EFFECT OF PYRACETAM* ON HABITUATION OF THE ACETYLCHOLINE RECEPTOR MEMBRANE OF Helix lucorum Neurons

A. S. Pivovarov, R. U. Ostrovskaya,
E. I. Drozdova, and S. A. Saakyan

UDC 612.821.2.014.46:[615.214.3:547.745]-019:594.8

KEY WORDS: pyracetam; habituation; acetylcholine receptor membranes; acetylcholine

Pyracetam (Nootropil) is a member of the group of nootropic compounds which are used in medicine to improve the higher integrative functions of the brain, including memory, when disturbed by various extremal factors [6]. Habituation to a repeatedly applied stimulus is a convenient experimental model with which to study the trace processes lying at the basis of memory. Habituation is generally regarded as the simplest form of learning, during which reversible weakening or complete suppression of responses takes place in the animal [2, 5, 8]. It is characteristic of animals at different phylogenetic levels [2]. Considering the relatively simple organization of the nervous system of invertebrates and, in particular, of mollusks, it was decided to use it in order to assess the cellular mechanisms of the action of pyracetam on trace processes. In this investigation the effect of pyracetam on the membrane potential of identified snail neurons and on habituation of the acetylcholine receptor membrane of these neurons to local repetitive application of acetylcholine (ACh) to the neuron body was studied.

EXPERIMENTAL METHOD

Experiments were carried out on identified neurons (for the classification, see [4]) of the subesophageal ganglion complex in a preparation of the isolated CNS of the snail Helix lucorum taurica Kryn. The preparation was placed in a continuous-flow chamber with a capacity of 1 ml, through which Ringer's solution of the following composition (in mM) flowed at the rate of 1 ml/min: NaCl 100, KCl 4, $CaCl_2$ 10, $MgCl_2$ 4, Tris-HCl 10 (pH 7.5). The cell potentials were recorded intracellularly by glass microelectrodes filled with 2 M potassium citrate solution (resistance of the electrodes 70-120 mΩ). Extracellular microiontophoretic application of ACh was used, by passing cationic currents (70-1440 nA, 0.1-10 sec) through electrophoretic pipets, filled with a solution of ACh chloride in distilled water (4 M, pH 4.0, from Serva, West Germany). The resistance of the pipets was 30-70 MΩ. To verify the specificity of the reactions to ACh electrophoresis, blockers of acetylcholine receptors of muscarinic (atropine, from Serva) or nicotinic (D-tubocurarine, from Fluka, Switzerland) type were injected with a micropipet into the continuous-flow chamber in concentrations of 10^{-6}-10^{-4} M. Pyracetam was injected into the chamber from a micropipet in a concentration of 10^{-6}-10^{-1} M or was diluted in the solution which was passed through the chamber (10^{-3}-10^{-2} M).

EXPERIMENTAL RESULTS

In the first stage of the work the neurons sensitive to pyracetam were selected among a series of identified cells: LPl1, Vl, V2, V3, V4, V5, V6, RPa2, RPa3, RPa4, and RPa5 (19 neurons). It was shown that pyracetam, if injected into the continuous-flow chamber, changed the membrane potential of most (57.9%) of the neurons tested, in a fairly high concentration ($5 \cdot 10^{-2}$ M). Pyracetam had an excitatory effect (membrane depolarization, an increase in the spike discharge frequency) in most (63.6%) of the neurons sensitive to it. These were cells V2, V3, V4, V6, and LPl1. The excitatory response lasted more than 30 min. The excitation which appeared in neuron RPa2 and an unidentified cell in the F zone (18.2%) was

*α-Pyrrolidone acetamide

Department of Physiology of Higher Nervous Activity, M. V. Lomonosov Moscow University. Department of Neuropharmacology, Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 7, pp. 51-53, July, 1987. Original article submitted June 29, 1986.

