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Site of Action of Phencyclidine

IV. Interaction of Phencyclidine and Its Analogues on Ionic Channels of the Electrically Excitable Membrane and Nicotinic Receptor: Implications for Behavioral Effects

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SUMMARY

The effects of 1-(1-phenylcyclohexyl)piperidine [phenycyclidine (PCP)] and three of its analogues were studied on the ionic channels of the electrically excitable membrane and of nicotinic acetylcholine (ACh) receptors in frog sciatic nerve-sartorius muscle preparations and in a behavioral paradigm in rats. Two of these analogues, i.e., 1-piperidinocyclohexanecarbonitrile (PCC) and 1-(1-*m*-nitrophenylcyclohexyl)piperidine (*m*-nitro-PCP) are not active behaviorally, whereas the third one has the same effects as PCP. Thus, like PCP, 1-(1-*m*-aminophenylcyclohexyl)piperidine (*m*-amino-PCP) blocked the indirectly elicited contraction and potentiated the directly elicited contraction while prolonging the half-decay time of the action potential in muscle. These effects were associated with a block of delayed rectification, a reduction in the amplitude, and an increase in the threshold of the action potential. Like PCP, *m*-amino-PCP increases the error ratio of alternation tasks in the behavioral paradigm. Both *m*-nitro-PCP and PCC depressed the amplitude and rate of rise of the action potential, but only *m*-nitro-PCP caused a small increase in half-decay time of action potentials. Delayed rectification is only partially blocked by *m*-nitro-PCP. Neither PCC nor *m*-nitro-PCP affects the error score in the behavioral tests. Although they differ behaviorally, the four compounds are quite similar in their action on the ionic channel of the nicotinic ACh receptor. Thus, all four compounds produced a concentration-dependent depression of peak end-plate current (EPC) amplitude. The time constant of EPC decay (τ_{EPC}) was shortened by *m*-amino-PCP and *m*-nitro-PCP, which induced a change in voltage sensitivity at higher concentrations. In contrast, PCC had no effect on τ_{EPC} but significantly depressed peak EPC amplitude. All four agents appear to interact with the open and closed conformations of the ionic channel of the ACh receptor. The marked potency of *m*-nitro-PCP and PCC on the ionic channel of the nicotinic ACh receptor is in contrast to their inactivity in the behavioral tests. We also show that the rank order of potency of these drugs on muscarinic receptors does not match their potency in the behavioral paradigm. These two cholinergic mechanisms are therefore not adequate to account for the behavioral effects of PCP and its other psychoactive analogues (e.g., *m*-amino-PCP). Rather, a blockade of potassium conductance in the electrically excitable membrane and subsequent effects on transmitter release at central synapses by these psychoactive drugs are here proposed to account for the observed behavioral alterations. PCC and *m*-nitro-PCP have only negligible effects on potassium permeability.

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INTRODUCTION

PCP[5] is a potent drug of abuse requiring emergency care and often psychiatric management. It causes profound changes in mental status in man, including euphoria, aggressive behavior, and psychotic disturbance (1–3) which may be difficult to distinguish from schizophrenia.

In previous studies we have shown that PCP is a potent inhibitor of potassium conductance of the electrically excitable muscle membrane, of the ionic channel associated with the nicotinic receptor (4, 5), and of muscarinic receptors (6–8). Studies on the behavioral effects of PCC, a synthetic precursor of PCP, and *m*-nitro-PCP (Fig. 1) revealed a much smaller response than that elicited by either PCP or *m*-amino-PCP. We therefore decided to study the action of these discriminant compounds at three different levels: (a) the voltage-sensitive sodium and potassium conductance of skeletal muscle, (b) the nicotinic receptor-ionic channel complex of frog sartorius muscle, and (c) the behavioral changes in rats evaluated by alterations of their performance in a spatial alternation test. The effects of these compounds on sodium and potassium conductance were assessed by examining the waveform of the muscle action potential and of delayed rectification. The actions of *m*-amino-PCP, *m*-nitro-PCP, and PCC on neuromuscular transmission were compared with those of PCP by analysis of their effects on muscle twitch, EPCs, and behavior. Results presented here indicate that the common behavioral effects of PCP and *m*-amino-PCP are more likely to be related to a decrease of potassium conductance than to blockade of the nicotinic ion channel of the cholinergic system or to blockade of muscarinic receptors.

