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Sites of Action of Phencyclidine

I. Effects on the Electrical Excitability and Chemosensitive Properties of the Neuromuscular Junction of Skeletal Muscle

MING-CHENG TSAI, EDSON X. ALBUQUERQUE, ROBERT S. ARONSTAM,[1] AMIRA T. ELDEFRAWI, MOHYEE E. ELDEFRAWI AND DAVID J. TRIGGLE*

*Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, Maryland 21201, and *Department of Biochemical Pharmacology, School of Pharmacy, State University of New York at Buffalo, Buffalo, New York 14260*

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SUMMARY

TSAI, M. -C., E. X. ALBUQUERQUE, R. S. ARONSTAM, A. T. ELDEFRAWI, M. E. ELDEFRAWI AND D. J. TRIGGLE. Sites of action of phencyclidine. I. Effects on the electrical excitability and chemosensitive properties of the neuromuscular junction of skeletal muscle. *Mol. Pharmacol.* **18:** 159–166 (1980).

The effects of phencyclidine (PCP) were studied on the electrical and chemosensitive properties of the neuromuscular junction of skeletal muscles as well as on the binding of ligands to acetylcholine (ACh) receptors in the electric organ membranes of the electric ray. PCP potentiated both the directly and the indirectly elicited muscle twitch, an effect which occurred with a simultaneous prolongation of the falling phase of the action potential blockade of delayed rectification and only a slight decrease in the rate of rise of spike activity. The prolongation of the action potential was also increased as a function of the frequency of nerve stimulation. In contrast to the marked potentiation of directly elicited muscle twitch, indirect muscle twitch was only transiently potentiated at concentrations lower than 60 μM and subsequently blocked. Indeed, at concentrations higher than 60 μM, blockade of neuromuscular transmission occurred with little or no potentiation of the indirectly elicited twitch. Resting membrane potential and passive electrical properties were little affected by PCP. At high concentrations of PCP the miniature endplate potentials were blocked, as were the ACh sensitivities of the junctional region of innervated muscles as well as the extrajunctional region of chronically denervated muscles. PCP decreased the sensitivity to repetitive microiontophoretic application of ACh. PCP did not prevent the irreversible effects of α-bungarotoxin on ACh sensitivity in junctional regions of the innervated and extrajunctional regions of chronically denervated muscles. At these effective concentrations (i.e., 1 to 100 μM) PCP caused negligible inhibition of ACh-esterase. In addition, since PCP did not inhibit the binding of [^{3}H]ACh or [^{125}I]α-bungarotoxin to the ACh receptors, it was suggested that the inhibition of ACh-receptor-regulated ionic conductances was not due to the inhibition of ACh-receptor binding sites. Inhibition was possibly due to an interaction with the ionic channel of the ACh receptor. Furthermore, the effect of PCP on the electrical excitability of muscle membrane, shown by the marked prolongation of the action potential and inhibition of delayed rectification, suggested that the agent caused significant blockade of potassium conductance. This effect most likely could account for the potentiation of the muscle twitch.

INTRODUCTION

Since its introduction by Chen and collaborators in 1959 (1), the anesthetic agent 1-(1-phencyclohexyl)-piperidine hydrochloride (PCP)[2] has been recognized as a psychologically reinforcing hallucinogen. When taken

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[1] Present address: Department of Pharmacology, Medical College of Georgia, Augusta, Georgia 30912.

[2] Abbreviations used: PCP, phencyclidine; ACh, acetylcholine; α-BGT, α-bungarotoxin; RMP, resting membrane potential; EPP, endplate potential; MEPP, miniature endplate potential; DFP, diisopropylfluorophosphate.

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chronically or in large doses, or given to individuals who are hypersensitive to the drug, PCP displays schizophrenomimetic and convulsant effects which are often associated with episodes of paranoia, unpredictable violent behavior, and psychosis. An extensive appraisal of PCP abuse has recently been published (2). The clinical symptomatology of PCP abuse suggests a number of different molecular actions, but evidently points to the involvement, either directly or indirectly, of the neurotransmitters 5-hydroxytryptamine, dopamine, norepinephrine, acetylcholine (ACh), and γ-aminobutyric acid as the mechanisms underlying the behavioral effects of the agent (3). In addition to the relatively well-described actions of PCP in terms of behavioral studies (4, 5), the drug has also been shown to have anticholinergic action (6–8). The precise mechanism of action of PCP on the ACh receptor–ionic channel complex at the nicotinic synapses remains to be clarified. Since the agent also possesses an anesthetic effect (9), the possibility exists that PCP might affect Na^+ and/or K^+ conductance of the electrically excitable membrane.

In this series of three papers, the sites of action of PCP on different cholinergic systems were studied in detail. In this paper, the effects of PCP on the electrical excitability and chemosensitive properties of the neuromuscular junction of skeletal muscles were studied. The second paper focuses on the interaction of PCP with the nicotinic ionic channel, and the third is a study on the interactions of PCP with the muscarinic receptor in brain and smooth muscle. We utilized biophysical, biochemical, and pharmacological techniques to understand the mode of action of PCP. In the present study, PCP was found to cause a significant prolongation of the falling phase of the action potential and blockade of delayed rectification, leading to the suggestion that potassium conductance was markedly decreased. This effect might explain the potentiation of muscle twitch. PCP also blocked postsynaptic events by inhibiting the ACh-receptor-regulated ionic currents without blocking the binding sites for ACh and α-bungarotoxin (α-BGT).

