Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 3523–3527, May 1986
Neurobiology

Voltage- and time-dependent effects of phencyclidines on the endplate current arise from open and closed channel blockade

(neuromuscular synapse/noncompetitive blockers/desensitization/perhydrohistrionicotoxin)

L. G. AGUAYO*, B. WITKOP†, AND E. X. ALBUQUERQUE*

*Department of Pharmacology and Experimental Therapeutics, University of Maryland, School of Medicine, 655 W. Baltimore Street, Baltimore, MD 21201; and †Laboratory of Chemistry, National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20205

Contributed by B. Witkop, January 2, 1986

ABSTRACT **The actions of phencyclidine [1-(1-phenylcyclohexyl)piperidine, PCP] and its morpholine analog [1-(1-phenylcyclohexyl)morpholine, PCM] on ionic currents of nicotinic acetylcholine receptors were studied at the neuromuscular junction of frog skeletal muscle and on embryonic rat muscle cells in tissue culture. PCP and PCM reduced the peak amplitude and the decay time constant of the endplate current (EPC). PCP produced a voltage-dependent curvature and a time-dependent hysteresis loop at negative potentials (at potentials from −50 to −150 mV). In contrast, PCM caused a depression of EPC peak amplitude, but the current–voltage relationship (+60 to −150 mV) remained linear. When PCP-modified EPCs were elicited in trains at hyperpolarized potentials the amplitudes of successive events were progressively decreased and the magnitude of the decrease was dependent on the level of hyperpolarization. At positive potentials the process was reversed; the amplitude increased with successive stimulations. The EPC decayed exponentially in the presence of PCP and PCM, with a shortened time constant of decay that was less dependent on membrane potential than control. PCP and PCM caused only a 20% decrease of the amplitude of the iontophoretically evoked acetylcholine potential, which was significantly different from that induced by the desensitizing alkaloid perhydrohistrionicotoxin. Both PCP and PCM reduced by 50% the mean channel open time obtained from rat myoballs, giving a potency ratio for PCP to PCM of 2.5. This relative potency was correlated with that obtained for the reduction in the decay time constant of the EPC (ratio = 2.2). The effects of PCP on the peak amplitude of the EPC seem to be related to a conformational change of the acetylcholine receptor occurring before channel activation and not to a receptor desensitization.**

Biochemical and electrophysiological experiments have established that 1-(1-phenylcyclohexyl)piperidine (phencyclidine, PCP) specifically binds to at least two sites of the acetylcholine receptor (AcChoR) (1, 2). PCP, histrionicotoxin, and perhydrohistrionicotoxin ($H_{12}HTX$) have provided a great deal of information about the interaction of noncompetitive blockers with the AcChoR ion channel (also referred to here as AcChoR). Similarly to $H_{12}HTX$, PCP produced a voltage- and concentration-dependent depression of the peak amplitude of the endplate current (EPC). In the present paper we describe these effects of PCP (Fig. 1) and compare them with the distinctly different effects of its morpholine analog, 1-(1-phenylcyclohexyl)morpholine (PCM). We also provide evidence indicating that the mechanism of action of PCP is different from that of $H_{12}HTX$, which is known to cause receptor desensitization (3, 4). Finally, we explain the pharmacological activity of PCP and its morpholine analog by suggesting a difference in affinity for the closed conformation of the AcChoR (5).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Acetylcholine (AcCho) Sensitivity of the Chronically Denervated Rat Muscle. The response to iontophoretically applied AcCho was studied in denervated (7–10 days) rat soleus muscles by using methods previously described (6). Micropipettes filled with 1 M AcCho and having resistance >100 MΩ were used to induce AcCho potentials of a few millivolts at 1, 2, 4, and 8 Hz. The muscles were perfused with the following solution (mM): NaCl, 135; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 1; $NaHCO_3$, 15; NaH_2PO_4, 1; and glucose, 11. This solution was bubbled with 95% O_2/5% CO_2 (pH of 7.2).

