The ionic dependence and the nature of conductance was examined at slowly inactivating inward current in metacerebral giant cells of Helix pomatia, induced by 50 mM pentylenetetrazol. Ramp and square wave depolarizations in voltage clamp mode revealed, that withdrawal of sodium ions prevented this current to flow. While TTX was ineffective, Mn, Co and Ni-ions and verapamil blocked the current. It is concluded that PTZ, especially in presence of TEA impairs calcium channels, which loose their specificity and transmit sodium ions, with very slow kinetics.

Keywords: Snail neuron — pentylenetetrazol — ionic currents — ion channel Blockers

INTRODUCTION

In a previous paper the effects of pentylenetetrazol (PTZ) were analyzed on the electric membrane parameters, action potentials, inward and outward currents on the metacerebral giant cell (MCC) of Helix pomatia /3/. At the same time paroxysmal depolarization shifts (PODs) elicited by the drug were also recorded. The most obvious effect of PTZ, the depression of the IA and IK currents, although proved to be a corollary of the convulsive actions, failed to provide an explanation for the PODs, all the more, because it did not modify substantially the inward Na- and Ca-currents. Our attention was therefore directed to the origin of those long-lasting depolarization plateaus, which underlie to the PODs and bear on the crest.
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normal or partially inactivated spike potentials. Our aim was to clear up i) what kind(s) of channels are used by this slow, scarcely inactivating current, and ii) what kind(s) of ions are carrying it.

MATERIALS AND METHODS

The experiments were carried out on the metacerebral giant cell (MCC) of the snail, Helix pomatia L. To prepare the identified cells, the ganglionic mass was dissected from the animal and the cerebral ganglion with its ventral surface upwards was fixed to the bottom of an organ bath, covered with Sylgard. After peeling off the connective tissue sheets the MCC was sought under binocular magnification (x40). For penetration of the neurone with a microelectrode a standard mechanical micromanipulator was used.

The preparation was continuously superfused with normal and modified Helix-physiological solution. The normal Helix-physiological solution contained (in millimoles) NaCl 80, KCl 4, CaCl₂ 7, MgCl₂ 5, Tris Cl 5, (pH 7.4). Sodium-free solution was prepared with equimolar substitution for NaCl with Tris-HCl or choline-Cl. In Ca-free solutions CaCl₂ was replaced by MgCl₂, equimolar. In some experiments 10 mmol/l NiCl₂ was used to block Ca-channels, 30-50 mmol/l tetraethylammonium chloride (TEA) to block potassium channels. PTZ was dissolved in 20 to 50 mmol/l concentrations without osmotic balance. All experiments were performed at room temperature (22–25 °C).

Current-clamp and voltage clamp recordings were made by use of a single-channel voltage clamp amplifier built according to the design of Wilson and Goldner /10/ and Merckel /6/. Glass microelectrodes were filled with one molar potassium citrate; their resistance ranged from 2 to 7 MΩms. Potentials and current records were visualized and photographed from the screen of a Tektronix storage oscilloscope. Occasionally, current-voltage curves were recorded with an X-Y plotter. A second oscilloscope was used for monitoring the sampling process. The duty cycle of the sample-and-hold amplifier was 50% and all current values were corrected according to this proportion.

RESULTS

Since PDs are generally introduced by a spike potential (at least the spontaneous ones) it was supposed, that the slow inward current underlying them is mediated by voltage dependent ionic channels. This was demonstrated previously by Gola /4/. Therefore cells operating in voltage clamp mode were depolarized in two ways: slow voltage ramps and square wave pulses were applied through the microelectrode.