MATERIALS AND METHODS

Electrophysiological techniques. All experiments were performed at room temperature (20–22°C) on sciatic sartorius muscle preparations of the frog, *Rana pipiens*, except for ACh sensitivity measurements, which were on 10- to 15-day denervated rat soleus muscles. The physiological solutions used had the following composition (mM): NaCl, 115.5; KCl, 2.0; $CaCl_2$, 1.8; Na_2HPO_4; 1.3; and NaH_2PO_4, 0.7—in the case of frog muscles—and NaCl, 135.0; KCl, 5.0; $CaCl_2$, 2.0; $MgCl_2$, 1.0; $NaHCO_3$, 15; NaH_2PO_4, 1.0; and glucose, 11.0—in the case of rat muscle. The frog solution was bubbled with 100% O_2 and had a pH of 6.9–7.1, while the rat solution was bubbled with 95% O_2 and 5% CO_2 and had a pH of 7.1–7.3. For the action potential studies, the solutions contained 400–600 mM glycerol to block muscle contraction (19).

For twitch tension studies, the nerve was stimulated with supramaximal pulses having a duration varying from 0.05 to 0.1 ms via a Ag–AgCl salt bridge electrode connected to a wet electrode (10). Direct stimulation of the muscle was accomplished by applying supramaximal rectangular pulses of 1.0- to 2.0-ms duration at a rate of 0.05 Hz through a platinum electrode placed around the middle portion of the muscle. The muscle tension generated by both direct and indirect stimulation was recorded by attaching the muscle to a Grass FT.03 force displacement transducer. The simple twitch was also displayed on a Grass polygraph and on the screen of a 502 oscilloscope with simultaneous Polaroid photography of the event.

The intracellular recording of the resting membrane potential (RMP), action potential, endplate potential (EPP), miniature endplate potential (MEPP), and delayed rectification, as well as ACh sensitivity, in chronically denervated muscle has been described elsewhere (10). In all the experiments on delayed rectification, the muscles were pretreated with tetrodotoxin (1 μM) to ensure complete block of Na^+ conductance. The maximum rate of rise of the action potential (dV/dT) was measured by means of an RC circuit (0 kΩ–100 pF) and was recorded on the oscilloscope. The membrane electrical constants were calculated as described elsewhere (10). All measurements of the junctional and extrajunctional ACh sensitivity of the chronically denervated muscle were made 2–3 mm away from the apparent endplate region. The half-decay time of the directly evoked spike was obtained from the peak amplitude of the potential to 50% of its amplitude as measured from the holding potential of −90 mV (10). Unless otherwise stated, all values are expressed as means ± SEM.

Biochemical techniques. Membranes were prepared from the electric organ of *Torpedo ocellata* (stored at −90°C) by homogenization in an equal volume of an ice-cold solution containing 1 mM disodium EDTA, 0.02% sodium azide, 0.1 mM diisopropylfluorophosphate (DFP), 0.1 μM phenylmethylsulfonylfluoride, and 10 mM Tris–HCl, pH 7.4, and centrifugation at 5000*g* for 10 min. The pellets were resuspended in the original buffer and spun again at 5000*g* for 10 min. The two supernatants were combined and centrifuged at 30,000*g* for 60 min. The resulting pellets were homogenized in 10 mM Tris–HCl, pH 7.4, 0.02% sodium azide, and 0.1 mM DFP at a concentration of 1 g original tissue wet weight/ml. The final protein concentration, as determined by the method of Lowry *et al.* (11), ranged from 1 to 2 mg/ml.

ACh-esterase activity was assayed by the spectrophotometric method of Ellman *et al.* (12), utilizing acetylthiocholine as the substrate. ACh-esterase was extracted from frog sartorius muscles by homogenization at 0°C in 1 M NaCl, 10 μM $NaHPO_4$, pH 7.4, with a Polytron (setting 7) for three 15-s bursts. After shaking for 30 min at 4°C, the suspension was centrifuged at 100,000*g* for 45 min. The tetram-sensitive ACh-esterase activity was 23 ± 6 mol/min/ml ($N = 6$).

Equilibrium dialysis was used to study the binding of [*acetyl*-^{3}H]acetylcholine (49.5 mCi/mmol, from New England Nuclear) to electric organ membranes as described previously (13). An aliquot of the membrane preparation was placed in a dialysis bag and shaken for 4 h at room temperature in Krebs original Ringer phosphate (25 ml) containing 10^{-7} M [^{3}H]ACh and 100 μM DFP. Triplicate samples of 50 μl were then taken from each dialysis bag and bath, the excess radioactivity in the former representing the amount of ligand bound.

As previously described, a filter assay was used to

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study the binding of [^{125}I]α-BGT (130 Ci/mmol, from New England) using Whatman GF/C glass-fiber filters (14). To 0.3 ml of the membrane preparation, typically containing 10 μg protein (≃5 pmol recepter sites/ml), [^{125}I]α-BGT was added to reach a final concentration of 10 nM. After exposure of the membranes (≃5 pmol/ml) to the toxin for various lengths of time at 21°C, a 200-μl aliquot was mixed with an equal volume of methylated bovine serum albumin (10 mg/ml) to reduce nonspecific [^{125}I]α-BGT binding to the glass filters. The mixture was then filtered and the filter washed with 20 ml cold Ringer's solution. Filters were counted in a Packard 5230 auto-gamma scintillation spectrometer. Specific binding of [^{125}I]α-BGT was taken as the binding inhibited by 1 μM unlabeled α-BGT. Each experiment was repeated three times using triplicate samples.