MATERIALS AND METHODS

Electrophysiological techniques. Experiments were performed at room temperature (20–22°) on sciatic nerve sartorius muscle preparations of the frog *Rana pipiens.* The physiological solution had the following composition (millimolar): NaCl, 115.5, KCl, 2.0, $CaCl_2$, 1.8; Na_2HPO_4, 1.3; and NaH_2PO_4, 0.7. The solution was bubbled with 100% O_2 and the pH was 6.9–7.1. For twitch studies, the nerve was stimulated with supramaximal rectangular pulses having a duration varying from 0.05 to 0.1 msec, via an Ag-AgCl salt bridge electrode connected to a wet electrode. Direct stimulation of the muscle was accomplished by applying supramaximal rectangular pulses of 1.0- to 2.0-msec duration through a bipolar platinum electrode placed around the middle portion of the muscle. Direct and indirect stimulation was applied alternately, each at a rate of 0.05 Hz. The muscle tension generated by both direct and indirect stimulation was recorded by attaching the muscle to a Grass force displacement transducer (FT.03) connected to a Grass Model 7 polygraph on which the twitch was displayed.

For the recording of intracellular potentials, the muscles were pinned under slight tension to the paraffin lining of a Plexiglas plate having a planoconvex lens in the center and then placed in a 20-ml capacity bath. Resting membrane potentials were recorded only from surface fibers using conventional intracellular recording techniques (4).

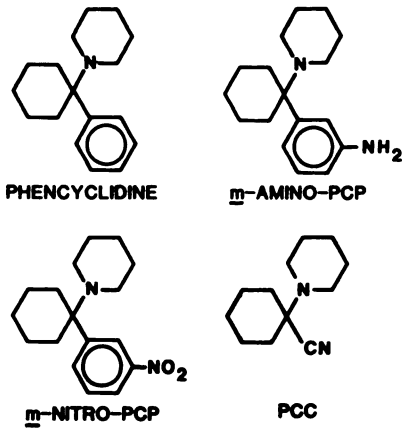

FIG. 1. *Structures of phencyclidine and its analogues*
The molecular weights are as follows: phencyclidine (PCP), 243.4; *m*-amino-PCP, 258.4; *m*-nitro-PCP, 290.4; PCC, 228.8.

Directly elicited action potentials were recorded with one microelectrode inserted into a surface fiber while passing a 30-msec depolarization pulse through another microelectrode inserted into the same fiber at an interelectrode distance of 50 μm (5). Delayed rectification was determined in surface fibers of frog sartorius muscle which were continuously exposed to tetrodotoxin (1.0 μM). The muscle fibers were polarized to −90 mV and stimulated with rectangular depolarizing and hyperpolarizing current pulses. The resulting electrotonic potentials were plotted against the injected current.

The effects of PCP and its analogues on EPCs were investigated at 22° using sciatic nerve-sartorius muscle preparations which had been treated with glycerol to disrupt excitation-contraction coupling. The voltage-clamp technique was similar to that described earlier (9). The DC output of a Tektronix oscilloscope, which displayed the nerve-evoked EPC and the membrane potential, was sampled at a rate of 100 μsec/point using an analog to digital converter and analyzed by a laboratory computer (PDP 11/40, Digital Equipment Corporation, Marlboro, Mass.). The value of τ_{EPC} was determined by linear regression of the logarithms of the digitized decay (20–80%) points against time.