Voltage Clamp Experiments. Voltage clamp experiments were carried out on surface fibers at the endplate region of sciatic nerve–sartorius muscle of *Rana pipiens* at 20°C, using a two-microelectrode voltage clamp described elsewhere (7, 8). Two voltage paradigms were used to study the effects of PCM and PCP on the current–voltage (*I–V*) relationship and on the time- and voltage-dependent blockade of the EPC. Voltage sequence I consisted of 10-mV steps starting from a holding potential of −50 mV. The command potentials were made sequentially in the depolarizing direction and then the hyperpolarizing direction between the voltage extremes of +50 and −150 mV at a frequency of 0.33 Hz. Voltage sequence II was used to test the effects of long conditioning pulses on the peak amplitude and the decay time constant of the EPC (τ_{EPC}). The voltage was held constant for several hundreds of seconds at +30, −50, −100, or −150 mV. The muscles were perfused with the following solution (mM): NaCl, 115; KCl, 2; $CaCl_2$, 1.8; Na_2HPO_4, 1.3; and NaH_2PO_4, 0.7 (pH 7.1–7.3).

Patch Clamp Experiments. Single channel currents were recorded from membranes of embryonic rat muscle cells that were grown in tissue culture (9–11). Myoballs were perfused with Hanks' solution of the following composition (mM): NaCl, 137; KCl, 5.4; $NaHCO_3$, 4.2; $CaCl_2$, 1.3; $MgSO_4$, 0.81; KH_2PO_4, 0.44; Na_2HPO_4, 0.34; and D-glucose, 5.5. The pH was adjusted to 7.1 with Hepes buffer, and 1.0 μM tetrodotoxin was added to abolish the contraction of the cells upon stimulation. Single-channel currents were recorded by using cell-attached and inside-out patch clamp techniques. The patch pipette was filled with PCP or PCM dissolved at the desired concentration in Hanks' solution. Where applicable, Student's unpaired *t* test was used to compare data from control and experimental conditions. Values of $P < 0.05$ were considered statistically significant.

Abbreviations: AcCho, acetylcholine; AcChoR, nicotinic AcCho receptor and its associated ionic channel; PCP, 1-(1-phenylcyclohexyl)piperidine (phencyclidine); PCM, 1-(1-phenylcyclohexyl)-morpholine; HTX, histrionicotoxin; $H_{12}HTX$, perhydrohistrionicotoxin; *I–V*, current–voltage; EPC, endplate current.

RESULTS

Effects of PCP and PCM on the *I–V* Relationship and the Decay Time Constant of the EPC. The *I–V* relationships, as measured by using voltage sequence I, in the presence of PCP and PCM showed significant differences (Fig. 1 *Left*). PCP (20 μM) produced a hysteresis loop at potentials above −60 mV and a negative conductance in the *I–V* relationship at potentials negative to −100 mV. PCM, even at much higher concentrations, did not exhibit either of these two actions on the *I–V* relationship of the EPC. The kinetics of the decay phase were also altered by both compounds (Fig. 1 *Right*) but remained a single exponential decay at all the membrane potentials and drug concentrations tested, thus suggesting a slow unbinding from open channels. The relationship between the τ_{EPC} and membrane potential can be described by two constants: $\tau(0)$, the time constant in the absence of electric field and H, the constant describing its voltage sensitivity (12). The control values for $\tau(0)$ ranged from 0.79 to 1.67 msec, and the values for H ranged from −0.0055 to −0.0072 mV^{-1}. PCP (30 μM) and PCM (80 μM) reduced H to −0.0043 mV^{-1} and to −0.0015 mV^{-1}, respectively. In the presence of the same concentrations of PCP and PCM, $\tau(0)$ was reduced to 0.44 and 0.47 msec, respectively.

Time- and Voltage-Dependent Blockade of the EPC. The peak amplitude of the EPC and the τ_{EPC} recorded when voltage sequence II was used are shown as a function of time in Figs. 2 and 3. The blockade of the EPC produced by PCP (Fig. 2) was different from that produced by PCM (Fig. 3). With PCP, the inward current decreased each time that the membrane potential was made more negative than the holding potential (−50 mV). When the membrane potential was depolarized from −50 mV, the peak amplitude of the EPC increased to a new steady-state value. Fig. 2 shows that the peak amplitude of the PCP-modified EPC was reduced exponentially when the membrane potential was changed from −50 to −100 mV. Upon return to the holding potential, the peak amplitude of the inward current increased from −20 to −40 nA. Similarly, at +30 mV, the peak amplitude of the EPC increased with time, reaching a value of +40 nA after 30 sec of stimulation. This plot also shows that the peak amplitude obtained at the holding potential after depolarizing the postsynaptic membrane to +30 mV was markedly larger than the one obtained after hyperpolarizing the membrane to −100 mV. Finally, repetitive stimulation at −50 mV decreased the peak amplitude from −75 to −40 nA, which appears to be the steady-state current at the holding potential. PCM, unlike PCP, did not produce any of these actions at the membrane potentials tested (Fig. 3). Neither PCP nor PCM caused time-dependent alterations in τ_{EPC}. This is particularly interesting with PCP because it reduced the peak amplitude without altering τ_{EPC} (Fig. 2).