RESULTS

Effect of PCP on the directly and indirectly elicited twitch of the frog sartorius muscle. While the directly elicited muscle twitch was potentiated within seconds of the addition of PCP (80 μM), this agent blocked the indirectly elicited muscle twitch. For example, at concentrations of 10, 20, 40, 60, and 80 μM, the directly elicited muscle twitch was potentiated by 25, 40, 64, 67, and 69%, respectively. PCP (10 μM) blocked the indirectly elicited muscle twitch by 50% in 20 min and, at the concentration of 80 μM, induced complete blockade within 5 min. As the concentration of PCP was raised above 40 μM, potentiation of the directly evoked twitch reached a plateau, which may be related to the decrease in Na^+ and K^+ conductances as reflected in the concomitant depression of the amplitude and rate of rise and prolongation of the half-decay time of the action potential. All these effects of PCP were slowly reversed by washing the muscle with drug-free physiological solution (Fig. 1). If PCP was not washed out, the directly elicited twitch tension remained potentiated for periods as long as 4 h. However, it should be noted that at these concentrations of PCP (10–100 μM), only a partial recovery of the indirectly elicited twitch was observed even when the washing period was extended to 2 h (Fig. 2), while the directly elicited twitch always recovered completely after washing for only 1 h.

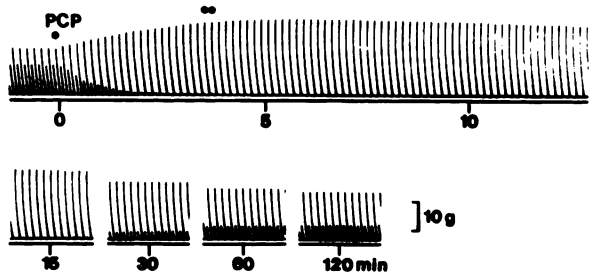

FIG. 1. *Effect of PCP (80 μM) on the isometric directly and indirectly elicited switch of the frog sartorius muscle*

At zero time (●) PCP was applied; the beginning of the washing phase with drug-free physiological solution began at 3 min (●●). The stimulus frequencies for direct and indirect stimulation were each 0.1 Hz.

Effect of PCP on membrane potential, membrane resistance, and action potential of the frog sartorius muscle. Exposure of the sartorius muscles to various concentrations of PCP (10–100 μM) did not change the junctional RMP (Table 1) and resting cable properties (Table 2). Indeed, as shown in Table 1, the values for RMP in the presence of PCP (10–100 μM) ranged from −98 to −91 mV, a variation which did not differ significantly from control values. Similarly, aside from a significant ($P < 0.01$) increase in input resistance from 0.58 ± 0.05 ($N = 10$)M in the control condition to 0.76 ± 0.08 M after exposure to 100 μM PCP for 60 min, the membrane time constant, transverse resistance of a unit area, and capacitance per unit area were unaltered (Table 2). When the directly evoked action potentials were recorded in the presence of PCP (10 to 100 μM), there was a decrease in amplitude and rate of rise and a marked prolongation of the falling phase (Table 1). Figure 3 demonstrates the effect of PCP (100 μM) on the falling phase of the directly evoked action potential measured 60 min after the application of the drug; a 15% decrease in the rate of rise of the potential is also evident. The prolongation of the falling phase of the action potential was dependent upon the frequency of nerve stimulation such that, with repetitive stimulation at a rate of 1 Hz, the prolongation would occur at an earlier period of exposure to the drug. In fact, at stimulus frequencies of 1.0 Hz in the presence of 100

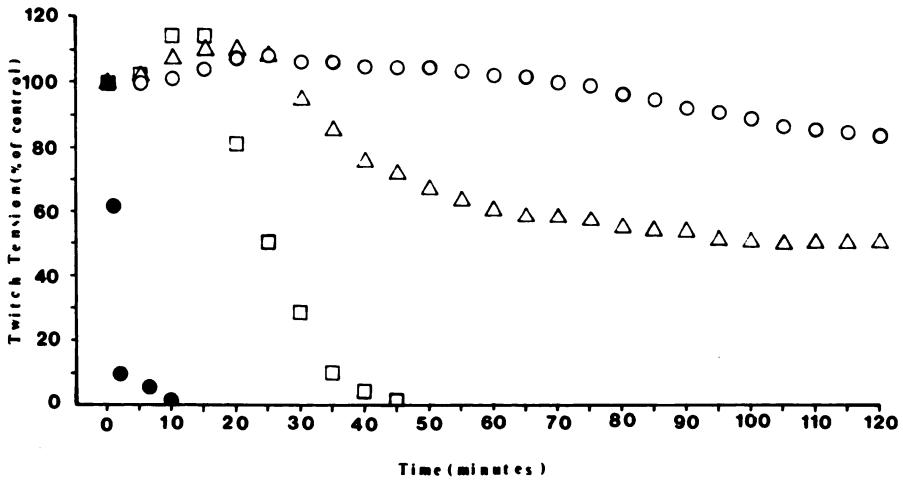

FIG. 2. *Dose-effect relationship of PCP on the indirectly elicited isometric twitch of the frog sartorius muscles*

PCP was added to the experimental chamber at time zero at the following concentrations (μM): 3 (○), 10 (△), 60 (□), 100 (●).

