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Anaesthetic phencyclidine, blocker of the ATP-sensitive potassium channels

Yuri M. Kokoz*, Alexey E. Alekseev, Alexander A. Povzun, Antonina F. Korystova, Hector Peres-Saad

Laboratory of Functional Biochemistry, Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Moscow Region, 142292, Russian Federation

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Abstract

The double sucrose gap and patch-clamp studies revealed that phencyclidine blocked the ATP-sensitive K^+ channel in isolated cardiac cells (half-maximal inhibition at ≈20 μM; Hill coefficient ≈1). 10 μM phencyclidine increased the inward Ca^{2+} current and blocked the outward K^+ current in the frog auricle trabeculae. The phencyclidine effects on the frog auricle trabeculae and the isolated cardiac cells proved to be quite reversible.

Key words: ATP-sensitive K-channel; Phencyclidine; Calcium current; Cardiomyocyte; Patch clamp

1. Introduction

Phencyclidine (PCP, 'angel dust') is a drug, originally applied as an effective anaesthetic also possessing some narcotic properties [1]. Euphoria, hallucinations or maniacal behavior, which is difficult to distinguish from schizophrenia, could be caused by PCP depending upon the dose. PCP was applied as a narcotic in various ways: from intramuscular injection to smoking [2]. Wide abuse of phencyclidine in some areas of the USA is a source of great social trouble [3]. Up to date the mechanism of PCP action has not been elucidated in detail. The typical behavioral reaction to PCP intoxication is assumed to be related to its interaction with a number of brain receptor complexes (acetylcholine, opiate, NMDA). This interaction is followed by the blocking of various types of receptor-associated K^+ channels [4,5], the inhibition of excitatory postsynaptic potentials [6], and the additional release of neurotransmitters [7,8]. This compound, apart from the behavioral reaction, has a variety of well known positive effects: it relieves psychic overload and protects against neuronal degeneration evoked by ischaemia, anoxia and hypoglycemia. One may expect that in addition to known K^+ currents PCP is able to inhibit ATP-sensitive K^+ channels, since the blockers of this type of channels prevent hypoxia and hypoglycemia [9]. The action of PCP on voltage-dependent Ca^{2+} channels is also of interest, because the release of neurotransmitters is mainly dependent on Ca^{2+} currents. The aim of this paper was to study the influence of PCP on the ATP-sensitive K^+ channels in isolated rat cardiomyocytes and on the inward Ca^{2+} current in frog auricle trabeculae.

2. Materials and methods

Isolated cardiac cells were obtained from rat heart (the Wistar line) by a previously described method [10] with some modifications. Ca^{2+} tolerance of the cells was estimated in the cell suspension by Trypan blue exclusion 20 min after the concentration of Ca^{2+} ions had elevated from 200 μM to 2 mM. The number of non-dyed cells was equal to 85 ± 5% of their total number and did not change significantly during the experiments. Isolated cardiac cells were kept in low-calcium medium (LCM) containing 100 mM NaCl, 10 mM KCl, 200 μM Ca^{2+}, 1.2 mM KH_2PO_4, 5 mM $MgSO_4$, 20 mM glucose, 50 mM taurine, 10 mM MOPS, pH 7.25.

At the beginning of the experiments the cells were placed into a bath solution (LCM + 2 mM EGTA). The Ca^{2+} concentration was minimized to decrease the probability of vesicle formation while withdrawing the patch pipette. The resistance of patch electrodes was 5–10 MΩ. After obtaining a seal (20 GΩ) and an inside-out patch formation, this solution was substituted by an 'intracellular' one. It contained 140 mM KCl, 5 mM $MgSO_4$, 10 mM MOPS, 200 μM $CaCl_2$. Pipette solution: LCM.