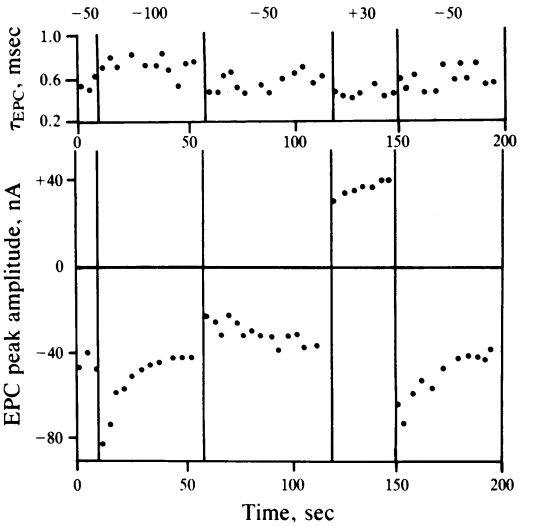

FIG. 2. Voltage-dependent effects of PCP (20 μM) on τ_{EPC} and the peak amplitude of the EPC. (*Upper*) Each point represents the τ_{EPC} obtained at an initial holding potential of −50 mV and a rate of 0.33 Hz (20°C). (*Lower*) Each point represents the current amplitude corresponding to the τ_{EPC} shown above at the same membrane potentials. After a voltage change to −100 mV, the peak EPC amplitude decreased exponentially as a function of time, with a time constant of approximately 15 sec. Stepping the membrane potential back to the holding potential increased the peak amplitude, which reached a new equilibrium at −40 nA. At +30 mV, the peak amplitude increased with time, reaching a value of +40 nA. Finally, the peak amplitude decreased from −70 to −40 nA with successive stimulations at −50 mV. Note the distinct behavior of the peak amplitude, at −50 mV, before and after the depolarization to +30 mV. In contrast to the changes with time in EPC amplitude, the time constant of decay remained unchanged at all the membrane potentials tested.