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TABLE 1

Effect of PCP on the directly evoked action potential

	Initial RMP[a]	Amplitude	Threshold	Rate of rise	Half-decay time
	mV	*mV*	*mV*	*V/s*	*ms*
Control	−94.6 ± 0.7	91.7 ± 2.6	45.4 ± 1.3	504 ± 20	0.57 ± 0.03
PCP, 10 μM	−92.8 ± 0.3	87.5 ± 2.8	45.7 ± 1.4	429 ± 13*	0.85 ± 0.09*
PCP, 30 μM	−96.5 ± 0.5	86.2 ± 2.9	47.8 ± 2.1	428 ± 15*	1.73 ± 0.31**
PCP, 40 μM	−97.1 ± 0.4	85.6 ± 3.1	48.2 ± 3.8	426 ± 23*	1.83 ± 0.33**
PCP, 60 μM	−98.2 ± 0.5	83.1 ± 3.3	48.2 ± 2.7	427 ± 24*	1.92 ± 0.34**
PCP, 100 μM	−92.2 ± 0.6	82.8 ± 3.0	45.8 ± 3.0	421 ± 24**	2.23 ± 0.54**

[a] Initial resting membrane potential (RMP) 1 min after impalement of the muscle fiber 1 mm from the endplate region. To obtain homogeneous responses, RMP was adjusted to −95 mV in every fiber. Each number is the mean ± SEM of 30–35 single surface muscle fibers from 5 sartorius muscles obtained after 30 min of exposure to PCP.

* $P < 0.05$ with respect to control.

** $P < 0.001$ with respect to control.

μM PCP, the half-decay time was increased to 2.5 ± 0.6 ms by the tenth directly evoked action potential and to 3.9 ± 1.0 ms by the twentieth spike.

To verify that the prolongation of the action potential was related to a block of K^+ conductance, the ability of PCP to block delayed rectification was investigated. This electrical property of the membrane can be used as an indication of the increase in K^+ conductance that occurs with a predictable delay after depolarization. Exposure to PCP (100 μM) for 30–60 min blocked delayed rectification (Figs. 4 and 5). The onset of the effects of PCP was dependent upon the frequency of stimulation of the cell membrane such that the faster the rate of stimulation, the faster the induction of the blockade. Thus, one may conclude that PCP prolongs the action potential by blocking active K^+ conductance (Fig. 5). A lesser effect on Na^+ conductance (Fig. 3, Table 1) was indicated by the decrease in amplitude and rate of rise of the action potential. The precise molecular sites of action of PCP on the Na^+ and K^+ channels of the electrically excitable membrane are not disclosed by the preceding analysis. Recovery to 60–70% of the control levels of the falling phase of the action potential was obtained 60 min after exposure to 80–100 μM PCP.

Effect of PCP on the amplitude and frequency of MEPPs. Since muscle fiber size affects the amplitude of the MEPP, we measured the effect of PCP on the MEPP amplitude in the same fibers of 15 different muscles throughout the experiment. The amplitude of MEPPS was corrected by the method of Martin (15). After 30 min of perfusion with PCP (3 μM), the mean amplitude of MEPPs was decreased to 83% of control, with no further decrease after continuous perfusion for another 30 min (Fig. 6). When the PCP concentration was increased to 10 μM, the MEPP amplitude was decreased to 67% of control within 15 min and was further decreased to within the noise level of the recording system 30 min after the start of perfusion. This suggests that the agent reacts with the postsynaptic receptor–ionic channel complex in a manner such that blockade occurs without local membrane depolarization.

The mean frequency of MEPPs in the control muscles was 0.67 ± 0.11 potential/s; after 60 min of exposure to PCP (1 μM), the MEPP frequency was 0.71 ± 0.15 potential/s. However, after 15 min, 10 μM PCP decreased the frequency to 0.23 ± 0.04 potential/s, probably because PCP attenuated the MEPP amplitude, rendering the smaller MEPPs undetectable within our noise level, i.e., <0.05 mV.

Effect of PCP on ACh-esterase. PCP was found to have negligible inhibitory effect on ACh-esterase of frog sartorius and rectus abdominis muscles at the concentrations that affect the excitability or chemosensitive properties of the muscle membrane. Indeed, at concentrations of 100 μM, PCP inhibited the Tetram-sensitive hydrolysis of 500 μM acetylthiocholine iodide by only 11 ± 3% ($N = 6$) after 5 min of exposure. On the other hand, the K_i of PCP on ACh-esterase of *Torpedo* membranes was 100 μM.