The steepness of the ramps (dv/dt) in both directions ranged from 20 to 25 mV/s; the maximal depolarization extended from the holding potential (typically -40 – 45 mV) to +30 mV. The I-V characteristics of a non-treated
THE IONIC MECHANISM OF THE PENTYLENETETRAZOL CONVULSIONS

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The ionic dependence and the nature of conductance was examined at slowly inactivating inward current in metacerebral giant cells of Helix pomatia, induced by 50 mM pentylentetrazol. Ramp and square wave depolarizations in voltage clamp mode revealed, that withdrawal of sodium ions prevented this current to flow. While TTX was ineffective, Mn, Co and Ni-ions and verapamil blocked the current. It is concluded that PTZ, especially in presence of TEA impairs calcium channels, which loose their specificity and transmit sodium ions, with very slow kinetics.

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Fig. 1. A: Current-voltage characteristic of an untreated metacerebral giant cell (MCC). Left top: voltage and current calibration. B: The same under the effect of 50 mM PTZ, after 20 min application. C: The effect of 50 mM PTZ and 30 mM TEA

MCC is presented in Fig. 1A as recorded during a voltage ramp under voltage clamp conditions. The hysteresis on the descending limb can be ascribed to a slowly activating outward current. On the hyperpolarizing limb only some leakage current is present. The superfusion of 50 mM PTZ brought about considerable modifications at this I-V characteristic (Fig. 1B). With the decrease of the membrane potential pari passu an inward current appeared, which attained an apparent maximum at -10 mV; then this went over into an outward current with decreased final amplitude and without hysteresis. At the beginning of the depolarization spike artifacts appeared from poorly clamped regions of the cell.

Since it was probable, that the slow inward current induced by the PTZ might be partially masked by outward currents, being activated simultaneously, 30 mM TEA was added to the PTZ, for depressing most part of the outward potassium currents. As it is demonstrated in Fig. 1C, under these conditions during depolarizing ramp no outward current appeared, and the negative resistance region of the I-V characteristic contained the slow inward current at its total amplitude. Its maximum was at 0 mV membrane potential and its reversal point was at +25 mV. At the start of the depolarization also spike artifacts could be seen. This state of the cell, when recorded in current clamp mode, was already strongly convulsive: PDs follow each other with irregular intervals. The spike potentials,
which initiate PDSs or ride on their crest were widened, due to the
presence of TEA and/or PTZ.

In further experiments the slow inward current, isolated in the
aforementioned way, was analyzed in view of the ions carrying it and the
kind of channels used by them.

The ion dependence was examined by use of ion-deficient solutions.
In presence of 50 mM PTZ and 30 mM TEA the substitution of the sodium ions
with Tris modified the I-V characteristic in a direction qualitatively well
defined, but at different extents. In some cases (Fig. 2) omission of Na-
ions resulted in complete disappearance of the slow inward current, indi-
cating that it was mediated exclusively by sodium ions. In this same
experiment, however, 11 min application of 15 mM CdCl₂ led to the same
result. Application of 15 mM NaCl₂ had the same effect (Fig. 2B).

Since participation of sodium ions in the slow inward current was
indisputable, it could not be excluded that part of them is conveyed
through tetrodotoxin (TTX) sensitive "fast" sodium channels. Therefore the
effect of 10 μM TTX was examined on the slow inward current induced by PTZ
and TEA, on several cells. As it can be seen in Fig. 3, this concentration
of TTX failed to modify the I-V characteristics also after 28 min of appli-
cation. At the same cell, withdrawal of sodium ions depressed the slow
inward current to a small fraction. Although there are observations /5/
that sodium channels in the Helix neuron membrane are TTX resistant, it is
worth of mention that after application of TTX no spontaneous or evoked
spike potentials were encountered.

The conductance, transmitting the slow inward current could be
measured quantitatively by use of square voltage pulses of 4 s duration in
voltage clamp mode. The current amplitude was measured at different command
potentials at 1000 ms after onset of the pulse, because by this time any
contamination from fast inward and outward currents (eventually not blocked
by TEA) could be excluded. The voltage dependence of the slow inward current
on four cells, treated with 50 mM PTZ and 30 mM TEA is demonstrated in
Fig. 4. The corresponding conductance values are represented in Fig. 5. The
conductances are uniformly and monotonically voltage dependent and along
with the applied voltage they increase practically linearly. The current,
however, diminishes over some level of depolarization because the membrane
potential gets near to the reversal potential of the ion carrying it.