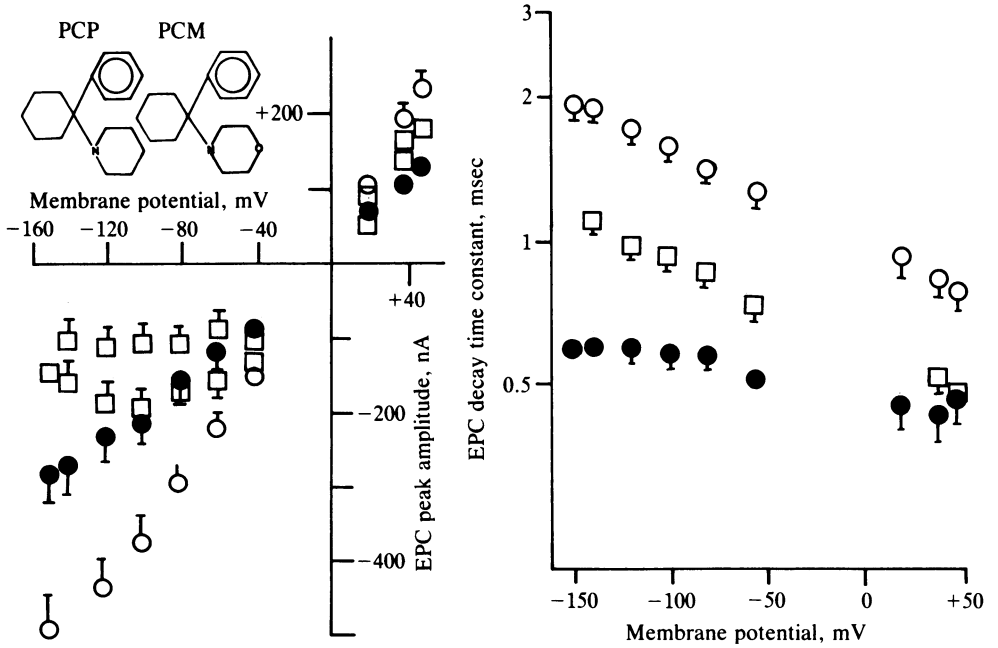

FIG. 1. Effects of PCP and PCM (structures in *Inset*) on the current–voltage relationship and the decay time constant of the EPC (τ_{EPC}). (*Left*) *I–V* relationship in the absence (○) and presence of PCP (□, 20 μM) and PCM (●, 80 μM). Each symbol and bar represents the mean (±SEM) obtained from at least five endplates from three or more muscles. (*Right*) Relationship between the τ_{EPC} and the membrane potential. These values were obtained by using voltage sequence I at a stimulation frequency of 0.33 Hz (20°C).

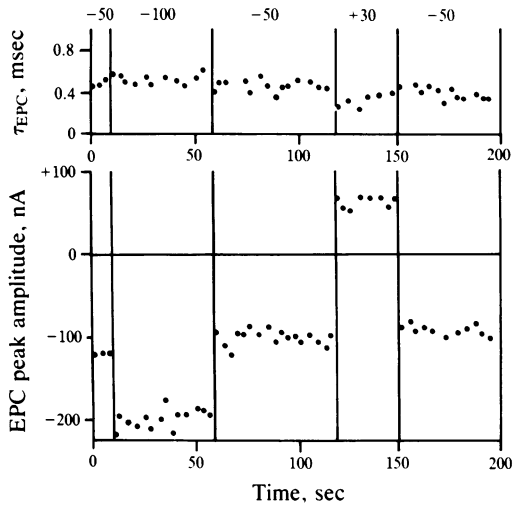

FIG. 3. Effects of PCM (80 μM) on τ_{EPC} and the peak amplitude of EPC. (*Upper*) Each point represents the τ_{EPC} obtained from a separate experiment using the same voltage paradigm as in Fig. 2. (*Lower*) Each point represents the amplitude of an EPC evoked (0.33 Hz) from a single muscle fiber, starting at a holding potential of −50 mV. Depolarizing the membrane to +30 mV did not remove the blockade produced by PCM.

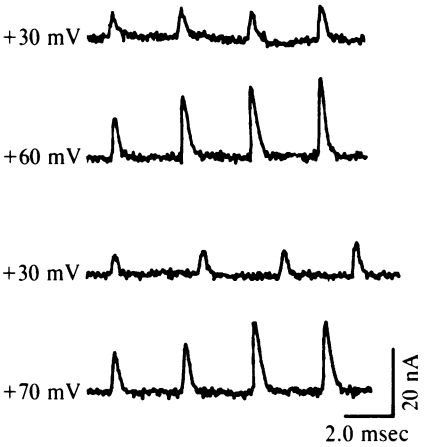

FIG. 4. Voltage- and time-dependent recovery of the peak amplitude during the exposure to PCP. The four groups of EPCs were recorded from a single cell that was initially held at −50 mV in the presence of 30 μM PCP. EPCs were elicited at 0.33 Hz until the EPC amplitude reached an equilibrium value (approximately 45 sec, mean amplitude of −38 nA). The membrane potentials were then stepped to the test membrane potential of +30, +60 and +70 mV. Between each test voltage the membrane potential was returned to −50 mV and held until equilibrium was reached. The four EPCs shown at each membrane potential are the 1st, 4th, 8th, and 12th pulses in the train.

PCP was able to produce this time-dependent blockade even in the absence of nerve stimulation. In the presence of PCP, the EPC measured at the holding potential after hyperpolarizing the membrane to −100 mV for about 60 sec was significantly smaller than the one measured immediately before.

Voltage-Dependent Recovery of the Outward EPC. The peak amplitude of outward currents increased in a voltage-dependent manner in the presence of PCP. The peak amplitude of the PCP-modified EPC measured with voltage sequence I displayed a small increase at positive potentials (see +40 mV in Fig. 1). When the postsynaptic membrane was depolarized for a longer period of time by using voltage sequence II, a significant increase on the outward current amplitude was observed (Figs. 2 and 4). Fig. 4 shows the typical response obtained at positive potentials in the presence of PCP (30 μM). In this experiment, the membrane potential was changed to +30, +60, and +70 mV from a holding potential of −50 mV. It shows that the rate and magnitude of recovery of the peak amplitude were greatly enhanced when the membrane potential was increased to +60 or +70 mV. The time course of the outward current did not undergo any change during the increase in the peak amplitude. The increase in the amplitude of the outward current contributed to the increase of the inward current found when the membrane potential was returned to −50 mV (Fig. 2). The outward current in the presence of PCM did not change at positive potentials (Fig. 3).