Effect of PCP alone and in combination with α-BGT on the indirectly elicited muscle twitch and ACh sensitivity of the chronically denervated skeletal muscle. PCP (10–60 μM) depressed the junctional ACh sensitivity of normal muscles and the extrajunctional ACh sensitivity of chronically denervated muscles. Marked depression of MEPP amplitude during exposure to PCP strongly suggested that this agent reacted with the postjunctional ACh receptor–ionic channel complex. Indeed, the appli-

TABLE 2

Effects of PCP (30 μM) on the membrane electrical constants of frog sartorius muscle[a]

	RMP	R_{in}	τ_m	R_m	C_m
	mV	*MΩ*	*ms*	*Ω·cm²*	*μF/cm²*
Control	−93 ± 1.3 (25)	0.58 ± 0.05	14.1 ± 0.7	4431 ± 248	3.23 ± 0.2
PCP (30 μM)	−98 ± 1.7 (27)*	0.64 ± 0.08*	15.7 ± 0.6*	5311 ± 515*	2.97 ± 0.3*

[a] Each value is the mean ± SEM, and the figure in parentheses is the number of fibers sampled from 4 muscles which were treated with PCP for 30–60 min. The symbols are: RMP, resting membrane potential; R_{in}, input resistance; τ_m, time constant; R_m, transverse resistance of a unit area; C_m, capacitance per unit area. The internal resistivity of the myoplasm was calculated to be 140 Ω·cm.

* $P < 0.05$ with respect to control.

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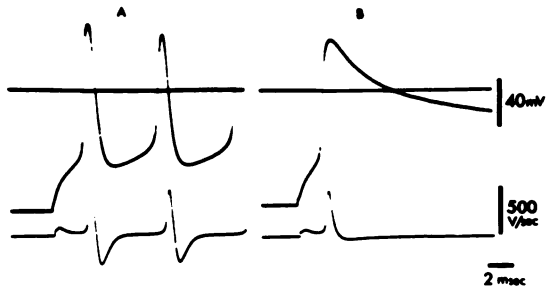

FIG. 3. *Effect of PCP on the action potential generating mechanisms*

Directly elicited action potentials (upper trace, single record) and their first derivative (dV/dT, lower trace) obtained from surface fibers of glycerol-treated frog sartorius muscle, before (A) and after (B) treatment with PCP (100 μM) for 60 min. The horizontal trace is the zero potential. Membrane potentials were held at −90 mV before each stimulus.

cation of PCP (60 μM) for a period of 30–60 min to the denervated soleus muscle significantly reduced its extrajunctional sensitivity to microiontophoretically applied ACh (Fig. 7). The mean value for controls was 1251 ± 327 mV/nC (mean ± SD of 26 surface fibers in 6 muscles) and, after 60 min of exposure to PCP (10 μM), was 45 ± 5.3 mV/nC (32 fibers/8 muscles). The effect of PCP on the denervated muscle was dependent upon its concentration, time of exposure, rate of stimulation, and duration of the pulse applied to release ACh from the micropipet onto the junctional membrane. While under control conditions the denervated muscle fibers reacted to iontophoretically applied ACh with regular and almost identical potentials even at high rates of stimulation (5 pulses/s), PCP reduced ACh sensitivity at concentrations ranging from 3 to 100 μM. In many cases subsequent stimulation at a rate of 1 Hz markedly reduced the ACh potentials such that by the fifth or sixth pulse a further reduction of the potential was recorded (Fig. 7b). A short interval of 30–60 s without stimulation recovered the amplitude to that of the first ACh potential. These actions of PCP were partially reversed after washing one preparation for 1 to 2 h.

The depression in extrajunctional ACh sensitivity pro-

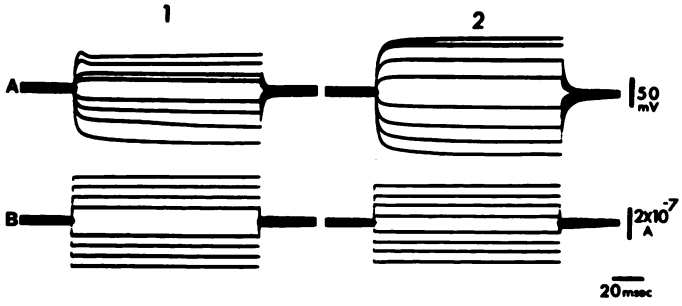

FIG. 4. *Effect of PCP (100 μM) on electrotonic potentials produced by anodal and cathodal pulses in glycerol-treated frog sartorius muscle fibers*

Tetrodotoxin (1 μM) was present at all times to block sodium conductance. A-1 shows control electrotonic potentials recorded after square pulses (B-1, B-2) of various intensities and signs were applied to the muscle membrane. The membrane potentials were kept at −90 mV. A-2 depicts the effect of PCP exposure for 30 min, where complete blockade of delayed rectification was achieved.