 0007-4888/87/0007-0934$12.50

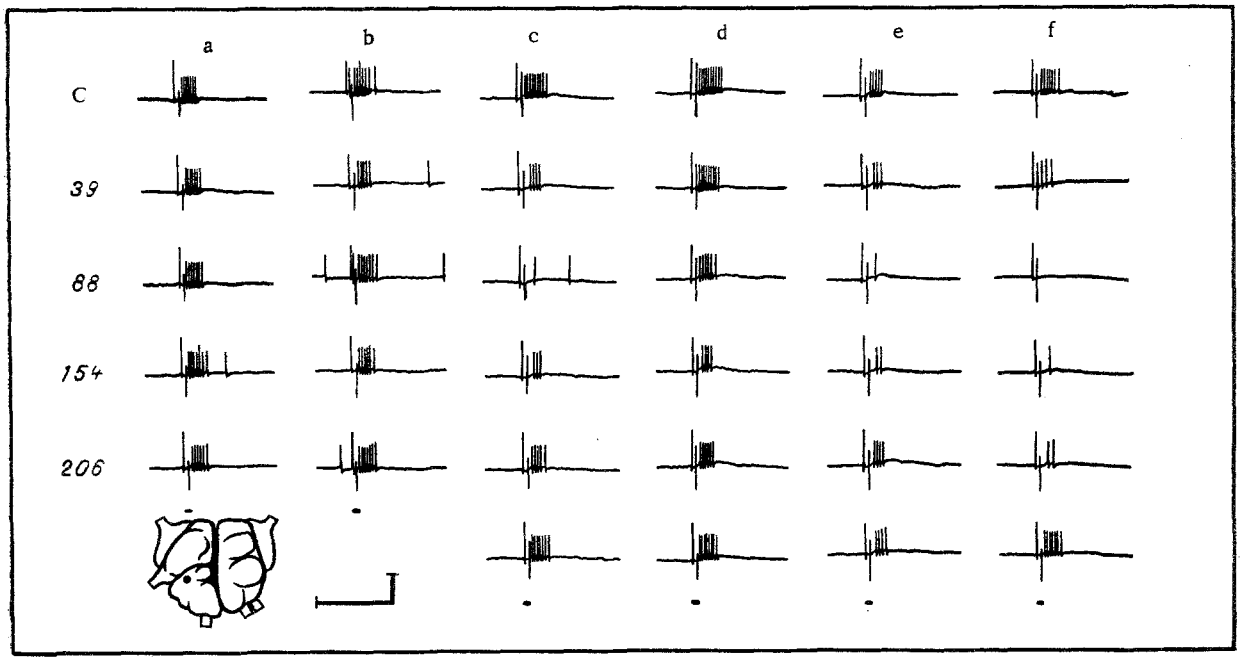

Fig. 1. Effect of atropine and D-tubocurarine on response of neuron V3 to ACh. a, c, e) Atropine in concentrations of 10^{-6}, $5 \cdot 10^{-5}$, and 10^{-4} M respectively; b, d, f) D-tubocurarine in concentrations of 10^{-5}, $5 \cdot 10^{-5}$, and 10^{-4} M respectively. C) Response to ACh before injection of the acetylcholine receptor blocker into the continuous flow chamber. Numbers on the left show time after injection of blocker (in sec). Calibration: 50 mV, 20 sec.