Effect of PCP, PCM, and H_{12}HTX on AcCho-Induced Desensitization of the Extrajunctional Receptors of the Chronically Denervated Soleus Muscle. The time-dependent effects produced by PCP on the peak amplitude of the EPC could be related to receptor desensitization of the kind seen with chlorpromazine (13). To determine the degree of receptor desensitization, if any, the response to iontophoretically applied AcCho was studied and compared to that observed in the presence of H_{12}HTX, an alkaloid that desensitizes AcChoR (14, ‡) (Fig. 5, trace D).

The amplitude of the control AcCho potential induced by short iontophoretic pulses (0.5 msec) was constant at frequencies between 1.0 and 4.0 Hz. The amplitude at the 60th pulse at 1.0 Hz for example, was about 95% of the first potential (Fig. 5, trace A). PCP at 5 μM caused only a 20%

‡Aracava, Y., Daly, J. W. & Albuquerque, E. X. (1984) Ninth International Congress of Pharmacology, July 30–August 3, 1984, London, England, abstr. no. 601P.

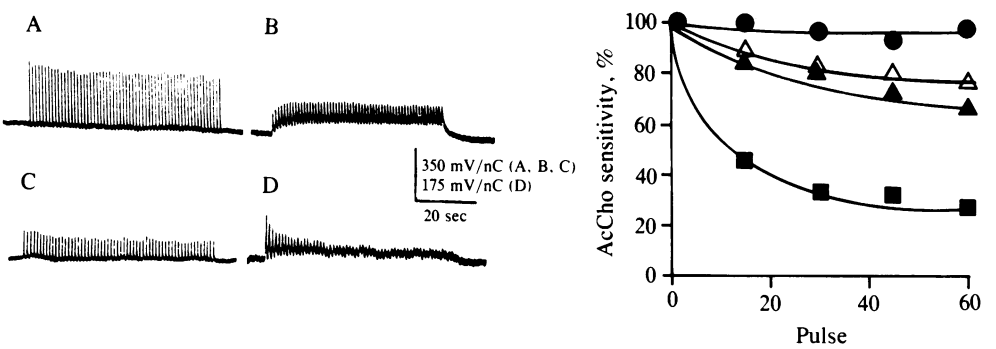

FIG. 5. Desensitization of the extrajunctional AcCho receptors of the rat soleus muscles induced by PCP, PCM, and H_{12}HTX. (*Left*) Typical AcCho potentials obtained in the absence (trace A) and presence of PCP (5 μM, trace B), PCM (50 μM, trace C), and H_{12}HTX (5 μM, trace D). (*Right*) Time course of desensitization, with sensitivity expressed as a percentage of control at the same drug concentrations. ●, Control; △, PCP; ▲, PCM; and ■, H_{12}HTX. Each symbol represents the mean from four or five determinations.

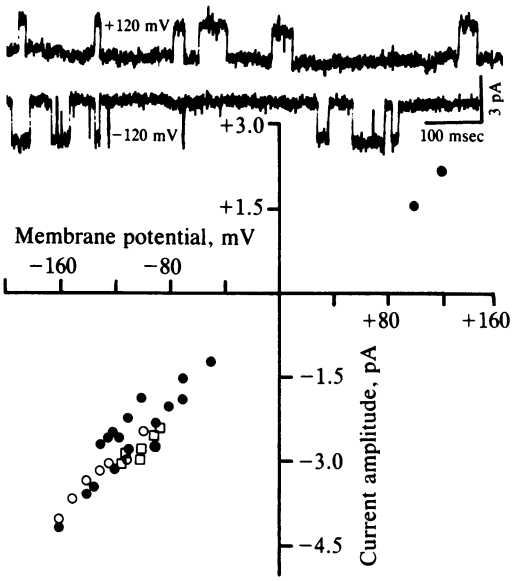

FIG. 6. Current–voltage relationships obtained in the presence of PCP and PCM. Single-channel currents were obtained from an inside-out patch in the presence of AcCho (0.2 μM) at ±120 mV (10°C). Bandwidth was 1.0 kHz. Each point in the lower graph represents a single determination of the single-current amplitude at the indicated membrane potential (inside-out and cell-attached conditions). The pipette contained AcCho alone (0.2 μM, ●), AcCho plus PCP (4 μM, ○), or AcCho plus PCM (10 μM, □). Linear regression analysis gave a channel conductance of 25 pS and a reversal potential of −5.0 mV for all the conditions (10°C).