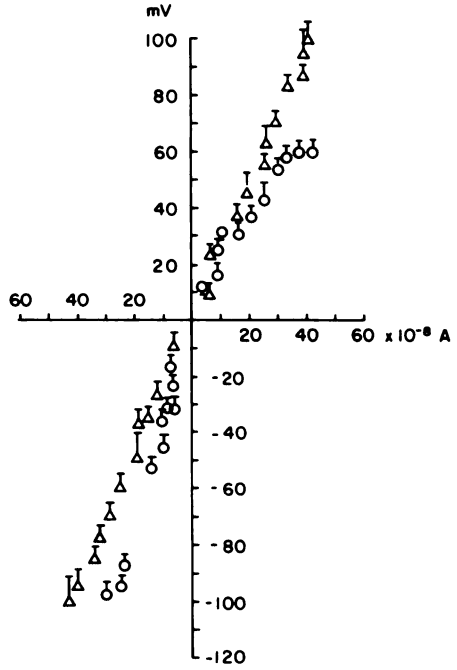

FIG. 5. *Voltage-current relations recorded from surface fibers of the frog sartorius muscle*

The delayed rectification remained unaltered after treatment with 1 μM tetrodotoxin (○) for 60 min and was blocked when the preparation was treated with 1 μM tetrodotoxin plus 100 μM PCP (△). Each point is the mean ± SEM of five to seven determinations from four muscles.

duced by PCP (Fig. 7) may result from an action of the agent on ACh-receptor sites or a reaction with its ionic channel sites or both. If PCP reacts with the ACh-receptor sites, it would be expected to compete with receptor ligands, e.g., α-BGT and ACh, for binding. PCP was

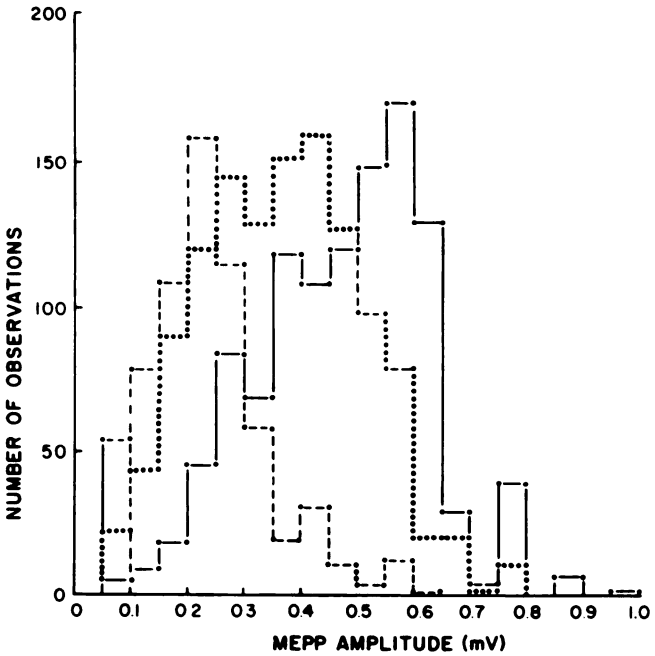

FIG. 6. *Distribution of mean amplitudes of the spontaneous MEPPs of frog sartorius muscle in normals (●——●) and after 30 to 60 min of exposure to PCP, 3 μM (●●●) and 10 μM (●··●)*

Although no major variations were found in membrane potentials, all amplitudes are corrected to a membrane potential of −95 mV.

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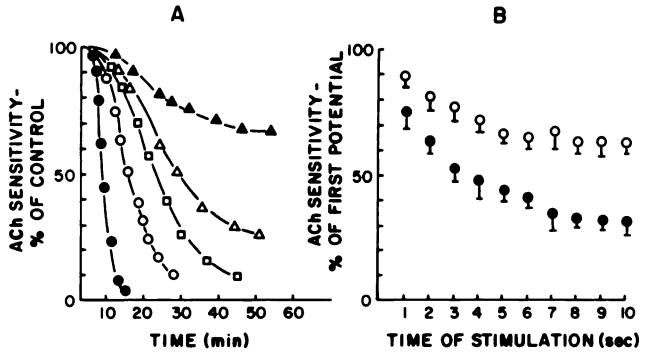

FIG. 7. *The influence of PCP (60 μM) on the ACh sensitivity of the extrajunctional region of the chronically denervated soleus muscle of the rat*

A shows the effect of various concentrations of PCP on the extrajunctional ACh sensitivity. Each point refers to 6–10 measurements obtained from four to six experiments. PCP concentrations were 1 μM (▲), 3 μM (△), 10 μM (□), 30 μM (○), and 60 μM (●). B depicts the increase in effectiveness of PCP as a function of the amount of ACh released from the micropipet when the duration of the electrical pulse applied to the micropipet was increased in duration. Each point refers to six measurements in three muscles. Stimuli rate was 0.5 Hz beginning at time zero, and ACh sensitivity is expressed as percentage of the first potential. The time of recording was during 20–30 min of exposure to the agent, and the durations of the stimuli delivering ACh from the micropipet were 5 ms (○) and 25 ms (●).

tested for its ability to prevent the quasi-irreversible action of α-BGT on the ACh receptor of the frog sartorius muscle (Fig. 8). The muscle was incubated initially with PCP (150 μM) for 30 min, followed by PCP and α-BGT (5 μg/ml) then PCP alone for another 60 min, and finally the muscle was washed with Ringer's for 20 min. Only a 5–10% recovery of the indirectly elicited muscle twitch was obtained. To verify that PCP reacted with the ACh receptor–ionic channel complex, the 10-day denervated soleus muscle of the rat was again used so as to eliminate possible presynaptic contributions. When the denervated soleus muscle was treated for 30 min with α-BGT (5 μg/ml), extrajunctional ACh sensitivity was blocked within 15 min (19). After washing of the excess α-BGT for 8 h, only partial ACh sensitivity, equivalent to 11.5 mV/nC, was observed. Thus, in one group of soleus muscles, an initial incubation for 60 min with PCP (100 μM) was made, followed by PCP and α-BGT (5 μg/ml) for 30 min and then PCP alone for 60 min, succeeded by 4 h of washing with Ringer's solution. Again a partial recovery of ACh sensitivity similar to that seen with α-BGT alone was observed (Fig. 9), indicating that PCP was unable to protect against the binding of α-BGT to ACh receptors.