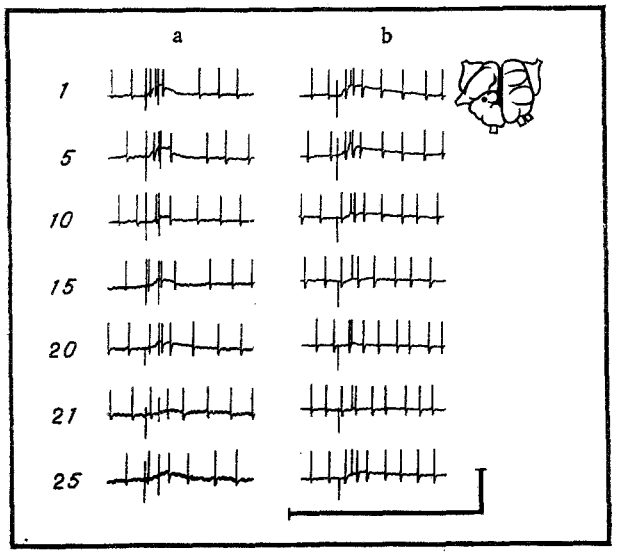

Fig. 2. Effect of pyracetam on depressor ACh response of V3 neuron to repetitive microiontophoretic applications of ACh. a) Increase in frequency in continuously flowing solution of normal composition; b) addition of 10^{-2} M pyracetam after running solution for 45 min through chamber with preparation. Numbers on left — serial numbers of ACh applications at intervals of 18-22 sec; 25th application of ACh, 8 min (a, b) after end of repetitive stimulation. Amplitude of current 760 nA. Scheme of dorsal surface of subesophageal ganglion complex, on which the dot marks the location of the neuron whose response is illustrated, is shown at the top of the figure. Calibration: 50 mV, 20 sec.

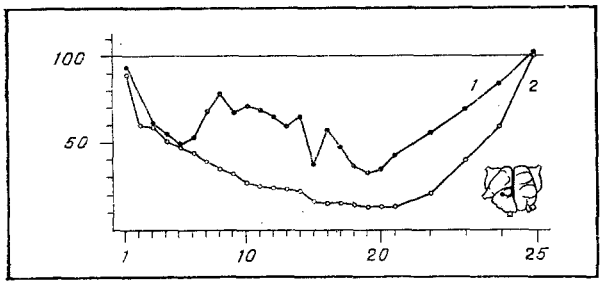

Fig. 3. Effect of pyracetam on time course of amplitude of depolarization potential of ACh response of V3 neuron to repetitive applications of ACh. Abscissa, serial No. of ACh application; ordinate, amplitude of depolarization potential of ACh reaction (in %, average amplitude of depolarization potentials of ACh responses to control stimulus 5 min before beginning of series and first stimulus in series taken as 100%). Amplitude of electrophoretic current 604 nA. Intervals between stimuli: from first through 21st 18-22 sec, from 21st through 25th 2 min. 1) Series in continuously flowing solution of normal composition, 2) with addition of pyracetam (10^{-2} M, 45 min). Each curve is the average of two graphs obtained in consecutive series with an interval of 45 min.

followed by an inhibitory response, in the form of hyperpolarization and a decrease in the spontaneous firing rate. In neuron RPa5 and an unidentified cell in the A zone (18.2%) pyracetam caused inhibition without previous excitation. On neurons V1, V5, RPa3, and Rpa4 (42.1%) pyracetam, in the concentrations used, did not change the membrane potential.

Since depolarization of the cell membrane was the most characteristic response to pyracetam, the effect of the drug on habituation to responses to ACh was tested on cells of one type (V3; 13 neurons) in the next experiments. Local application of ACh to the body of these neurons caused high-amplitude (up to 30 mV) depolarization with superposed spikes (Figs. 1 and 2). Injection of acetylcholine receptor blockers (atropine, D-tubocurarine) into the flow reversibly reduced the ACh response of the neurons or completely blocked it (Fig. 1c-f), evidence of specific activation by ACh of the muscarinic and nicotinic acetylcholine receptors of the membrane.

Repetitive application of ACh at constant time intervals, which varied from 20 to 80 sec in different experiments, led to a gradually developing diminution of the ACh response (compare Figs. 1 and 2a; Fig. 3). The response was reduced as a rule by 50-70%. On cessation of the repetitive stimulation the magnitude of the ACh response was completely restored after 7-10 min.

The effect of pyracetam on habituation of the acetylcholine receptor membrane was studied during prolonged exposure (45 min) by passing a solution containing pyracetam in a concentration of 10^{-3}-10^{-2} M, below the threshold for changing the membrane potential of the cells, through the chamber. Pyracetam in a concentration of 10^{-2} M was found not to change the primary response to ACh (evidence that it had no direct effect on sensitivity of the acetylcholine receptors to ACh), whereas it accelerated and deepened habituation to ACh responses (Figs. 2b and 3).

