The Effect of Pentylenetetrazol on the Metacerebral Neuron of *Helix pomatia*

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Abstract. The effects of Pentylenetetrazol (PTZ) on the metacerebral giant cell (MCC) of the snail, *Helix pomatia* were studied. Actions on membrane resistance, time constant, resting and action potentials, outward and inward ionic currents were examined. Superfusion with PTZ in concentrations of 25 to 50 mmol/l, induced a gradually evolving convulsive state, which could be studied by intracellular recording from the MCCs. In the pre-convulsive state an acceleration of the spontaneous activity developed and was followed by paroxysmal depolarization shifts (PDSs), in the convulsive phase. PTZ prolonged the membrane time constant by about 10 percent, but this could not be traced back to alterations in membrane resistance or capacity. The resting membrane potential was not significantly altered; the action potentials were prolonged by slowing down of both the rising and decaying phases. The outward potassium currents were repressed by PTZ in a voltage dependent manner. The decrease of the I_A current became more pronounced at increasingly positive command pulses, while I_K was relieved from depression especially at longer pulse durations. Inward currents were isolated with the aid of suppression of outward currents by 50 mmol/l TEA. Under these conditions sodium currents, measured in calcium deficient Ringer solution were moderately depressed, while the calcium currents, examined during sodium-free superfusion, were mildly enhanced by PTZ. It is concluded that PTZ effects on ionic conductances, on membrane parameters, on the resting potential and ionic currents explain only modifications of spike potentials occurring in the convulsive state and do not account for the PDS, the central phenomenon of the convulsive electrographic activity, at least in this thoroughly examined type of neuron.

Key words: Pentylenetetrazol — Ionic currents — Convulsant

Introduction

The metacerebral cell (MCC) of *Helix pomatia* has proven to be a good experimental object for the study of chemical convulsants such as pentylenetetrazol or

aminopyridines. Its electrophysiological characteristics, as described by Kandel and Tauc (1966) and Weiss and Kupfermann (1976) offer a good opportunity for the study of changes in membrane parameters and ionic currents induced by convulsant drugs. In the series of experiments presented herein the effects of pentyleneterazol (PTZ) were examined on MCC of *Helix pomatia*. As described earlier by Klee et al. (1973) and Williamson and Crill (1976a, b), PTZ exerts a strong convulsant action on Helix and Dorid neurons, respectively and has proved a good model for the convulsive electrographic phenomena occurring in mammals. However, there has been no comprehensive study about PTZ effect on all electric membrane parameters and ionic currents in correlation with the convulsant action. In this paper PTZ effects on spontaneous activity, membrane input resistance, time constant, voltage dependent outward and inward currents are described in relation to the convulsive action of the drug.

Materials and Methods

Experiments were carried out on the metacerebral giant cells (MCC) of the snail, *Helix pomatia* L. To prepare the cells, the ganglionic mass was dissected from the animal and the cerebral ganglion was fixed with its ventral surface upwards to the bottom of an organ bath, covered with sylgard. After peeling off the connective tissue sheats, MCC was sought under binocular microscope at a magnification of 40 ×. A standard mechanical micromanipulator was used to penetrate the neurons with microelectrode.

The preparation was continuously superfused with normal or modified Helix-Ringer solution (further Helix-Ringer). The normal Helix-Ringer contained (in millimoles) NaCl 80, KCl 4, $CaCl_2$ 7, $MgCl_2$ 5, Tris-HCl 5, pH 7.4. Sodium-free solution was prepared with equimolar substitution for NaCl with Tris-HCl or choline-HCl. In Ca-free solutions $CaCl_2$ was replaced by equimolar concentration of $MgCl_2$. In some experiments $NiCl_2$ (10 mmol) was used to block Ca channels and tetraaethylammonium chloride (TEA, 30 to 50 mmol) to block potassium channels. PTZ was dissolved in Helix-Ringer at 25 to 50 mmol/l without osmotic compensation. All experiments were performed at room temperature (22—25 °C).