Binding experiments. For the characterization of the muscarinic properties of PCP and its analogues we carried out competition binding experiments with radiolabeled atropine. These were done as described earlier (10) under equilibrium conditions at 25°, pH 7.4, in 100 mOsM Tris-HCl. A portion (0.3 mg) of the P_2 fraction (rat whole brain without cerebellum) was incubated for 90 min with 3.5 nM ^{3}H-labeled (−)-atropine (4.6 Ci/mmole) with six to nine concentrations of the tested drug. Blanks (nonspecific binding) were determined in the presence of 0.5 μM (±)-quinuclidinyl benzilate. The free ^{3}H-labeled (−)-atropine was separated from bound by the rapid filtration method (GF/B Whatman filters), and the amount of radioactivity trapped was assayed by a Beckman 9000 scintillation counter (43% efficiency), using NEN 963 scintillation fluid.

[5] The abbreviations used are: PCP, 1-(1-phenylcyclohexyl)piperidine (phencyclidine); ACh, acetylcholine, PCC, 1-piperidinocyclohexanecarbonitrile; *m*-amino-PCP, 1-(1-*m*-aminophenylcyclohexyl)piperidine; *m*-nitro-PCP, 1-(1-*m*-nitrophenylcyclohexyl)piperidine; EPC, end-plate current; τ_{EPC}, time constant of EPC decay.

Behavioral studies. Previous studies have established that PCP impairs spatial alternation performance (11). In this task a rat is required to alternate responding on left and right levers in a two-lever operant chamber. The subjects are naive female Sprague-Dawley rats, approximately 90 days old and weighing 225–250 g at the beginning of testing. All testing is conducted in BRS/LVE operant test cages, each containing two levers and each enclosed in a sound-attenuated cubicle. The task is programmed and run by a NOVA 1200 minicomputer. Initially, rats deprived of water for 23 hr are shaped by delivering a noncontingent water reward every minute during testing; every bar-press on either lever is also rewarded with water (0.01 ml). When a rat makes a total of 100 responses during a 30-min daily test session, the noncontingent water is eliminated and rats are then maintained on a schedule of continuous reinforcement for five more sessions. A forced alternation procedure is then begun. The levers, which are retractable, are made available one at a time, alternating after each reward (left-right-left, etcetera). When rates of responding stabilize, usually within 1 week, the task is finally changed to "learned" alternation. Both levers are now always available and the rat must still alternate responding on each lever in order to be rewarded. Excess responses (errors) are counted but have no consequences. After performance stabilizes, usually within 7–10 days, the rats are tested for an additional 2 weeks before drug administration is begun. Rats are tested daily for 30 min, and drug or control solutions (usually saline) are administered 15 min before test sessions on Tuesdays and Fridays. Although different groups of rats (N = 6 rats per group) are used to evaluate the effects of different drugs, the same rats receive all doses of a given drug; drugs are ordinarily administered in a sequence of increasing dosage. In addition, the effects of PCP are determined in all groups of animals, usually both before and after testing the effects of a derivative. Normally, rats perform the spatial alternation task with considerable accuracy and consistency. After training is complete and performance is asymptotic, the average rat makes only about 15% more responses than necessary; that is, the ratio of responses to rewards per 30-min test session is 1.15 (mean value of 36 rats on at least 45 test sessions per rat). Although in the most extreme case, response rates may fluctuate up to 12–13% from day to day, the error ratio hardly fluctuates at all; it varies no more than ±3% in the most variable of rats. As shown in detail elsewhere (7), PCP (administered i.p. 15 min before testing) also affects the rate of response. In contrast to the effect of PCP on alternation performance, the effect on the rate of the response is nonspecific (7).

Drugs. PCP and PCC (as the hydrochloride salts) were obtained from the National Institute of Drug Abuse (Rockville, Md.). Ketamine (as the hydrochloride salt) was obtained from Bristol-Meyers (Syracuse, N. Y.); atropine (as the sulfate salt) and scopolamine (as the hydrobromide salt) were obtained from Sigma Chemical Company (St. Louis, Mo.). All other drugs and chemicals were either synthesized in the laboratory or obtained from usual laboratory sources. All drugs, for electrophysiology and contractile studies, were maintained as refrigerated stock solutions (10^{-2} M) in physiological solution without calcium for 1 week and diluted just prior to use. Tritiated atropine (as the racemate, sulfate salt) was custom-made by general tritiation and obtained from Dr. O. Buchman, Radiochemistry Department, Nuclear Research Center (Negev, Israel).