Effect of PCP on binding of radiolabeled ligands to Torpedo ACh receptors. Membranes from *Torpedo* electric organ were utilized in the biochemical studies because of their high concentration of ACh receptors, an important factor for measurement of stoichiometric reactions, and their similar drug specificity to the receptors in skeletal muscle (16). Binding of [^{3}H]ACh (0.1 μM) to *Torpedo* membranes was unaffected by PCP at concentrations even up to 0.3 mM PCP (Fig. 10). Binding of 4 nM [^{125}I]α-BGT, 85% of which was inhibited by 100 μM *d*-tubocurarine (the K_i for *d*-tubocurarine determined from inhibition of [^{125}I]α-BGT is 0.4 μM), was unaffected by up to 0.1 mM PCP, but was reduced by higher concentrations, 20% by 0.3 mM and 35% by 3 mM. This suggested that PCP up to 0.1 mM did not interact with the well-characterized binding sites of the ACh receptor.

DISCUSSION

The present investigation demonstrates that PCP is able to affect both the chemosensitive and the electrical properties of skeletal muscles. Its effects are initially disclosed by a potentiation of the direct, and blockade of the indirect, muscle twitch (Fig. 1). The potentiation of the direct muscle twitch is not related to depolarization of the junctional or postjunctional membrane, a phenomenon seen with application of agonists (17) or partial agonists (18) to neuromuscular synapses. Rather, the increase in twitch tension appears to be related to the fact that PCP markedly prolongs the duration of the action potential of the muscle membrane, as has been seen by others with different agents (10, 19).

Measurement of the delayed rectification of the muscle membrane (Figs. 4 and 5) revealed that the prolongation of the action potential produced by PCP was most likely related to a block of K^+ conductance and possibly to a partial inhibition of Na^+ inactivation. Indeed, voltage clamp experiments (performed in the laboratory of Dr. Clay Armstrong by Dr. Randy Swansson) with internally perfused giant axons of the squid indicate that PCP (30–100 μM) and its quaternary salt, PCP methiodide, partially block open K^+ channels (personal communication). In addition, we found that PCP causes a small but significant depression of the amplitude and rate of rise of

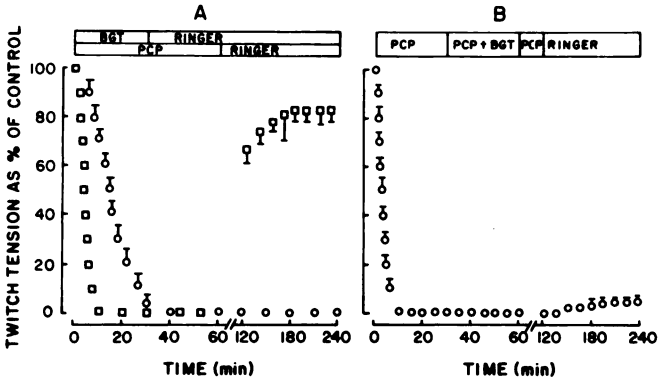

FIG. 8. *Effect of PCP and α-BGT on the indirectly elicited muscle twitch of the frog sartorius muscle*

(A) Blockade of the indirectly elicited twitch of the frog sartorius muscle produced by α-BGT and PCP. This figure combines two different sets of experiments. In one series of experiments (○) the muscles were exposed to α-BGT (5 μg/ml) for 30 min, then washed for 210 min, and no recovery of the muscle twitch was achieved. In the other set of experiments (□), the muscles were exposed to PCP (100 μM) for 1 h, then washed for 2 min. Recovery was observed to occur after 60 min, and the control condition was achieved at 90 min. (B) The absence of protection of neuromuscular transmission by PCP from the blocking effect of α-BGT. The muscles were initially exposed to PCP (100 μM) for 30 min, followed by a combination of PCP (100 μM) and α-BGT (5 μg/ml) for 30 min, then to PCP for 60 min, followed by washing in Ringer's for 120 min. Each point for the experiments shown in A and B is the mean ± SEM from at least three muscles. Where no bars are shown, the SEM was smaller than the symbols. Temperature, 22°C; stimulation rate, 0.1 Hz.