Current clamp and voltage clamp recordings were made by using a single channel voltage clamp amplifier, built according to the design of Wilson and Goldner (1975) and Merickel (1980). Glass microelectrodes were filled with 1 mol/l potassium citrate; their resistance ranged from 2 to 7 MΩ. Potential and current records were visualized and photographed from the screen of a Tektronix storage oscilloscope. Occasionally, current-voltage curves were recorded with the aid of and X—Y plotter. A second oscilloscope was used for monitoring the sampling process. The duty cycle of the sample-and-hold amplifier was 50 percent and all current values were corrected according to this proportion.

Results

The convulsive action of PTZ on the metacerebral cells. The electrophysiological parameters of MCCs corresponded to those reported by Kandel and Tauc

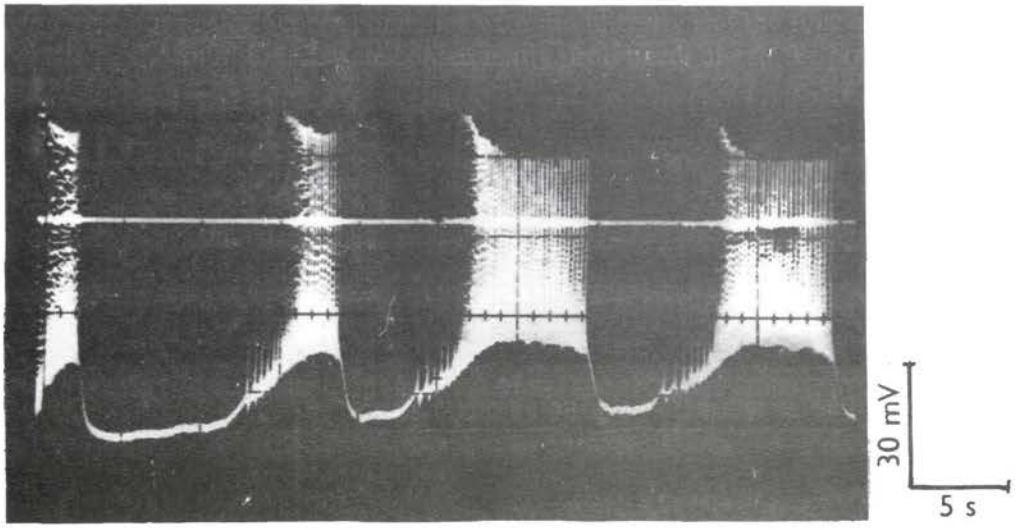

Fig. 1. Paroxysmal depolarization shifts recorded from a metacerebral giant cell of *Helix pomatia* after 10 min of application of 50 mmol/l PTZ.

(1966). The silent neurons or neurons driven by rare synaptic impulses, had resting potentials between −45 and −55 mV, while the amplitudes of spike potentials went up to 70—85 mV. The control rise time was between 8 and 9.5 ms while the half decay time ranged between 3 and 4 ms. On superfusion with 25—50 mmol PTZ the cells exhibited convulsive phenomena, lasting for several minutes: the rare spontaneous activity turned into frequent spiking, with paroxysmal depolarization shifts (PDSs) occurring at irregular intervals as long as superfusion with the drug continued. The parameters of PDSs will not be detailed here; only some characteristic examples are shown in Fig. 1.

PTZ effects on membrane resistance and time constant. The membrane resistance was measured with hyperpolarizing current pulses in current clamp mode and evaluated from steady state values of the voltage steps. The current pulses were recorded on the other beam of the oscilloscope. Superfusion with 25 and 50 mmol/l PTZ was not associated with any clear-cut effects on membrane resistance: both resistance increase and decrease could be observed, these changes being not in strict correspondance with modifications of the time constant, which, as shown in Fig. 2, were consequently prolonged, due to the presence of PTZ.

