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Effect of Picamilon on Inhibitory Postsynaptic Responses of Cortical Neurons

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In experiments on immobilized anesthetized rats, we intracellularly recorded neuronal responses in the motor cortex before and after application of picamilon (PM) on the cortical surface; the responses were evoked by intracortical stimulation. Aplications of PM in the 5, 20, 50, and 100 μM concentrations noticeably increased, while that in the 10 μM concentration decreased the amplitude of IPSP in the cortical neurons. Probable mechanisms of the effect of PM on a cellular level are discussed.

INTRODUCTION

At present, pharmacological characteristics of a group of the GABA–vitamin conjugates are being extensively studied; picamilon (PM) is a member of this group. These agents demonstrate GABA-ergic properties [1, 2], but possibilities for their therapeutic use have not been adequately studied, and a number of the mechanisms of their effects remain undiscovered. In our study, we tested the effects of PM on postsynaptic responses in neocortical neurons.

METHODS

The experiments were carried out on 15 adult rats weighing 200-300 g. Surgery was performed under urethane anesthesia (i.p., 1.0 g/kg). The motor cortex was opened. During recording, an animal was immobilized with i.m. injected d-tubocurarine (10 mg/kg) and artificially ventilated.

The activity of neocortical neurons was recorded intracellularly with glass microelectrodes (30-60 MΩ) filled with 3.0 potassium acetate. Responses of these neurons were evoked by intracortical stimulation (ICS) applied through a needle nichrome electrode.

In close proximity to the stimulating electrode, a strip of the filter paper was laid on, and a cannula touching the surface of the strip and connected to a microsyringe was positioned. The microsyringe was filled with the PM solution (Vitaminy, Russia); the PM concentrations of 5, 10, 20, 50, an 100 μM were tested. When administered systemically, PM was i.p. injected in the doses of 5, 10, 20, 50, and 100 mg/kg.

The results were treated using Student's *t*-test for small sample groups.

RESULTS AND DISCUSSION

In the first series of experiments, we studied the influence of local superficial applications of PM on ICS-evoked inhibitory postsynaptic responses in the cortical neurons. In this series, 39 neurons were recorded; they responded to ICS by reactions of three types: primary IPSP, EPSP-IPSP, and EPSP-spike-IPSP (Fig. 1A).

Application of the lowest tested concentration of PM (5 μM) resulted in a mild, but significant increase in the IPSP amplitude (by 0.9 mV, on the average). In most cases, the background spike activity (BA) of the neurons was preserved (Fig. 1B). The effect of a higer PM concentration (10 μM) was different: this evoked a clear decrease in the IPSP amplitude and gradual inversion of hyperpolarizing components of the responses into depolarizing reactions; early IPSP components underwent the most expressed reversion (C). The mean amplitude of these early depolarizing components under the above conditions was 1.7 ± 0.8 mV, and their mean duration was 45.2 ± 4.5 msec. At the same time, late IPSP components in some cases preserved their hyper-

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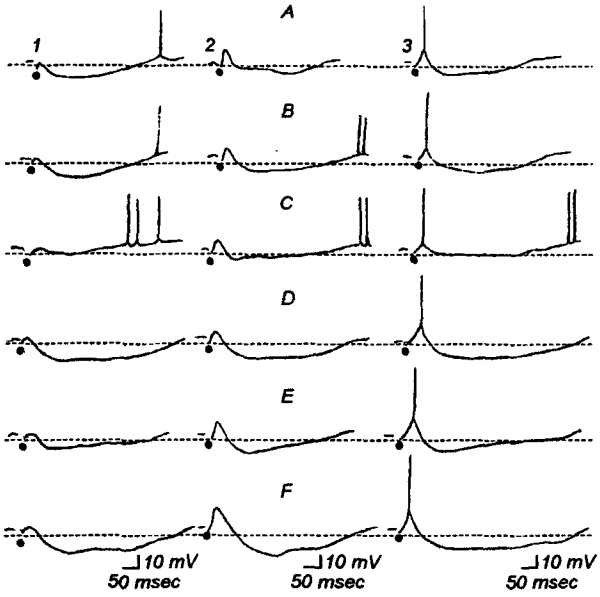

Fig. 1. Effects of picamilon (PM) in different concentrations on postsynaptic responses of three cortical neurons. A) Neuronal responses before PM application; B-F) after applications in the doses of 5, 10, 20, 50, and 100 μM, respectively. A 50 mV level of the membrane potential is shown by dashed lines; dots indicate the moments of intracortical stimulations. 1) Primary IPSP; 2) EPSP-IPSP; and 3) EPSP-spike-IPSP.