Whole cell Ca^{2+} and K^+ currents were recorded in isolated auricle trabeculae of the frog (*Rana ridibunda*) using the voltage-clamp mode and the double sucrose gap technique [11] with modifications described earlier [12]. Before measurement of the ion currents, the trabeculae were adapted for 20–30 min using current impulses with a duration of 5 ms and frequency of 0.1 Hz to measure the action potentials. The shape

*Corresponding author.
E-mail. kokoz@venus.iteb.serpukhov.su

Abbreviations: ATP, adenosine-triphosphate; MOPS, (3-[*N*-morpholino]propanesulfonic) acid; EGTA, ethylene glycol bis(2-aminoethylether)-*N,N,N',N'*-tetraacetic acid; PCP, phencyclidine, (1-[1-phenylcyclohexyl]-piperidine).

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of the action potentials in the stationary regime after the adaptation virtually did not change for 1–1,5 h. While measuring Ca^{2+} currents, a rapid Na^+ current was blocked by tetrodotoxin (10^{-6} M). To measure the resting potential, the depolarizing solution was used. It contained 148 mM monosubstituted aspartate potassium, 8 mM NaCl, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, 5.8 mM NaOH, 1.5 mM $NaHCO_3$, 7 μM KH_2PO_4, pH 7.4. Bathing solution: 110 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5.8 mM NaOH, 1.5 mM $NaHCO_3$, 7 μM NaH_2PO_4, 5 mM glucose, pH 7.4. All experiments were performed at room temperature, 20–22°C.

Membrane ion currents in frog auricle trabeculae and the currents through single channels in rat cardiac cells were recorded using a PCL-718 data acquisition card (Advantech Co., Ltd.) and an IBM/AT 286 computer. The data were analyzed using 'BioQuest' software developed by Dr. A.E. Alekseev and Dr. Yu.M. Kokoz.

3. Results and discussion

3.1. PCP action on outward and slow inward currents in frog auricle trabeculae

The current–voltage relationship of the outward current in frog trabeculae is shown in Fig. 1 (top). This current was mainly due to the efflux of K^+ ions [13]. The outward current measured at the end of clamp step of 1 s duration was plotted as a function of the membrane potential. It has been found that 10 μM PCP blocked the outward current at potentials from 10 mV to 40 mV (Fig. 1). This finding has been in good agreement with the data on PCP influence on the K^+ current obtained previously [4,14].

In the presence of 10 μM PCP, the slow inward current increased more than twofold as compared to the control one (Fig. 1, bottom). The current–voltage relationship for the peak of the slow inward current was plotted. 1 μM nifedipine, a specific blocker of the Ca^{2+} currents, reduced the inward current both in controls and in the

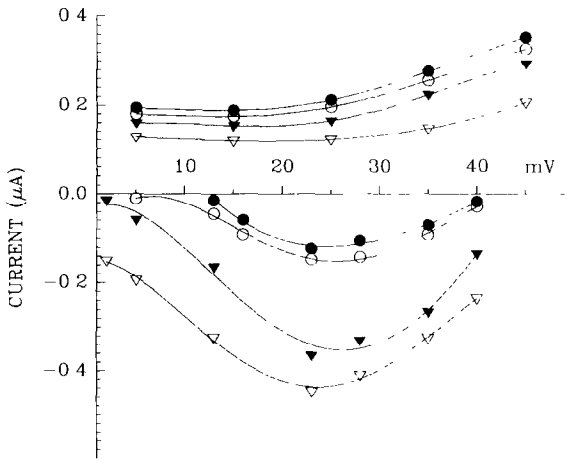

Fig. 1. The blocking of outward (K^+) and the activation of inward (Ca^{2+}) currents in auricle trabecula under the action of 10 μM PCP. Top: current–voltage relationship of the outward current. Bottom: current–voltage relationship of the inward current. Filled circle, control; filled triangle and hollow triangle, 7 min and 15 min of PCP action respectively; hollow circle, washing out.