reduction of the AcCho potential after 60 sec of repetitive AcCho application at the frequency of 1.0 Hz. This effect was almost indistinguishable from that of PCM (50 μM). The effects of PCP and PCM, however, were different from those of H_{12}HTX (1–15 μM), which reduced the response by about 70% (Fig. 5, trace D). These results demonstrate that PCP and PCM have similar desensitizing properties, but both are much weaker than H_{12}HTX.

Effects of PCP and PCM on Single-Channel Activity. Single-channel currents were recorded from myoballs in the presence of 0.2 μM AcCho (Fig. 6). The amplitude of single-channel currents activated by 0.2 μM AcCho was linearly related to the membrane potential across the membrane patch, and the *I–V* plot yielded a channel conductance of 25 pS and a reversal potential of −5 mV (n = 3, 10°C). Neither the single-channel conductance nor the reversal potential was changed by PCP (2 μM) and PCM (4 μM).

With AcCho alone (0.2 μM) channel activity was observed as single- and double-current events, and this suggests that at least two channels were being activated by AcCho. PCP caused two effects on single-channel activity: it reduced the mean channel lifetime and the frequency of channel opening (Fig. 7). For example, PCP, at concentrations between 4 and 10 μM, caused a large reduction in the number of channel openings at the same AcCho concentration (0.2 μM). With PCP (10 μM), channel openings were rare and only a few openings were detected. This reduction, which occurred without changes in channel conductance, may well represent blockade of the closed conformation of the ionic channel. PCM (10 μM) also caused a reduction in the channel opening frequency, but to a smaller extent (Fig. 7). No complex behavior (bursting) was observed in the closing kinetics of these AcCho-activated channels. PCP (4 μM) and PCM (10 μM) caused a reduction in channel lifetime and channel opening frequency without changing the mean burst time. The best correlation with EPC studies was obtained in the reduction of channel lifetime (which may represent open channel blockade). It was reduced by about 50% when the patch pipette containing AcCho had in addition PCP (4 μM) or PCM (10 μM). This represents a potency ratio of about 2.5, which compares rather well with that obtained for the reduction in τ_{EPC} (ratio of 2.2).

DISCUSSION

This study discloses two important features of noncompetitive blockers of the nicotinic receptor: (*i*) the unique voltage- and time-dependent alterations of the *I–V* relationship produced by PCP were eliminated when an oxygen atom was introduced in the piperidine ring, and (*ii*) H_{12}HTX produces a much larger desensitization than either PCP or PCM (Fig. 5).

PCP and its morpholine analog had two effects on the ionic current induced by the binding of AcCho to the recognition site on the AcChoR: a depression of the peak amplitude and a shortening of τ_{EPC} (Fig. 1). They reduced the peak amplitude of the EPC by about 50% at 15 μM (PCP) and 90 μM (PCM); their corresponding values for 50% decrease of τ_{EPC} were 25 and 55 μM, respectively. The blockade produced by PCP increased with time as the membrane potential was maintained at a hyperpolarized level and decreased when the

