Analysis of the Slow Inward Current Induced by Pentylenetetrazol

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Introduction

It is generally accepted that paroxysmal depolarization shifts (PDSs) are the most important phenomena in chemically induced (and, possibly, naturally occurring) epileptic activity (Ducreux and Gola 1975; Matsumoto and Ajmone-Marsan 1969; Speckmann and Caspers 1973). Generation of PDSs is most probably explained by assuming that chemical convulsants, such as pentylenetetrazol (PTZ), induce an abnormal, slow inward current (SIC). Membrane current phenomena, induced by PTZ, have been found and described by several authors (Ducreux and Gola 1975; Walden et al. 1988). Characteristic changes in intracellular free calcium and calcium distribution following administration of PTZ are also well-documented (Sugaya et al. 1987). Previously, we described the effects of PTZ on normal membrane parameters and currents in central neurons of *Helix pomatia* (Fehér et al. 1987) and some properties of the SIC with respect to ionic dependence and sensitivity to blocking agents (Papp et al. 1987). The experiments detailed below focussed on the effect of organic Ca antagonists and antiepileptic drugs on the SIC and on possible parallels between effects of various treatments on SIC and fast inward current (FIC).

Materials and Methods

Details of the applied methods have been given elsewhere (Papp et al. 1987). In short, the connective tissue sheath was peeled off the subesophageal ganglion group and the latter was fixed in a Sylgard-plated chamber continually superfused with Ringer's solution (in mM): NaCl 80; KCl 4; $CaCl_2$ 7; $MgCl_2$ 5; Tris-Cl 5. The pH was 7.4, at room temperature. The drugs applied were: PTZ 50 mM, tetraethylammonium-Br (TEA) 30 mM, tetrodotoxin (TTX) 10 μM, phenobarbital 5 mM, diphenylhydantoin (DPH) 1 mM, verapamil 1 mM, and diltiazem 1 mM.

Voltage clamping was performed using a single electrode device. Neurons were impaled with a microelectrode of 2 - 7 MΩ resistance, filled with 1:1 M KCl : K citrate. Slow ramp as well as square pulses were used as commands. Ramps were used for directly obtaining current - voltage characteristics on an X-Y plotter. Membrane currents evoked by square pulses were visualized on a storage scope screen and photographed.

Results

A great majority of *Helix* neurons were sensitive to PTZ. On application of 50 mM PTZ the I-V curves underwent characteristic changes. At 15 - 25 mV depolarization a negative slope region appeared, corresponding to a slowly inactivating inward current. Simultaneous administration of 30 mM TEA depressed the slow K currents and the SIC could be seen free of interferences.

The SIC was found to be sensitive to various blocking agents. Although it proved to be resistant to 10 μM TTX (Papp et al. 1987), it was readily depressed by Ca channel blocking cations like

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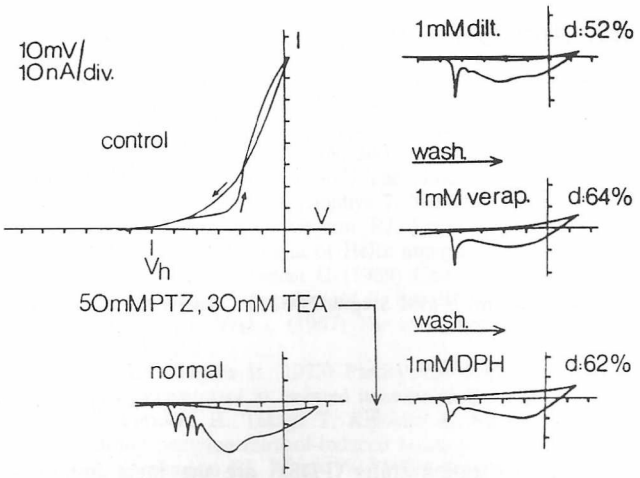

Fig. 1. Current-voltage characteristics of neuron RPa 77, obtained with ramp commands ranging from -60 to +20 mV with 20 mV/s steepness. The SIC induced by PTZ+TEA (*normal*) was reduced by 1 mM diltiazem, verapamil, and diphenylhydantoin

Ni^{2+}, Co^{2+}, Mn^{2+} (8 - 15 mM). Thus, supported by findings of others (Walden et al. 1988), it was plausible to test the effects of organic Ca antagonists on the SIC induced by PTZ. Fig. 1 displays I-V curves of the neuron RPa77 (numbered after Kerkut et al. 1975). On administration of PTZ+TEA the I-V curve showed changes indicating negative slope resistance (first and second curves, vertically). The maximum of the SIC was at about -20 mV.

Treatment with 1 mM diltiazem greatly reduced the SIC. The intensity of depression (d) is given as a percent, calculated from the areas enclosed by the voltage axis and the upstroke portion of the I-V curve. A similar effect was obtained with 1 mM verapamil.

Effects of several antiepileptic drugs were then examined on the SIC and compared to those of Ca antagonists. Using the same neuron, a substantial depression of the SIC was obtained with 1 mM DPH. On another neuron, 5 mM phenobarbital, also a known antiepileptic, was applied and led to the same depression as DPH. It could be seen that different drugs, known either as Ca antagonists or antiepileptics, exert the same effect on the SIC. They do not shift the maximum point of SIC on the voltage axis and the depression caused by them is stronger with higher depolarizations.