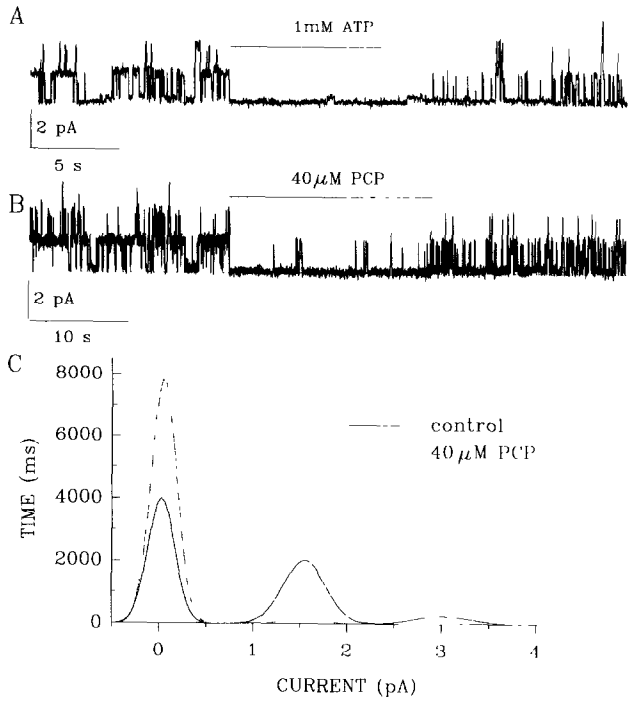

Fig. 2. Recording of the current through single ATP-sensitive K^+ channels (at least 3 independent channels functioned in the patch). A: the effect of 1 mM ATP (10 mM K^+ in pipette solution, 140 mM K^+ in bath solution; holding potential, 0 mV; filter, 300 Hz). B: the effect of 40 μM PCP (filter, 1 kHz, other parameters as in A). C: the amplitude distribution of channel currents. The data were taken from experiment shown in Fig. 2B and fitted by a sum of several Gauss functions. The total recording time (in the control conditions and under the PCP action) was 71.3 s. The open state probability was calculated using the parameters of binomial distribution fitted to areas under the peaks of the histogram.

presence of PCP to comparable values. Therefore, PCP mainly activated the Ca^{2+} inward current. Meanwhile, it was difficult to explain the augmentation of the inward current by a decrease in the outward current because the changes of that current were much smaller than those of the inward current at the same PCP concentration (Fig. 1). PCP action on the inward and outward currents was reversible; after washing out the currents returned to their control values.

3.2. The PCP action on the ATP-sensitive K^+ channels in rat cardiac cells

The ATP-sensitivity of K^+ channels was tested at the beginning of each experiment by blocking the channel activity induced by the addition of 1 mM ATP to the bath solution. When ATP was washed out, the channel activity completely recovered (Fig. 2A). Under asymmetric K^+ conditions (bath solution, 140 mM; pipette solution, 10 mM) the single channel conductance determined from the linear part of the current–voltage relationship was equal to 24 ± 1.3 pS. The reversal potential coincided with one predicted by Nernst equation. Thus, the

recorded channel could be identified as an ATP-sensitive K^+ channel.

The effects of PCP on the channel were studied by the addition of PCP at various concentrations to the bath solution. To wash out PCP, the patch was perfused by control solution for 2–3 min. Fig. 2B demonstrates that PCP inhibited the channel at a significantly lower concentration than ATP. Fig. 2C shows that 40 μM PCP decreased the open-state probability more than twofold.

The dose–response relationship for the blocking action of PCP, obtained in the same patch by stepwise increases of the PCP concentration, is shown in Fig. 3. The degree of channel blocking was plotted as a function of the logarithm of the PCP concentration and calculated as:

$$b = [1 - (p/p_c)]\ 100\%,$$

where p and p_c are average open-state probabilities under the PCP action and in controls, respectively. The average probabilities were estimated by the same equation as in [15]:

$$p = I/(N \cdot i),$$

where I is the arithmetic mean of the currents passing through the channels in the patch membrane, N is the total number of channels present in the patch, and i is the unit amplitude of the single channel current.