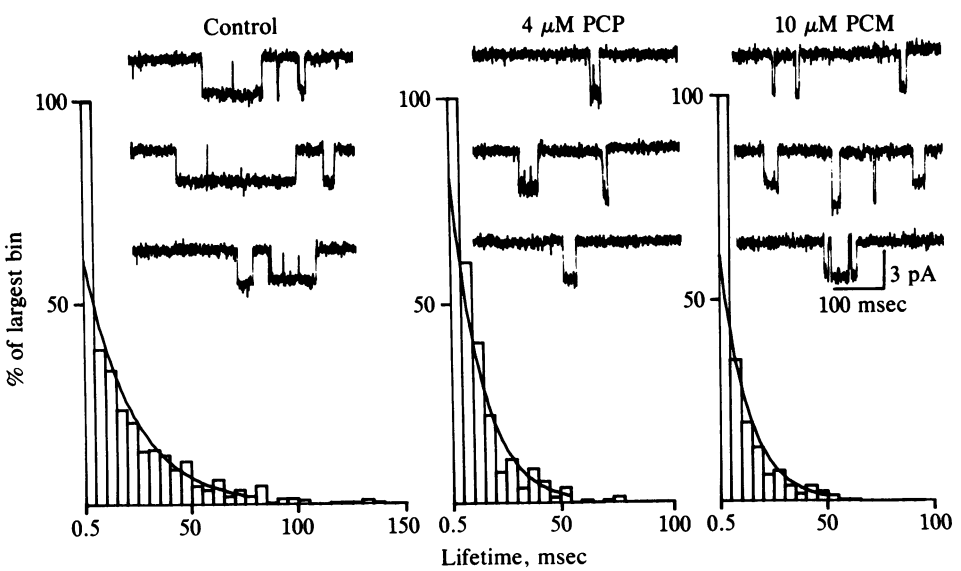

FIG. 7. Single-channel currents recorded in the presence of PCP (4 μM) and PCM (10 μM). Single-channel currents were recorded in the presence of 0.2 μM AcCho and in the presence of AcCho plus the indicated drug concentration (−120 mV, 10°C). PCP caused a decrease in the channel frequency and channel lifetime without channel flickering. Bandwidth was 1 kHz. The histograms represent open time distributions obtained from cell-attached patches in the presence of PCP and PCM. The mean open time for control was 21.0 msec, and it was reduced to 9.65 and 8.95 msec by PCP and PCM, respectively.

membrane potential was subsequently held at positive values (Fig. 2). In addition, we found a voltage- and time-dependent unblock of the EPC at positive potentials (Fig. 4). Such an increase of the peak of the outward current contributed to the larger amplitude upon return to negative potentials. Under the same experimental conditions, PCM did not produce a voltage- and time-dependent blockade of the EPC.

Our results suggest that PCP can block the EPC by a slow inactivation of the EPC, which appears to correspond to the high-affinity binding observed in biochemical experiments on the AcChoR in the closed state (2, 4), and by open channel blockade. The blockade of the closed state of the ionic channel was highly dependent on the membrane potential: as the membrane potential was hyperpolarized the blockade increased even in the absence of nerve stimulation. The macroscopic *I–V* relationship showed a negative conductance. Single-channel conductance, on the other hand, was not affected, suggesting that this voltage-dependent action may result from the large reduction in channel frequency observed with PCP (10 μM). The decrease in the number of channel openings (Fig. 7), which appears to represent closed channel blockade, may effectively reduce the EPC amplitude. These results, together with the single exponential EPC decay produced by PCP and PCM, indicate that there exists a strong binding between the drug and the open channel of the AcChoR.

The effects of PCP on the single-channel current were markedly different from those observed during AcChoR desensitization (15). For example, under our experimental conditions, PCP reduced channel activity without producing grouping of single currents as observed with desensitizing agonist concentrations. This action, therefore, appears to be caused by a blockade of the closed state of the ionic channel prior to channel opening and not by a desensitizing effect.

Previous studies have demonstrated that histrionicotoxin (HTX) and H_{12}HTX are able to cause a marked desensitization-like action on the AcChoR while producing voltage- and time-dependent effects on the EPC (14, ‡). Biochemically, these toxins initially increase affinity of the binding site for AcCho and subsequently the receptor is desensitized. By using the patch clamp technique, it was demonstrated that HTX and H_{12}HTX caused no alteration of channel conductance or lifetime at concentrations as high as 4 μM.‡ At low concentration, they produced an initial increase followed by a marked decrease in the frequency of channel openings such that at concentrations >5 μM no openings could be recorded. Because PCP also increases the affinity of AcCho for its receptor and causes desensitization (1), we compared the degree of receptor desensitization produced by the two drugs. The small desensitization of the AcCho response observed with PCP and PCM, but not with H_{12}HTX, suggests a different mechanism of action for the blockade of the EPC, with H_{12}HTX reducing the EPC by desensitizing receptors rather than by blocking ionic channels.