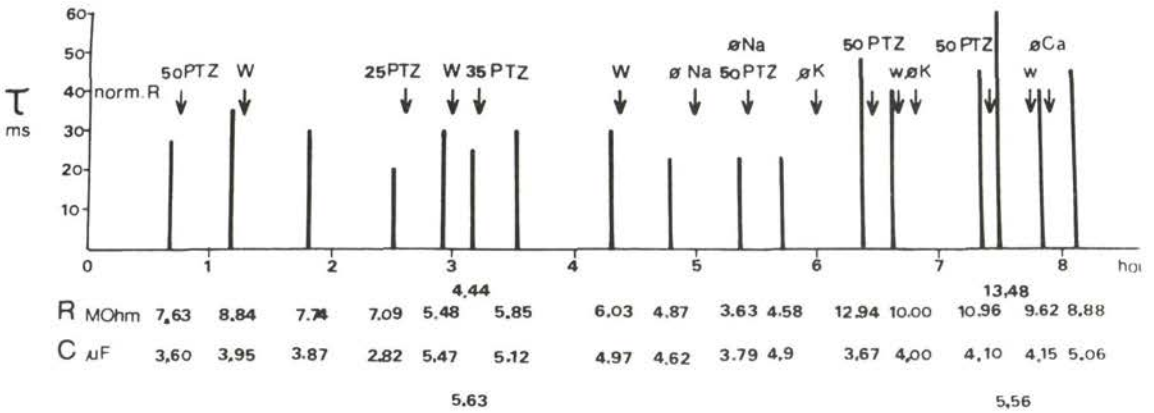

Fig. 2. The action of PTZ on membrane time constant, resistance and capacity in course of a single experiment. W: washing with normal Helix-Ringer solution.

Membrane time constants were measured in current clamp mode. Hyperpolarizing current pulses of 400 ms duration were applied and the ensuing voltage steps were recorded. Times necessary to reach 63 percent of the maximal amplitude on the ascending limb and times at the 37 percent value on the descending limb were measured on photographs at standard magnification. The values of time constants, as measured in course of a single experiment, are summarized in Fig. 2. Initial values of 27.5 ms were measured in normal Helix-Ringer. On superfusion with 50 mmol/l PTZ this parameter rose to 35 ms; after washing out of PTZ it fell to 30, then to 20 ms. At repeated applications of PTZ, 25 mmol prolonged it to 30 ms. 35 mmol/l PTZ, applied somewhat later, had the same effect. However, this moderate but consequent effect of PTZ was observable only in normal ionic environment. In sodium- or potassium-free solution the time constants changed independently of the presence or absence of the drug and were not further analysed.

PTZ effects on resting membrane potentials and action potentials. In the pre-convulsive phase (i.e. before appearance of PDSs) PTZ had no effect on the resting membrane potential. In the convulsive phase, repolarizations between PDSs were not always complete and the membrane potential remained at a somewhat depolarized level. This can be considered an indirect effect of PTZ.

Essentially the same holds for the ation potentials. In the pre-convulsive phase, when the PTZ action manifested itselt as an enhancement of the spontaneous activity, the amplitude and time course of the action potentials did not differ from normal. However, as PDSs appeared, the action potentials riding on

the crest of the depolarization plateaus, showed gradually evolving changes. Their rise time was prolonged from 3.2 ms (first spike) to 5.8 ms (the last spike in the series). The amplitude decreased form 85 mV to 73 mV after several discharges and remained so until the end of the burst. The most conspicuous changes concerned the descending limb: it was prolonged from 4 ms to 8—12 ms and the hump sitting on it was growing with each discharge. Repolarizations between the spikes were rather incomplete. In later phases of PTZ action also PDSs with depolarization plateaus and spike inactivation occurred. All these PTZ effects could be suspended by washing with normal Helix-Ringer.

PTZ effects on ionic currents. The ionic currents were studied under voltage clamp conditions.

Potassium currents. I_A and I_K currents were isolated as described by Connor and Stevens (1971a, b). I_A currents were elicited with depolarizing voltage steps, applied after conditioning hyperpolarization to −100 mV. The peak amplitude and the steady state amplitude after 300 ms were measured and plotted in