Statistics. All values are expressed as the mean ± standard error of the mean. Student's t-test was used to examine the difference between control and drug-induced changes. Probability (p) values ≤0.05 were considered statistically significant.

RESULTS

Effects on muscle twitch. The m-nitro derivative of PCP (10–160 μM) caused a concentration-dependent block of neuromuscular transmission (i.e., indirectly elicited twitch) in frog sartorius muscle (Fig. 2). The directly elicited twitch was transiently potentiated and then depressed by m-nitro-PCP (100 μM) (Fig. 3), an effect which may be related to the suppression of action potential generation in muscle (see below). Peak potentiation of the directly elicited twitch with m-nitro-PCP (100 μM) occurred at 6–7 min, when it reached 110 ± 10% of control—an insignificant effect ($p > 0.05$). The subsequent depression of both the directly and indirectly elicited twitches by m-nitro-PCP was reversed by washing the drug from the bath. When exposed to m-amino-PCP (100 μM), the indirectly elicited muscle twitch was also blocked about 5 min after application. Like PCP (12), m-amino-PCP produced a concentration-dependent potentiation of the directly elicited twitch. For example, at 80 and 100 μM, m-amino-PCP produced a significant ($p < 0.01$) and sustained potentiation of the directly elicited twitch which reached peak values of 138 ± 8% (N = 3 muscles) and 161 ± 14% (N = 4 muscles; range 133–199) of control within 6–7 min, respectively (Fig. 4). The direct response recovered 1 hr after washing with drug-free physiological solution. PCC (10, 40, and 100 μM) reduced

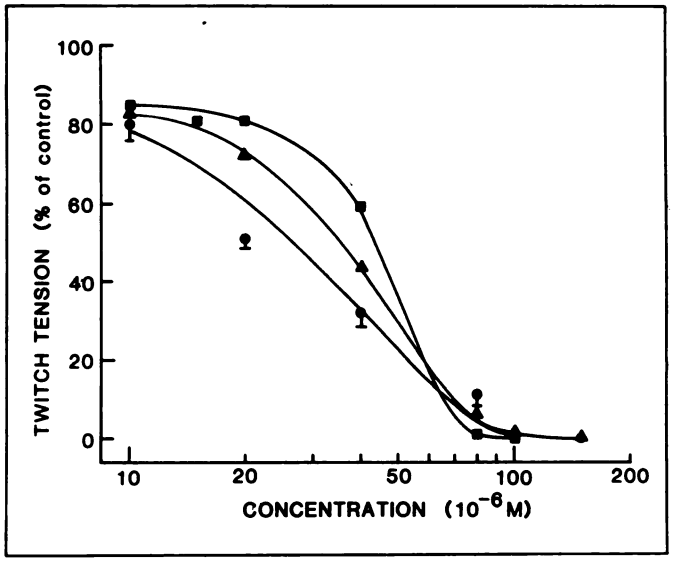

FIG. 2. *Concentration-dependent depression of the indirectly elicited twitch in frog sartorius muscle by PCP analogues*

Each point and bar represent the mean (± standard error) twitch tension relative to control values of three to five muscles after exposure to the compound for 20–30 min. ●, m-Nitro-PCP; ■, m-amino-PCP; ▲, PCC. The curves drawn were best approximation of the data.