The effectiveness of action of pyracetam only when used in high concentrations was evidently due to the fact that in the whole animal this preparation exhibits its activity only in high doses — up to 1000 mg/kg, corresponding to a blood level of up to 10^{-3} M. A similar concentration of pyracetam was found in the blood serum of patients receiving this drug in a therapeutic dose [7]. The fact that pyracetam has a mainly depolarizing action, described in this paper, is interesting from the standpoint of the hypothesis of synergism of pyracetam with the glutamate—pyroglutamate system and its antagonism with proline [3], for we know that a shift toward membrane depolarization is characteristic of the action of glutamate.

The ability of pyracetam to accelerate and potentiate habituation at the neuronal level, revealed by these experiments, is evidently the analog, at the cellular level, of those effects of pyracetam which have been found in relation to integral behavioral responses, such as its ability to restore habituation of rats to stress factors in the open field test,

weakened by cycloheximide [1]. This suggests that experiments on identified neurons of Helix lucorum is an adequate model for the pharmacological analysis of trace processes in the nervous system.

LITERATURE CITED

1. Yu. V. Burov, R. U. Ostrovskaya, N. M. Smol'nikova, et al., Byull. Éksp. Biol. Med., No. 4, 447 (1986).
2. D. Dewsbury, Comparative Animal Behavior, New York (1978).
3. R. U. Ostrovskaya, S. S. Trofimov, N. M. Tsybina, et al., Byull. Éksp. Biol. Med., No. 3, 311 (1985).
4. D. A. Sakharov, The Genealogy of Neurons [in Russian], Moscow (1974).
5. R. A. Hine, Animal Behavior: A Synthesis of Ethology and Comparative Psychology, 2nd edn., New York (1970).
6. C. Giurgea, Acta Pharmacol., 25, 115 (1972).
7. R. M. Nalbandian and R. L. Henry, International Symposium on Nootropic Drugs, Rio de Janeiro (1979), pp. 265-273.
8. W. H. Thorpe, Learning and Instinct in Animals, London (1964).

EFFECT OF VERAPAMIL ON FOCAL EPILEPTIC ACTIVITY IN THE RAT CEREBRAL CORTEX

M. N. Karpova, R. N. Glebov, G. N. Kryzhanovskii,* and O. L. Agatova

UDC 616.831-009.24-031.84-092.9-085.217.24

KEY WORDS: penicillin; Ca^{++} ions; epileptic activity; generator of pathologically enhanced excitation; verapamil

An important role in the hyperactivation of neurons which collectively constitute a generator of pathologically enhanced excitation (GPEE) [3] is played by a number of factors, including Ca^{++} ions. During the development of epileptic activity (EA), produced by different methods, the inflow of Ca^{++} into neurons from the extracellular medium and its release into the cytoplasm from the internal depots are intensified [1, 2]. Under these conditions blockers of intracellular calcium inflow may have a protective action.

The writers showed previously [4] that the development of generalized EA is accompanied by inactivation of the Ca pump of the synaptic membranes, with which an increase in the intracellular Ca^{++} concentration and the development of pathological hyperreactivity of the neurons may be connected. It has been suggested that inhibitors of Ca channels may be anticonvulsants.

The aim of this investigation was to study the effect of verapamil (izoptin), a blocker of voltage-dependent Ca channels, on focal EA induced by application of penicillin to the rat cerebral cortex.

EXPERIMENTAL METHOD

Experiments were carried out on 32 control and 32 experimental male Wistar rats weighing 200-220 g. Under hexobarbital (150 mg/kg, intraperitoneally) and local procaine anesthesia, burr-holes measuring 2 × 4 mm were drilled in the animal's skull in symmetrical regions of the sensomotor cortex of both cerebral hemispheres 24 h before the experiment, the dura was removed from these regions, and monopolar silver cortical electrodes were applied to record

*Academician of the Academy of Medical Sciences of the USSR.

Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow.

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 7, pp. 54-57, July, 1987. Original article submitted December 18, 1986.