The dose–response relationship was fitted using the least square method by the Hill function:

$$b = \frac{b_m(X/K_d)^n}{1 + (X/K_d)^n}$$

where b_m is the maximum blocking; K_d, the dissociation constant; X, the PCP concentration; n, the Hill coefficient.

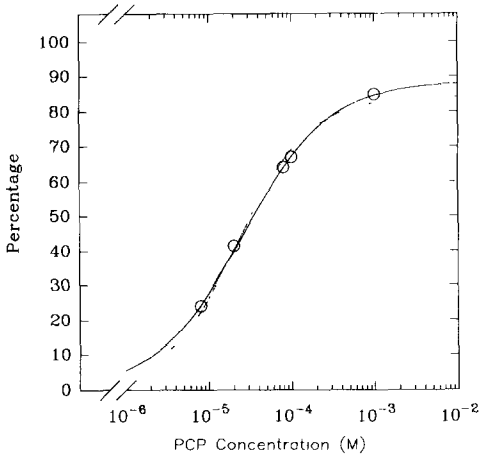

Fig. 3. Dose–response relationship of PCP inhibition of ATP-sensitive K^+ channels. The relationship was obtained on the same inside-out membrane patch by a stepwise increase of PCP concentration in the bath. The degree of channel blocking was plotted as a function of logarithm of PCP concentration. Data were fitted by Hill equation (see text for the explanation).

The following results were obtained at this approximation: $b_m = 89 \pm 1.6\%$; $n = 0.83 \pm 0.041$; $K_d = 24.5 \pm 1.57$ μM (mean ± S.E.). The solid line in Fig. 3 was drawn using these parameter values. For comparison, the above equation was fitted to the data using fixed Hill coefficient, $n = 1$ (Fig. 3, dotted line). The values of other parameters were: $b_m = 84.3 \pm 2.2\%$; $K_d = 21.9 \pm 2.3$ μM.

M. Kakei and co-workers [15] carried out similar calculations for the ATP action on the ATP-sensitive K^+ channel, and a Hill coefficient of around 3 was found suggesting a high cooperatively. It was concluded from our data that PCP, in contrast to ATP, interacted with the channel protein in a non-cooperative manner. The marked difference between the Hill coefficients for ATP and PCP binding probably implied that these compounds did not compete for the same binding site in the membrane.

We could not perform a more detailed kinetic analysis of PCP action as it was not possible to obtain the membrane patch containing only one active ATP-sensitive K^+ channel. As a rule, the activity of 3 to 5 individual channels was recorded simultaneously. These multiple transitions was unlikely to be attributed to substates of one channel. We have analyzed the relative areas under the peaks of amplitude histogram and found out that this distribution was well explained by the binomial law which indicated the presence of several independent channels.

It is generally accepted that effects of PCP are caused by its binding to the receptors in the central nervous system [4–8]. In this study the interaction of PCP with ion channels in cardiac cells has been found, it indicates a broader spectrum of PCP action. The PCP influence on the ATP-sensitive K^+ channel may be of physiological importance, since this channel has been identified in nervous tissues. For instance, the contribution of the channels to the anoxic response of CA3 hyppocampal neurones has been reported [16]. From this point of view, the protection of a neuron from degradation as well as the behavioral reaction induced by PCP are likely to be caused not only by the PCP interaction with a number of brain receptors but also by PCP inhibition of the ATP-sensitive K^+ channel. Besides, PCP activation of the Ca^{2+} inward current has been found in this study. Therefore, the additional release of neurotransmitters observed in the presence of PCP [7,8] can be a consequence of an increase in intracellular Ca^{2+} concentration. It is well known that the ATP-sensitive K^+ channels provide an important link between metabolism of a cell and its excitability. Thus, the hypoglycemic effect of PCP and its ability to maintain the cardiac activity during hypoxia, at least in the early stage [17], are probably related to the PCP influence on the ATP-sensitive channels.

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