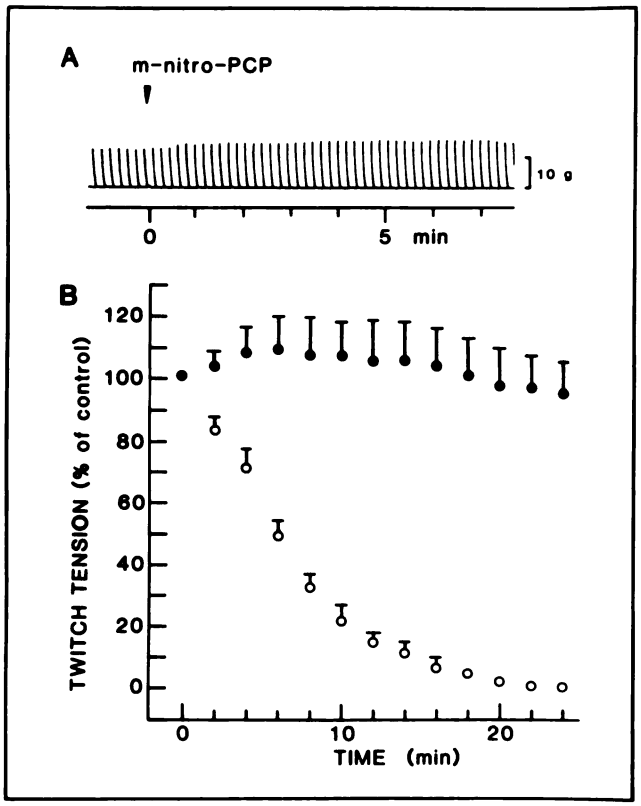

FIG. 3. *Effect of m-nitro-PCP on twitch tension of frog sartorius muscle*

In A, the indirectly elicited response was first blocked with α-bungarotoxin (5 μg/ml) prior to applying *m*-nitro-PCP (100 μM). B, Effects of *m*-nitro-PCP (100 μM) on directly (●) and indirectly (○) elicited muscle twitches. Each point and bar represent the mean (± standard error) of results with four muscles.

the amplitude of the indirectly elicited twitch by 16 ± 8%, 57 ± 8%, and 100% (N = 3 to 5 muscles each), respectively (Fig. 5). The directly elicited twitch was depressed less than 10% after 1 hr of exposure to PCC (100 μM), a value similar to control. The concentrations causing 50% blockade of the indirectly elicited muscle twitch with *m*-nitro-PCP, *m*-amino-PCP, and PCC were approximately 28, 45, and 36 μM (Fig. 2).

Effects on the resting membrane and action potentials. The mean control for the resting membrane potential of surface fibers was −90.1 ± 0.8 mV (N = 36 fibers). Neither PCP nor PCC, *m*-amino-PCP, or *m*-nitro-PCP at 100 μM concentration had any effect on the resting membrane potential of surface fibers, which remained at about

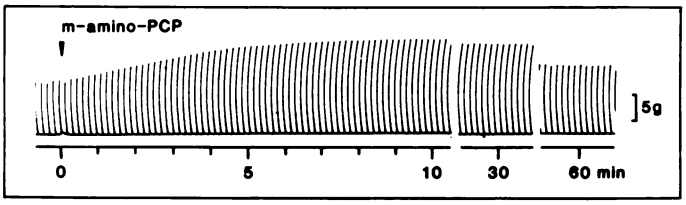

FIG. 4. *Time course of effect of m-amino-PCP (100 μM) on the directly elicited twitch of frog sartorius muscle*

The indirectly elicited response was first blocked with α-bungarotoxin (5 μg/ml) and then *m*-amino-PCP was applied. Comparable results were obtained in three additional experiments.

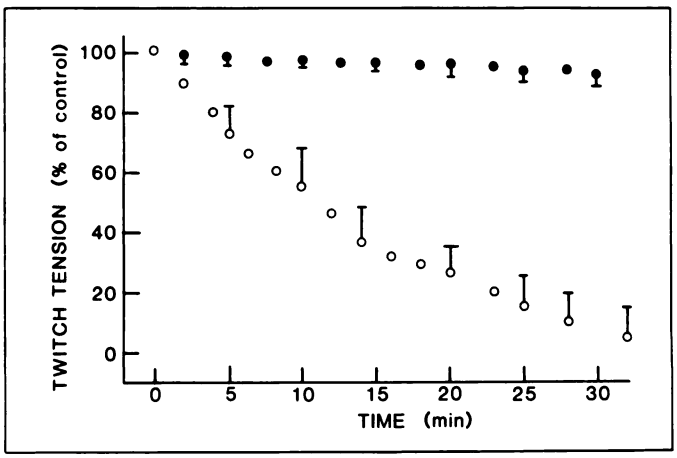

FIG. 5. *Effect of PCC (100 μM) on directly and indirectly elicited twitches of frog sartorius muscle*

Note that PCC had no effect on the directly elicited twitch (●), but blocked the indirectly elicited muscle twitch (○). Each point and bar represent the mean (± standard error) of results with four muscles.