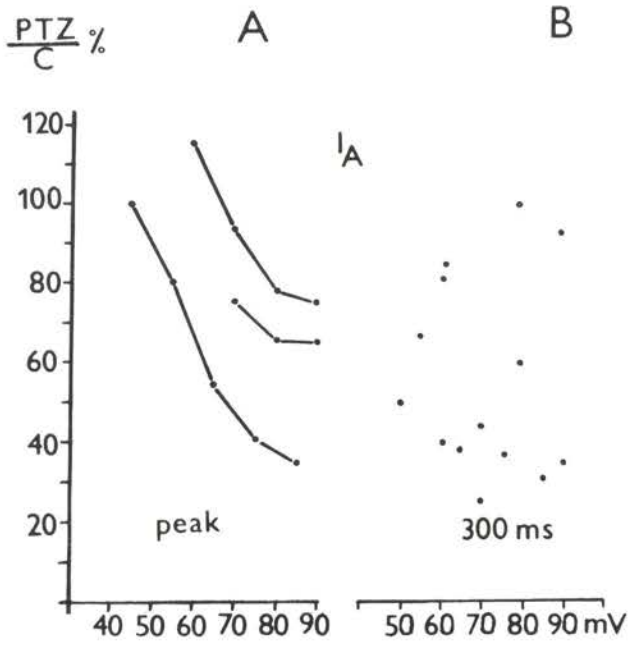

Fig. 3. PTZ effects on the peak (*A*) and steady state values of outward I_A potassium currents as measured at 300 ms after the current onset (*B*). Ordinate: normalized values of I_A expressed as percents of control.

dependence on the voltage steps as shown in Fig. 3. Currents recorded under PTZ action were normalized to the control values.

PTZ at 50 mmol depressed the peak amplitude of the I_A current in a voltage dependent manner: the larger the depolarization shift, the deeper the depress-

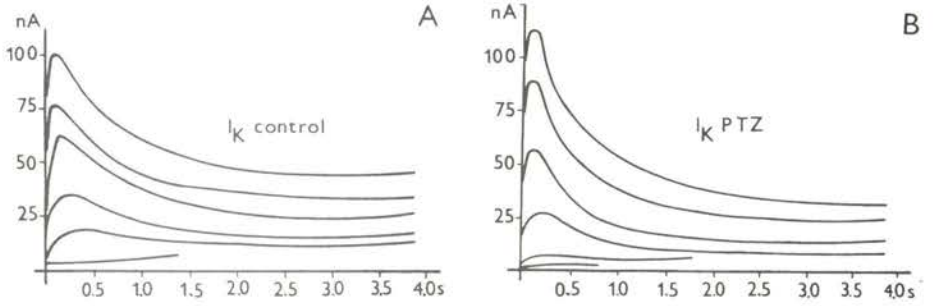

Fig. 4. PTZ effects on I_K potassium currents at depolarization steps of 80—90—100—110—120—130 mV. Abscissa: time in seconds; ordinate: current in nA. *A*: control *B*: after 50 mmol/l PTZ.

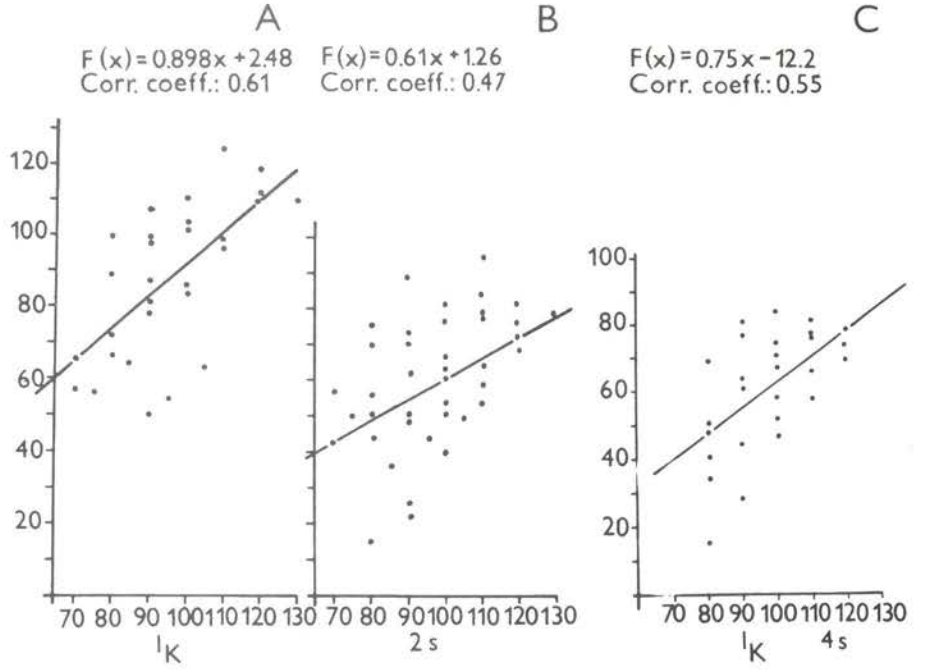

Fig. 5. The effect of 50 mmol l PTZ on the peak (*A*) and steady state values of outward potassium current, measured 2s (*B*) and 4s (*C*) after the onset. Ordinate: normalized values of I_K, expressed as percents of control.

