $BaCl_2$.

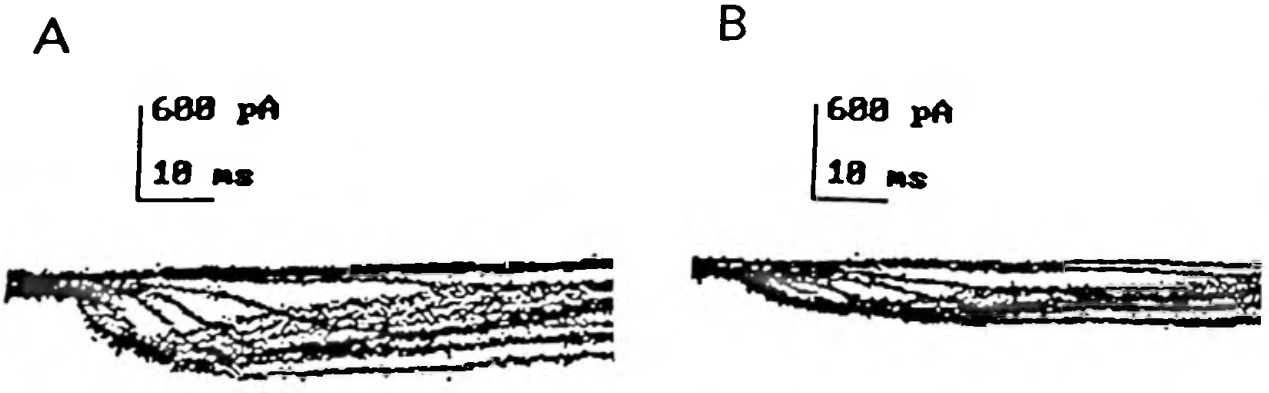

Figure 1. Whole cell Ca^{2+} current recording. The holding potential was -90 mV (A) and -60 mV (B) and the membrane was stepped in 10 mV steps from -80 to 50 mV in (A) and from -50 to 40 mV in B.

In cortical astrocytes co-cultured with neurons, depolarizing pulses from a holding potential of -90 mV activated two currents, first a rapidly inactivating inward current and then a slowly inactivating current (Figure 1A). However, when the membrane was held at -60 mV, only the slowly inactivating current could be activated (Figure 1B). Figure 2 shows the current-voltage relationship. It can be seen that the rapidly inactivating current is activated at above -60 mV, reaching peak activation of 700 pA at 0 mV. Such a current

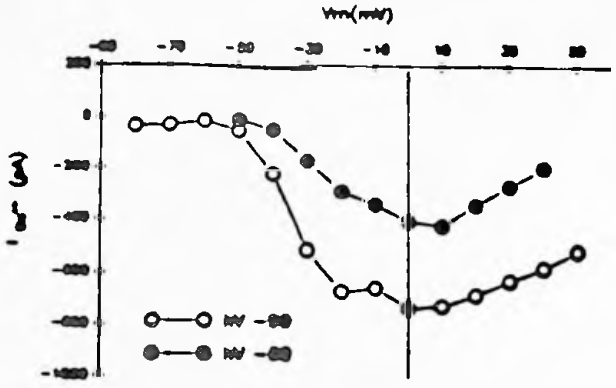

Figure 2. I-V relationship of Ca^{2+} channels recorded in Fig.1 indicating the possibility of two types of Ca^{2+} channels.

could be analogous to the "T" or transient Ca^{2+} current. The slowly inactivating current is activated at -30 mV, reaching the peak current of 500 pA at 10 mV. This current could be analogous to the "L" or long lasting Ca^{2+} current.

Sudden elevation of proton (H^+) concentration or step decreases of extracellular Ca^{2+} concentration in various cell types produce a transient inward sodium current flowing through Ca^{2+} channels that are transformed from a voltage-gated Ca^{2+} permeable state to a H^+-gated Na^+ permeable state. (Konnerth et al., 1986, J. Physiol., 386: 601; Davies et al., 1988, J. Physiol., 400: 159; Hablitz et al., 1986, Biophys. J., 50: 753). When a "step" change of extracellular pH from 7.9 (2mM Ca^{2+}) to 6.0 (0.2 mM Ca^{2+}) was applied in astrocytes, at holding potentials of -80 mV, an inward current was induced (Fig. 3).

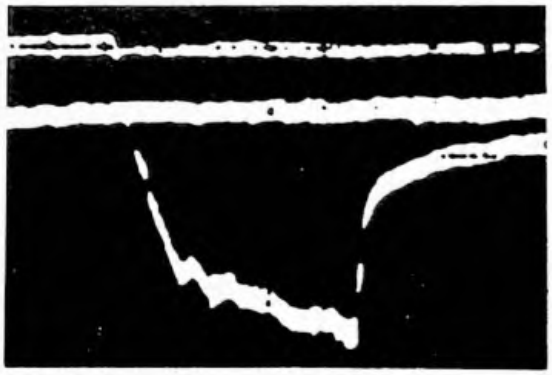

Figure 3. Proton-induced inward current. The extracellular pH was changed from 7.9 (2 mM Ca^{2+}) to 6.0 (0.2 mM Ca^{2+}).

This suggests that Ca^{2+} channels in astrocytes could exist in a voltage gated Ca^{2+} permeable state and a H^+-gated Na^+ permeable state.

Our results show that astrocytes express voltage dependent Ca^{2+} and H^+-activated Na^+ channels when grown in the presence of neurons.

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