−90 mV (i.e., within 1 mV of the mean control). The ability of PCP (at 100 μM) to suppress repetitive firing of action potentials in the muscle membrane and increase the half-decay time of the action potential (5) was confirmed in the present investigation. Thus, the threshold of the action potential increased to 108% of control, amplitude decreased to 82% of control, rate of rise decreased to 75% of control, rate of fall decreased to 10% of control, and the half-decay time of the action potential increased to 542% of control (Table 1). Similarly, *m*-amino-PCP (100 μM) markedly prolonged the muscle action potential; the half-decay time increased to 212% of control (Table 1), the rate of rise decreased to 75% of control ($p < 0.001$) and the rate of fall decreased to 33% of control ($p < 0.02$). When stimulated repetitively at a frequency of 1 Hz, the half-decay time of the first and tenth action potentials in a train should be identical. In the presence of PCP (100 μM), the half-decay times of the first and tenth action potentials increased to 405 and 454% of control (5); with *m*-amino-PCP (100 μM), the half-decay time increased to 210 and 380% of control, respectively (Fig. 6).

After a 30-min exposure to *m*-nitro-PCP (100 μM) or PCC (100 μM), the threshold of the action potential was significantly increased, the rate of rise was unaffected but the rate of fall was significantly decreased ($p < 0.02$) (Table 1), and secondary spikes normally present were abolished. Although *m*-nitro-PCP significantly prolonged the half-decay time of the action potential, the extent of the increase was small when compared with PCP and *m*-amino-PCP under similar conditions (Table 1), and the rate of fall decreased to 62% of control at 100 μM. PCC was without effect on the half-decay time of the action potential but increased the threshold and decreased the amplitude and rates of rise and fall of the action potential significantly.

In the presence of *m*-nitro-PCP (100 μM), the half-decay time of the first and tenth action potentials in a train evoked at 1 Hz were prolonged to 133 and 215% of control, values approximately 30% and 50% of those with PCP under similar circumstances. Additionally, the pro-

TABLE 1

Effects of PCP and its analogues on the directly elicited action potential of frog sartorius muscles

Values presented are means ± standard error; numbers in parentheses are the numbers of fibers from four muscles exposed to 100 μM of each drug for 30 min.

Condition	Threshold	Amplitude	Half-decay time	Rate of rise
	mV	*mV*	*msec*	*V/sec*
Control	44.0 ± 0.5 (43)	84.7 ± 1.0	0.65 ± 0.01	472 ± 14
PCP	47.3 ± 2.3 (45)[a]	69.1 ± 2.8[a]	3.52 ± 0.63[a]	352 ± 21[a]
m-Amino-PCP	46.9 ± 0.4 (33)[b]	76.6 ± 1.4[a]	1.38 ± 0.03[a]	375 ± 10[a]
m-Nitro-PCP	47.5 ± 0.6 (23)[a]	76.2 ± 1.1[a]	0.87 ± 0.03[a]	471 ± 15
PCC	56.8 ± 1.4 (11)[b]	68.3 ± 2.2[a]	0.70 ± 0.04	380 ± 23[a]

[a] $p < 0.001$ with respect to control.
[b] $p < 0.02$ with respect to control.

longation of the action potential under these conditions was less frequency-dependent with *m*-nitro-PCP than with *m*-amino-PCP, and the depression of rate of fall more profound (Fig. 6).