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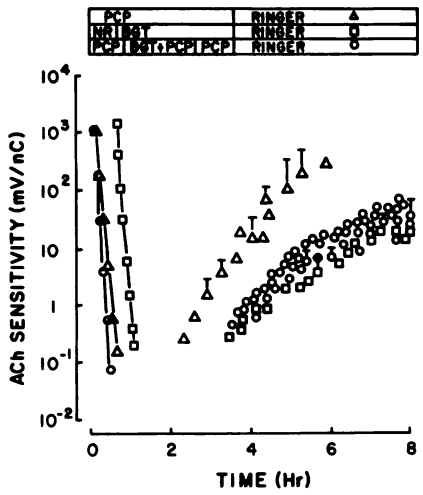

FIG. 9. *Effect of PCP and α-BGT on extrajunctional ACh sensitivity of chronically denervated rat soleus muscle*

In controls (△) the muscle was exposed for 90 min to PCP (100 μM) and subsequently washed with complete recovery. In one set (□), muscles were exposed to α-BGT (5 μl/ml), then washed with Ringer's (NR) for up to 8 h with little recovery of ACh sensitivity. In the other experiments (○), muscles were exposed to PCP (100 μM) for 30 min, followed by a combination of PCP (100 μM) and α-BGT (5 μg/ml) for another 30 min and then PCP alone for 30 min, succeeded by washing with Ringer's. There was little recovery of ACh sensitivity, only slightly more than that seen when α-BGT was used alone. Each symbol and vertical bar represent the mean ± SEM of three experiments. Where no bars are shown, the SEM was smaller than the symbols.

the action potential, thus suggesting a decrease in Na^+ conductance. A more detailed analysis of the action of PCP on the Na^+ and K^+ channels using the voltage clamp technique is indicated. Although these effects of PCP on the action potential may be of little consequence in the peripheral nervous system, it is interesting to speculate that such a depression in small neurons (especially inhibitory fibers) might be responsible for some of the clinical signs of PCP intoxication. These actions of PCP on K^+ conductance, which are similar to those observed with histrionicotoxin (10) and tetraethylammonium (10, 20), were not seen when the muscle was exposed to a physiological solution containing PCP methiodide (unpublished results). It, therefore, appears that PCP affects the ionic conductance of the excitable membranes by reacting with sites located inside the cell membrane.

If the action potential of nerve terminals is prolonged by PCP to the same extent as that seen to occur in the muscle membrane, the evoked release and storage processes of other transmitters whose mechanisms of release depend upon the invasion of the action potential into the nerve terminal will be affected. It has been demonstrated that agents which prolong action potentials by blocking K^+ conductance (20) and/or Na^+ inactivation (24, 25) in muscle and nerve alter evoked transmitter release (21). The blockade of K^+ conductance appears to be involved in the abnormal behavior induced by PCP in experimental animals. Indeed, the PCP analog *m*-nitro-PCP while qualitatively retaining the chemosensitive actions of PCP did not block K^+ conductance (unpublished results) and caused no abnormal behavior pattern in mice and rats (26, Stanley Grick, personal communication).

Even though PCP is a strong inhibitor of plasma butyrylcholinesterase (K_i = 0.1–1 μM), its effects on ACh-esterase of human red blood cells (K_i = 100 μM) (6) and *Torpedo* electroplax (K_i = 100 μM) are much weaker. It is even less potent on ACh-esterase of frog muscles (11% inhibition with 100 μM PCP). Therefore, the postsynaptic inhibition in muscles caused by PCP may be due to its inhibition of sites on the ACh receptor or its ionic channel. In contrast to previous conclusions (6, 7), and in support of our recent findings (22), PCP does not interact with the ACh-receptor ligand binding sites and is devoid of agonist activity. First, it does not protect against the quasi-irreversible inhibition by α-BGT of the indirectly elicited muscle twitch (Fig. 8) or of the extrajunctional ACh sensitivity (Fig. 9). In addition, up to 0.1 mM PCP cannot inhibit binding of [^{3}H]ACh or [^{125}I]α-BGT to sites on the ACh receptor of *Torpedo* electric organ membranes (Fig. 10).

The effects of PCP on ACh sensitivity are due to its inhibition of ACh receptor-regulated ionic conductances, but not through inhibition of ACh-receptor binding sites. We suggest that these PCP effects are due to its inhibition of ionic channel sites. This is clearly demonstrated in the following paper.

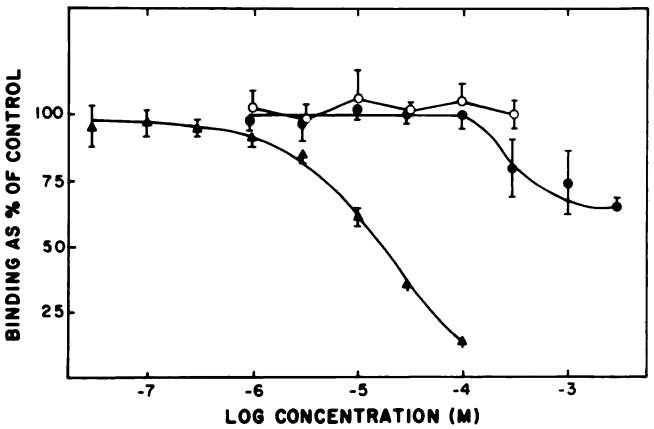

FIG. 10. *Influence of PCP and d-tubocurarine on ligand binding to the nicotinic ACh-receptor on Torpedo membranes*

The specific binding of 0.1 μM [^{3}H]ACh (○) and 4 nM [^{125}I]α-BGT (● and ▲) is plotted as a function of the concentration of PCP (○ and ●) and *d*-tubocurarine (▲) present. The binding is expressed as a percentage of specific binding in the absence of any competing ligand. Each symbol and vertical bar represent the mean ± SD of three experiments.

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Send reprint requests to: Edson X. Albuquerque, Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, Md. 21201.

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