In a series of experiments the pharmacological responsiveness and ionic dependence of the SIC and the FIC (underlying spikes) were compared. Fig. 2 shows current records (A) and I-V plots (B) based on those (see legend). Both currents proved sensitive to Ni^{2+}, verapamil and Na removal. In Na-free Ringer, the FIC completely disappeared and the SIC was reduced strongly, but not fully. Changes in the currents using Ca-channel blockers also showed some differences between the SIC and the FIC. Ni^{2+} (8 mM) caused a total block of the SIC whereas a small fraction of the FIC persisted. Reduction of both currents by verapamil was unequivocal. In other experiments both currents proved resistant to 10 µM TTX. Records of the SIC in Fig. 2A suggest that currents remaining after Ni^{2+} and verapamil treatment are kinetically different. When blocked by Ni^{2+}, the SIC became more constant in time and its initial, steep phase disappeared. This unmasked a rapidly inactivating outward current with a relatively high activation threshold. Under influence of verapamil, however, the initial phase was unchanged, but the plateau inactivation increased. These kinetic features need further investigation.

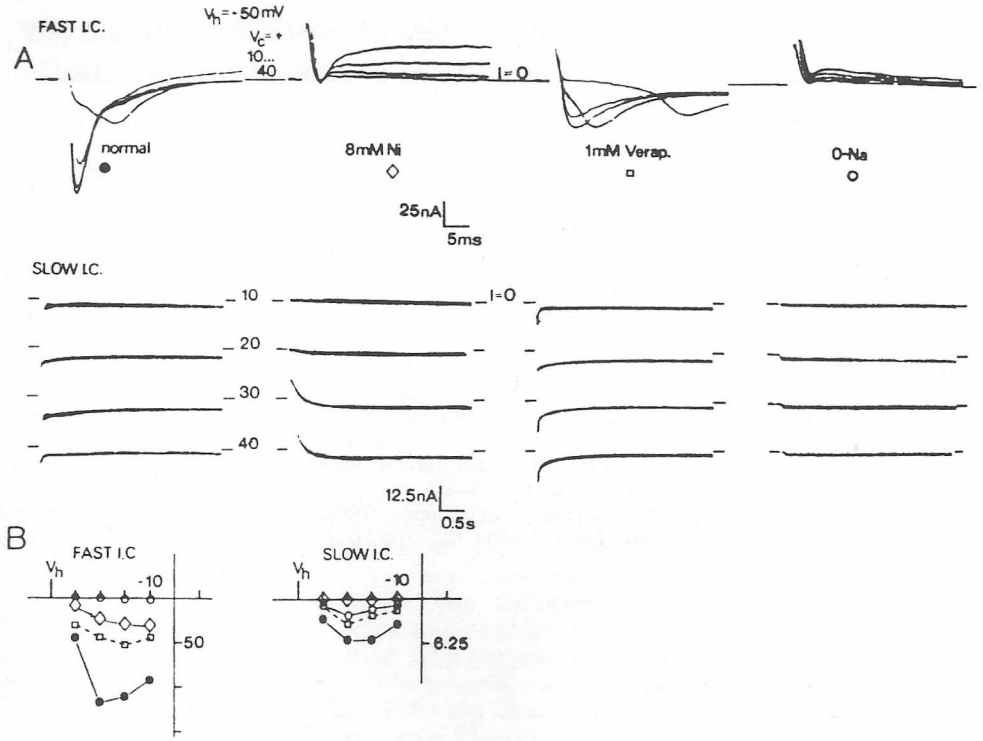

Fig. 2. A Records of FIC (*upper line*) and SIC (*lower lines*) in the presence of 50 mM PTZ and 30 mM TEA. Zero current level, holding and command potentials and calibration are given in the insets. Effects of Na removal and two different Ca channel blockers are shown. **B** Current-voltage curves plotted on the basis of currents in part **A**. For FIC, the peak current and for SIC, the current level at 1 s after pulse onset, was plotted against the actual membrane potential

Discussion

PDS has been considered as a general mechanism by which chemical - and probably other - convulsants exert their action (Ducreux and Gola 1975; Matsumoto and Ajmone-Marsan 1969; Speckmann and Caspers 1973). In invertebrates PDS is probably a non-synaptic event (Faugier-Grimaud 1974; Speckmann and Caspers 1973) in which voltage-gated currents play a central role.

Effects of various channel blockers on SIC, the current underlying PDSs have been investigated for a long time (Witte et al. 1987). In accordance with several authors we found that inorganic and organic Ca channel blockers can greatly reduce the SIC. We also compared the effects of the blocking agents on the SIC and the natural FIC. Based on the similarities discovered, one can propose that channels transmitting SIC are a subtype of the channels of the FIC, modified by PTZ. This channel subtype in *Helix* is relatively slow and permeable mostly to Ca. On administration of PTZ, these channels become slowed down, predominantly in their inactivation process, and become permeable for Na (and other cations). The strong and long-lasting inward current of the PDS would thus be carried mainly by Na ions through channels which are still sensitive to Ca channel blockers.

Two drugs known in mammals as antiepileptics were found also to be effective in *Helix* on PTZ-induced convulsive activity. Their effect was similar to that of organic Ca antagonists, except that they left the FIC intact. Thus, the mode and/or site of their action must be different.

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