Although in transmission of the slow inward current both sodium and calcium
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Fig. 2. A: Current-voltage characteristic of a MDC under control conditions (1st row), after application of 50 mM PTZ and 30 mM TEA (2nd row), after withdrawal of sodium ions (3rd row), after returning to PTZ+ TEA superfusion (4th row), and under the effect of 15 mM MnCl₂ (5th row). B: Another MDC under the same conditions but after 6 min application of 15 mM NiCl₂ (3rd row) and after 14 min (4th row).

Ions take part, as reversal potential for computation of conductances +30 mV was chosen.

In some part of the experiments the slow inward current, elicited with ramp voltage pulses, showed not only one maximum (as in Fig. 1) but two. During superfusion of PTZ and TEA (at the usual concentrations) the slow inward current had two maxima: one at -13 mV and one at +8 mV membrane potential (Fig. 6). 15 mM MnCl₂ depressed this current strongly. The first maximum disappeared and the second maximum seemed to be shifted to higher potential values. There appeared, that the membrane potential shift, provided by the ramp was not enough to reach the voltage at which this
Fig. 3. The effect of 10 µM tetrodotoxin (TTX) on the current-voltage characteristic of a MCC, during application of 50 mM PTZ and 30 mM TEA, and during superfusion of Na-deficient solution, containing the same drugs. The notation "normal" refers to the sodium concentration.

The current could attain its maximum. This might lie over +30 mV. After washing out the manganese, practically the initial situation was restored, but with the two maxima better separated. Replacement of the sodium with Tris destroyed the first maximum almost completely, keeping the second one intact. Application of MnCl₂ in this situation led to complete removal of any conductance.

A similar sequence of events could be reproduced with Ni- and Co-ions with interpolated withdrawal of sodium ions, not presented here.

The time relations of activation and inactivation of the slow inward current is rather difficult to analyze. The process of activation may suffer interferences from activation of fast sodium and calcium currents. Use of TTX is not of great use, because calcium channels, taking part in generation
of spike potentials are not blocked by the drug. The process of inactivation was examined by computation of the time constants. The descending phase of the currents, elicited by square wave voltage pulses in voltage clamp mode seem to decline along two time constants: an initial shorter and a late longer one (Fig. 7). Finally the current has a time independent plateau, without any sign of inactivation. The logarithmic plots of the descending phases and time constants calculated from them are comprised in Fig. 6.

The slow inward current proved to be largely sodium dependent also in this experimental paradigm: on withdrawing the Na-ions from the superfusing fluid PTZ failed to evoke any slow inward current also in presence to TEA (Fig. 7). With omission of calcium ions no experiment was made, because lack of calcium might damage the membrane seriously.
Fig. 5. Plot of conductances (g) as a function of membrane potential (V) at the same MCCs which were presented in Fig. 4. Notations are the same. Voltage calibration: 10 mV. Calibration for conductance: 100 nS for A and B, 200 nS for C and D.

Blockers of the calcium channels proved to be effective also in inward current, elicited with square wave voltage pulses: Co-, Ni- ions and verapamil decreased the slow inward current largely or completely (Fig. 9).
Fig. 6. Current voltage characteristic of a MEC under control conditions, under the effect of 50 mM PTZ and 30 mM TEA, then treated with 15 mM MnCl₂, and exposed to sodium deficient solution. The inward current had two maxima, which responded to Mn ions in different manner.