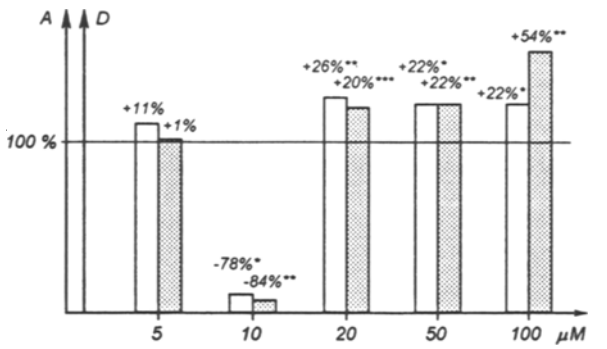

Fig. 2. Effects of superficial application of picamilon in different concentrations on the IPSP amplitude and duration. Horizontal scale) Concentration of the drug, μM; vertical scale) normalized mean amplitude (open columns) and duration (dashed columns) of EPSP. The IPSP parameters under initial conditions are taken as 100%. One, two, and three asterisks indicate the significance levels $P < 0.05$; $P < 0.01$; and $P < 0.001$, respectively.

polarizing pattern, but their amplitude considerably dropped; in these neurons an early IPSP component was inverted (C, 2, 3). In other cases, the late IPSP component followed the early component with its reversion: it gradually decreased and possessed a depolarizing pattern (C, 1). This phenomenon was accompanied by intensification of the BA of neurons.

Picamilon in the next increased concenration (20 μM) evoked opposite effects, which were to a certain extent similar to those evoked by the lowest PM concentration but more intensive. The amplitudes of both early and late IPSP components considerably increased (the former by 2.0 mV, on the average). Besides this, the total duration of inhibitory responses also significantly increased (by 70.1 msec, on the average; Fig. 1D). In some cases, about 15 min after application of the drug the membrane potential of the cells noticeably decreased and their BA was intensified. In a high concentration (50 μM), PM also prolonged IPSP (by 62.3 msec, on the average), but this concentration usually was less effective than 20 μM (E). At application of PM used in a "superhigh" concentration (100 μM), the duration of hyperpolarizing IPSP components in many cases dramatically increased (up to 450 msec; F).

Thus, it is obvious that under conditions of superficial application of PM its minimum concentration, which provides facilitation of inhibitory postsynaptic responses of cortical neurons, equals 5 μM. Yet, in a higher, 10 μM concentration PM significantly suppressed IPSP in these neurons. In higher concentrations, superficially applied PM again began to considerably intensify inhibitory postsynaptic effects. In a "superhigh" concentration (100 μM), PM significantly increased both the amplitude and, especially, duration of ICS-evoked IPSP. This was manifested both in primary IPSP and inhibitory components of complex responses.

Thus, our results demonstrate a complex pattern of the dose dependence of the effects of PM on the inhibitory processes in cortical neurons and, respectively, of its effects on the postsynaptic membranes of these neurons. In some doses, the drug exerted an activating influence on neuronal populations of the neocortex. This was expressed as a drop in the IPSP amplitude, depolarization of the neuronal membranes, and BA intensification. It should be mentioned that similar cases of generation of depolarization-directed IPSP under the influence of synaptically released GABA were reported earlier for pyramidal cells of the rat hippocampus [3, 4]. The cited authors hypothesized that generation of depolarizing responses is related to activation of $GABA_A$ receptors localized on the neuronal dendrites. It seems natural that, considering that PM is a GABA derivative, we can suppose that in our experiments we also met a similar variant of generation of depolarizing GABA-ergic responses. This is rather probable because these modifications are accompanied by general intensification of the neuronal BA and, consequently, by spreading of activating influences through neuronal populations within the zone of drug application. In higher doses, PM exerted uniform intensifying influences upon inhibitory processes in the cortex. Yet, it should be noted that the drug in such doses can provide

some undesired effects on the systemic processes in the neuronal networks.

The second series of experiments with systemic administration of PM showed that, similarly to what is observed after PM superficial applications, this drug can either intensify, or suppress postsynaptic inhibition in cortical neurons, and the effect direction depends on the dose used.

The PM concentration providing the maximum facilitatory effect on inhibitory postsynaptic potentials was equal to 5 mg/kg. At the same time, this dose was in general near-threshold: lower doses of PM did not influence the IPSP parameters, and these lower doses were not used in the experiments. The effect of PM in a higher dose (10 mg/kg) did not significantly differ from the effect of the 5 mg/kg dose. Higher doses of systemically administered PM (20 and 50 mg/kg) could produce undesirable effects, because in these cases the equilibrium between the processes of inhibition and excitation could be easily shifted toward the prevalence of the latter, i.e., the effect was opposite to what was expected. "Superhigh" PM doses (100 mg/kg) were ineffective and did not modify the studied parameters.

Therefore, doses of the drug providing a specific effect, i.e., facilitation of the inhibitory processes, were within the range of PM threshold doses (5-10 mg/kg). In addition, results of systemic PM administration demonstrated that the doses noticeably exceeding those used at superficial PM application are necessary to provide manifestation of this effect of the drug. This is obviously related to the well-known fact: PM effects are realized only after certain storage of the agent in the brain tissue [5].

Our results allow us to conclude that under conditions of systemic PM administration the dose ranges providing the specific effect of facilitation or inhibition usually are excessively low, and their efficacy is insufficient. The drug in the specific above-described dose range can exert activating influences upon neuronal populations in the neocortex.

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