ion. At 300 ms after the current onset, the effect was qualitatively the same, but without voltage dependence (Fig. 3.). I_K currents were elicited with voltage steps starting from the holding potential (usually −40 mV) and lasting for 4.5 ms. Amplitudes at the peak, at 2 s and 4 s after the onset were measured and plotted against voltage steps (Fig. 4.). Fifty mmol/l PTZ also depressed I_K current. This was not as conspicuous at the peak as after 2 or 4 s. Although this depression was voltage dependent as well, in contrast to I_A it could be observed not only at the peak but even more markedly after 2 and 4 s (Fig. 5.). The depression of I_K by PTZ seemed in inverse relation with the voltage step amplitude: at larger depolarization the I_K amplitude was closer to the control values, despite the presence of PTZ. The regression lines in Fig. 5 and the correlation coefficients suggest that this voltage dependence reflects an inherent feature of PTZ action.

The PTZ-induced depression of I_K appeared to consist mainly of an enhancement of inactivation of this current: this process seems to follow two exponentials, with a shorter and a longer time constant, respectively (Fig. 6).

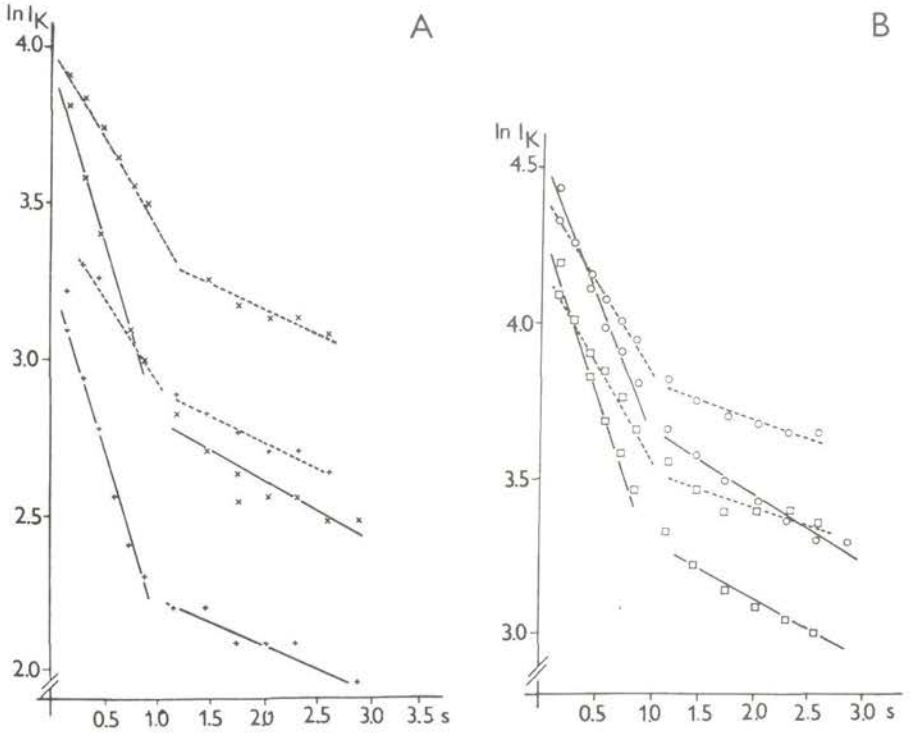

Fig. 6. Logarithmic plots of I_K values against time in seconds. Dotted lines: control values. Solid lines: currents after 50 mmol/l PTZ. ×: 80 mV, ○: 100 mV, □: 110 mV depolarization steps.

PTZ acted by curtailing the shorter one but was without similar effect on the longer one.