Fig. 7. Records of the slow inward current obtained with depolarizing steps of +20 to +60 mV from the holding potential (-45 mV), and with 4.5 s duration. The neuron was exposed to 50 mM PTZ and 30 mM TEA, over the whole recording period. At low depolarizations the current reached its maximum rapidly, decayed slowly and had a considerable steady state value. At depolarizations to 0 mV or more positive potentials the current showed a second maximum and faster inactivation. Pulse amplitudes are shown at right. Calibration for current: 12.5 nA, for time: 0.5 s.
Fig. 8. Logarithmic plots of the current curves presented in Fig. 7, and the time constants calculated from them. Numerical values of time constants are tabulated at top right.

Fig. 9. Effect of sodium withdrawal (A), NiCl₂ (B) and verapamil (C) on the slow inward current of the MCC demonstrated in Fig. 6. The neuron was treated with 50 mM PTZ and 30 mM TEA over the whole recording time. Calibrations are the same as in Fig. 6.
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DISCUSSION

It has been widely accepted that spontaneous bursting behaviour, at least in Mollusca, finds its basis in two special modifications of membrane conductance: (i) a slow, scarcely inactivating inward current and (ii) depression of potassium conductance. Although the same has been established for mammalian neural substrates, we shall deal here only with findings obtained on Gastropoda. Smith and coll. /7/ observed in neurons of Otala and Aplysia that diminution of the sodium concentration in the superfusing fluid from 100 nM to 25 nM destroyed bursting pacemaker activity. The authors presume that bursting pacemaker activity relies upon a slowly inactivating (τ = 16 s) sodium conductance, which together with a cyclically changing potassium conductance, becomes enabled to generate a bursting pacemaker activity. Swandulla and Lux /8/ report, that such a non-specific cationic conductance can be activated by elevation of the intracellular calcium concentration. TTX was found ineffective on these slow inward conductances. Inorganic and organic Ca-channel blockers, on the other hand, were highly effective in inactivating these channels (Walden and coll. /9/). In bursting pacemaker neurons this current is permanently present in the pacemaker range of membrane potential and at moderate depolarizations it causes a negative slope resistance, as revealed in voltage clamp situation. The carriers of this current may be Ca-ions /2/ or Na-ions /1/.

The MCC neuron is not a bursting pacemaker and under natural conditions neither slow inward current, nor cyclically decreasing potassium conductance is present in it. This is indicated by I-V characteristics taken by use of ramp- or square wave voltage steps. On effect of PTZ, however, they begin to operate which results in a membrane behaviour, comparable in all respects to that of the bursting pacemaker neurons, as it was characterized by Swandulla and Lux /8/. In view of its ionic dependence, it is not surprising that it is carried mainly by sodium ions, although in some cases also Ca-ions may take a considerable part in it. This supposition seems to be justified in cases, when the slow inward current exhibits two maxima, as it was described by us in this paper. The first of them vanishes on withdrawal of sodium ions, while the other one, at more positive potentials becomes depressed by calcium channel blockers. There appears, that although sodium ions use most part calcium channels also in these cases, another part is penetrated by Ca-ions.

The present experiments have succeeded in clearing up several other
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properties of this conductance. Thus, its voltage dependence proved to be almost linearly proportional to the depolarization, without attaining any maximum, as it is usual at conductances participating in the normal neuronal activity. This points to a basically different channel kinetics and cannot be dealt with as the "normal" ones. The inactivation properties are much more similar to those e.g. of the potassium, although its final stage with the time independent inward conductance appears to be unique. It is probable, that the final, time independent conductance causing long-lasting moderate depolarization gives opportunity for the gradually activating potassium currents to finish the paroxysmal depolarizations. At least at POs, which have an after-hyperpolarization this seems to be the case.

The "abnormality" of the conductance invoked by PTZ is indicated also by its poor specificity: this channel, which has retained its vulnerability by calcium channel blockers, has lost its specificity to calcium: it transmits sodium ions with unusual intensity. These distortions seem to be the central moments of the membrane behaviour, characteristic to the neurons, made convulsive by chemical, physical or metabolic factors.

REFERENCES

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