The introduction of an oxygen atom into the piperidine ring of PCP may cause the reduction in the potency of PCM in lowering the peak amplitude of the EPC, probably through an increase of the polarity of the molecule. Together with this reduction, the voltage-dependent blockade was abolished, and PCM caused neither a negative slope conductance in the *I–V* relationship nor a hysteresis loop at negative potentials (Fig. 1). The observed decrease in the potency for blocking the peak amplitude of the EPC appears to be associated with a smaller reduction of channel opening frequency. The polar oxygen in the piperidine ring may effectively reduce the partition of the molecule into a hydrophobic environment such as the interior of the AcChoR (4, 16), thus rendering PCM unable to alter closed channels.

The reduction of τ_{EPC} produced by PCP and PCM can be explained well by open-channel blockade. Direct evidence for this was provided by the reduction of the mean channel lifetime obtained with PCP and PCM. PCP was about 2 times more potent than PCM in reducing the channel lifetime, and the potencies of both were well correlated with the potencies obtained from EPC experiments (Fig. 7). The open-channel blockade is consistent with what we know about the geometry of the AcChoR (16, 17). Its large outer mouth can accept both PCP and PCM (largely protonated at physiological pH) which have diameters of about 11 Å. The channel gate, however, with a cross section of 6.5 × 6.0 Å (17), does not allow for their permeation through the channel at negative potentials. The hydrophobicity given by the cyclohexane and piperidine rings likely provides a strong binding affinity within the channel. This is supported by the finding that the removal of the piperidine ring or the hydroxylation of the cyclohexane ring increased the rate of drug dissociation, thus producing double exponential decays of EPCs (5, 18).

In summary, this study demonstrates that a discrete molecular modification of PCP changes the affinity for the closed state of the AcChoR and H_{12}HTX is a more potent desensitizing agent than PCP.

We are grateful to Dr. Karen Swanson for her comments on this manuscript. This work was supported by U.S. Public Health Service Grants NS 12063 and DA 02804 and by U.S. Army Research and Development Command Contract DAMD-17-84-C-4219.

1. Albuquerque, E. X., Tsai, M.-C., Aronstam, R. S., Eldefrawi, A. T. & Eldefrawi, M. E. (1980) *Mol. Pharmacol.* **18**, 167–187.
2. Eldefrawi, M. E., Miller, E. R., Murphy, D. L. & Eldefrawi, A. T. (1982) *Mol. Pharmacol.* **22**, 72–81.
3. Eldefrawi, M. E., Aronstam, R. S., Bakry, N. M., Eldefrawi, A. T. & Albuquerque, E. X. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2309–2313.
4. Heidmann, T., Oswald, R. G. & Changeux, J.-P. (1983) *Biochemistry* **22**, 3112–3127.
5. Aguayo, L. G. & Albuquerque, E. X. (1985) *Biophys. J.* **47**, 259 (abstr.).
6. Albuquerque, E. X. & McIsaac, R. J. (1970) *Exp. Neurol.* **26**, 183–202.
7. Takeuchi, A. & Takeuchi, N. (1959) *J. Neurophysiol.* **22**, 52–67.
8. Kuba, K., Albuquerque, E. X., Daly, J. & Barnard, E. A. (1974) *J. Pharmacol. Exp. Ther.* **189**, 499–512.
9. Neher, E. & Steinback, J. H. (1978) *J. Physiol. (London)* **277**, 153–176.
10. Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. (1981) *Pflügers Arch.* **391**, 85–100.
11. Akaike, A., Ikeda, S. R., Brookes, N., Pascuzzo, G. J., Rickett, D. L. & Albuquerque, E. X. (1984) *Mol. Pharmacol.* **25**, 102–112.
12. Magleby, K. L. & Stevens, C. F. (1972) *J. Physiol. (London)* **223**, 173–197.
13. Carp, J. S., Aronstam, R. S., Witkop, B. & Albuquerque, E. X. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 310–314.
14. Albuquerque, E. X., Barnard, E. A., Chiu, T. H., Lapa, A. J., Dolly, J. O., Jansson, S.-E., Daly, J. & Witkop, B. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 949–953.
15. Sakmann, B., Patlak, J. & Neher, E. (1980) *Nature (London)* **286**, 71–73.
16. Changeux, J.-P., Devillers-Thiery, A. & Chemouilli, P. (1984) *Science* **225**, 1335–1345.
17. Dwyer, T. M., Adams, D. J. & Hille, B. (1980) *J. Gen. Physiol.* **75**, 469–492.
18. Aguayo, L. G. & Albuquerque, E. X. (1985) *Neurosci. J.* **11**, 844 (abstr.).