Inward currents. These currents were also studied with the aid of depolarizing voltage steps but in the presence of 50 mmol TEA, in order to prevent interference by outward potassium currents. In normal TEA-Ringer, 50 mmol/l PTZ

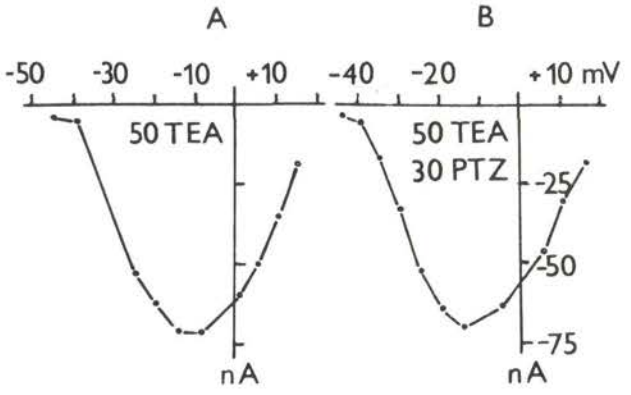

Fig. 7. *A*: inward currents in normal Helix-Ringer containing 50 mmol/l TEA; *B*: the same after the application of 50 mmol/l PTZ. Depolarization steps ranged from +10 to +70 mV. Holding potential was −45 mV.

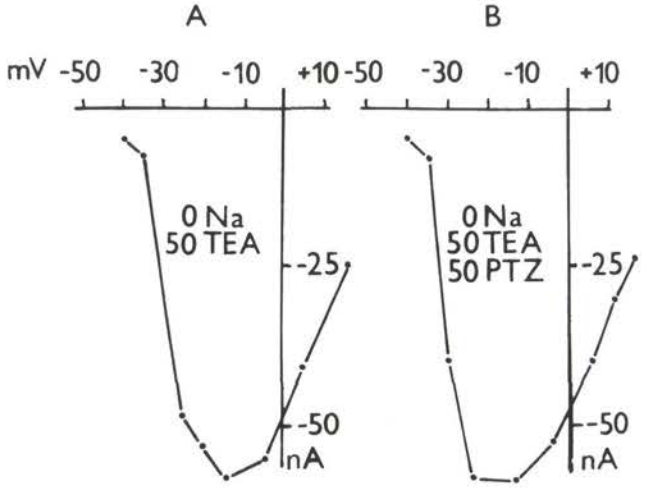

Fig. 8. The effect of 50 mmol/l PTZ on inward currents in sodium-free solutions, in the presence of 50 mmol/l TEA. Depolarization steps ranged from +10 to +70 mV. Holding potential was −50 mV.

scarcely influenced inward currents (Fig. 7). If sodium was replaced by Tris, the remaining, mainly Ca current was not remarkably changed by the same concentration of PTZ (Fig. 8). In Ca-free solution the remaining inward current, carried mainly by sodium ions was depressed mildly by 50 mmol/l PTZ (Fig. 9).

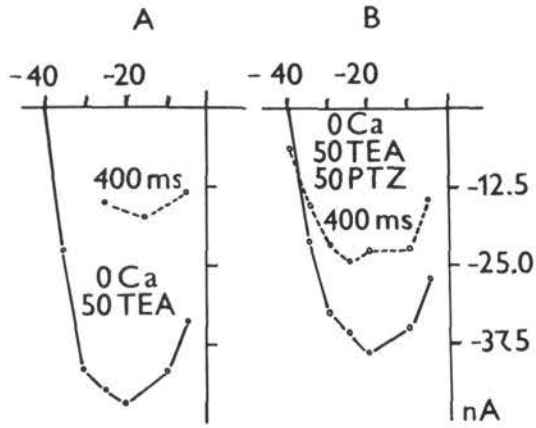

Fig. 9. The effect of 50 mmol/l PTZ on inward currents in calcium-free solutions in the presence of 50 mmol/l TEA. Depolarization steps ranged from +10 to +60 mV. Holding potential was −45 mV.

Discussion

Faber and Klee (1972) concluded that PTZ bursts might be a consequence of anomalous rectification and gradual depression of I_A currents. Klee et al. (1973) found PTZ to be ineffective on synaptic transmission, but to depress spike overshoot, hyperpolarizing afterpotentials and to cause depolarization and frequent firing. The drug increased membrane resistance, depressed the total inward current. Essentially the same was reported in a later publication by Klee (1976) based on experiments on R2 cells of *Aplysia californica*. Williamson and Crill (1976a, b) studied PTZ effects on central neurons of some Archidoris and Anisodoris species. At 140 mmol/l PTZ the spike amplitude was reduced, both spike duration and time-to-peak were prolonged and the afterhyperpolarization disappeared. PTZ depressed the leakage current and both voltage dependent potassium currents (I_A and I_K) by way of reducing the respective conductances. The convulsive action of PTZ was ascribed by these authors to the depression of the I_A current. PTZ effects on inward currents were not examined in this experimental series.

Our data obtained on *Helix pomatia* are in good agreement with those

derived from other Gastropoda species. PTZ prolonged the membrane time constant, although no consequent elevation of the membrane resistance was observed. It is worth of being mentioned that absence of potassium ions in the bathing medium remarkably increased both the membrane resistance and time constant. The membrane capacity as calculated from the resistance and time constant, was between 3.5 and 4 μF and suffered no consequent alteration by PTZ. The causes and mechanism of PTZ action on membrane time constant remains unclear; however it seems certain that they are not closely related to the convulsive action of the drug.

In the pre-convulsive phase PTZ did not alter the resting membrane potential. However, in the convulsive phase, when PDSs emerged, the membrane potential did not return to its resting level in the silent periods but remained depolarized by 5—10 mV. This may have contributed to the initiation of the next PDS if not being the immediate cause of it.

The PTZ effects on the ionic currents, observed by us are mostly in accordance with those reported by other authors. PTZ effect on I_A current was qualitatively and quantitatively the same as described by Williamson and Crill (1976a). The depression of I_A was strongly voltage dependent: it became deeper as depolarization commands increased. Depressing effect on I_A current was also obvious without any sign of voltage dependence. PTZ seems to attach to I_A channels in their open state.

I_K current was depressed by PTZ both at its peak value and at later steady state values. The depression was voltage dependent as well, with the direction of voltage dependence being inverse: with increasing depolarizing command pulses the depression was reduced. One is led to think that PTZ is removed from I_K channels by depolarization.

In view of PTZ action on inactivation time constants of I_K our findings are essentially in agreement with those of Klee (1976) and Williamson and Crill (1976a).

The total inward currents were not depressed in our experiments by 50 mmol/l PTZ (in the presence of 50 mmol/l TEA), but occasionally enhanced. (These rare instances were not shown in the Results.) This contrasts with the observation reported on Aplysia by Klee et al. (1973) and on Helix (Klee 1976). After removal of calcium ions the remaining sodium current was moderately depressed by the drug. The calcium current, after replacement of Tris for sodium ions, was somewhat enhanced by PTZ. The probable cause for the difference between our data and those obtained on Aplysia is that calcium ions participate more intensively in the generation of spike potentials in Aplysia than in Helix.

As to the mechanism of modification of spike potentials under the effect of convulsant drugs, our interpretation is supported by the data of Aldrich et al. (1979). At prolonging the spike potentials during repetitive firing the authors

observed a reduction in the I_K current. They have ascribed some role to the calcium current in delaying the repolarization: it becomes unmasked by the depression of potassium currents in the repolarization phase and by slow kinetics it may be able to considerably prolong the spike. Essentially the same was observed by Holden et al. (1982) after application of 10 mmol/l 4-aminopyridine, a potent blocker of I_K potassium channels. All these data show a close correspondance with our data and interpretations. PDS as a central phenomenon of the of the convulsive state cannot immediately be traced back to changes in membrane parameters and physiologically operating ionic conductances. Rhythmic firing, bursting pacemaker activity and PDSs may have their common background in a slowly inactivating inward current observed first by Smith et al. (1975) in bursting pacemaker neurons of Aplysia. This current carried mainly by sodium ions and entering the cell during PDS has not been investigated in this work. Its importance however, is suggested by the fact, that Williamson and Crill (1976b) were compelled to suppose a steady inward current in their model in order to maintain PDS-like depolarizations. This means that depression of potassium currents and some enhancement of normal inward currents are not satisfactory for building up the electrographic picture of drug-induced convulsions.

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