Effects on delayed rectification. To determine whether the prolongation of the muscle action potential was possibly related to a blockade of potassium conductance, as has been observed with PCP (5), we examined the property of delayed rectification in the muscle membrane in the presence of all four agents. Experiments on frog sartorius muscles were conducted in the presence of tetrodotoxin (1 μM) to block sodium conductance. At concentrations as low as 10 μM, PCP and *m*-amino-PCP caused a significant blockade ($p < 0.02$ or greater) which became more complete as the applied depolarization was increased (Fig. 7). At 10 μM, *m*-nitro-PCP (Fig. 7) and PCC (data not shown) were ineffective; at 100 μM, PCC still had no effect on delayed rectification, but 100 μM *m*-nitro-PCP did produce a partial blockade of delayed rectification whereas the effect of 100 μM *m*-amino-PCP, like that of PCP, was complete (Fig. 8). Similar to observations with PCP, the onset of blockade of delayed rectification was frequency-dependent, occurring with a decreased latency when the frequency of membrane activation was increased (data not shown). These frequency-dependent effects were similar to the prolongation observed in the decay phase of the muscle action potential (Table 1 and Fig. 6). Only partial recovery of delayed rectification (to 50% of control) was observed after washing either PCP or *m*-amino-PCP from the bath for 60 min, but complete recovery was observed with *m*-nitro-PCP.

Effects on the EPC. When the frog sartorius muscle was exposed to *m*-nitro-PCP (30 μM), the amplitude of the EPC at −90 mV decreased to 63% of control and the τ_{EPC} was shortened to 38% of control (Fig. 9). Similarly, *m*-amino-PCP (30 μM) decreased the peak EPC amplitude recorded at −90 mV to 26% of control and τ_{EPC} was shortened to 43% of control. Figure 9A illustrates the current-voltage relationships for EPCs in control fibers and in the presence of the three concentrations of *m*-nitro-PCP. There was a concentration-dependent depression of peak EPC amplitude by *m*-nitro-PCP (Fig. 9A); at 5 μM, for example, the EPC amplitude was significantly reduced between −100 and −160 mV, but there was little alteration of EPC amplitude between +50 and −85 mV. With higher concentrations, the EPC amplitude was depressed even at positive membrane potentials. At more negative membrane potentials, the current-voltage relationship of the EPC in the presence of 5–50 μM *m*-nitro-PCP showed a marked upward curvature and a region of negative conductance where the EPC amplitudes decreased in spite of an increase in the driving force.

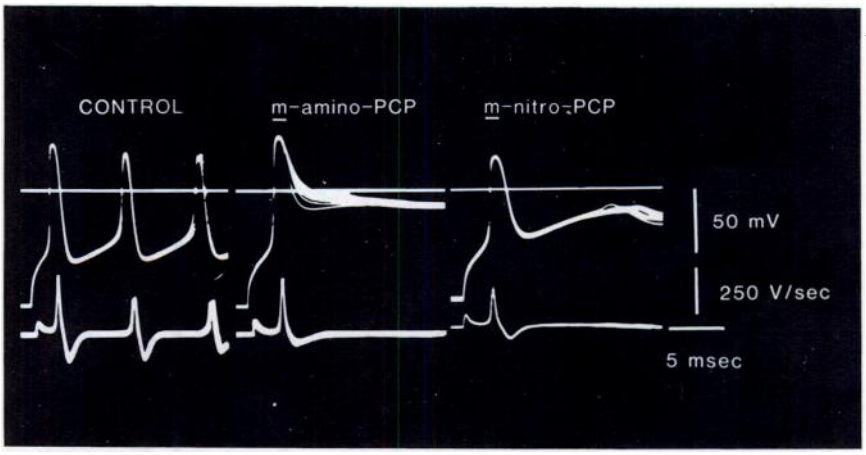

FIG. 6. *Effect of m-amino-PCP and m-nitro-PCP (100 μM) on the directly elicited action potentials in surface fibers of glycerol-shocked frog sartorius muscle.*

The upper traces are 10 superimposed action potentials and the lower trace their first derivative (dV/dt); the horizontal line is the zero potential. Membrane potentials were held at −90 mV before each stimulus. Stimulus frequency: 1.0 Hz. The compounds were applied for 30 min before recordings were obtained.

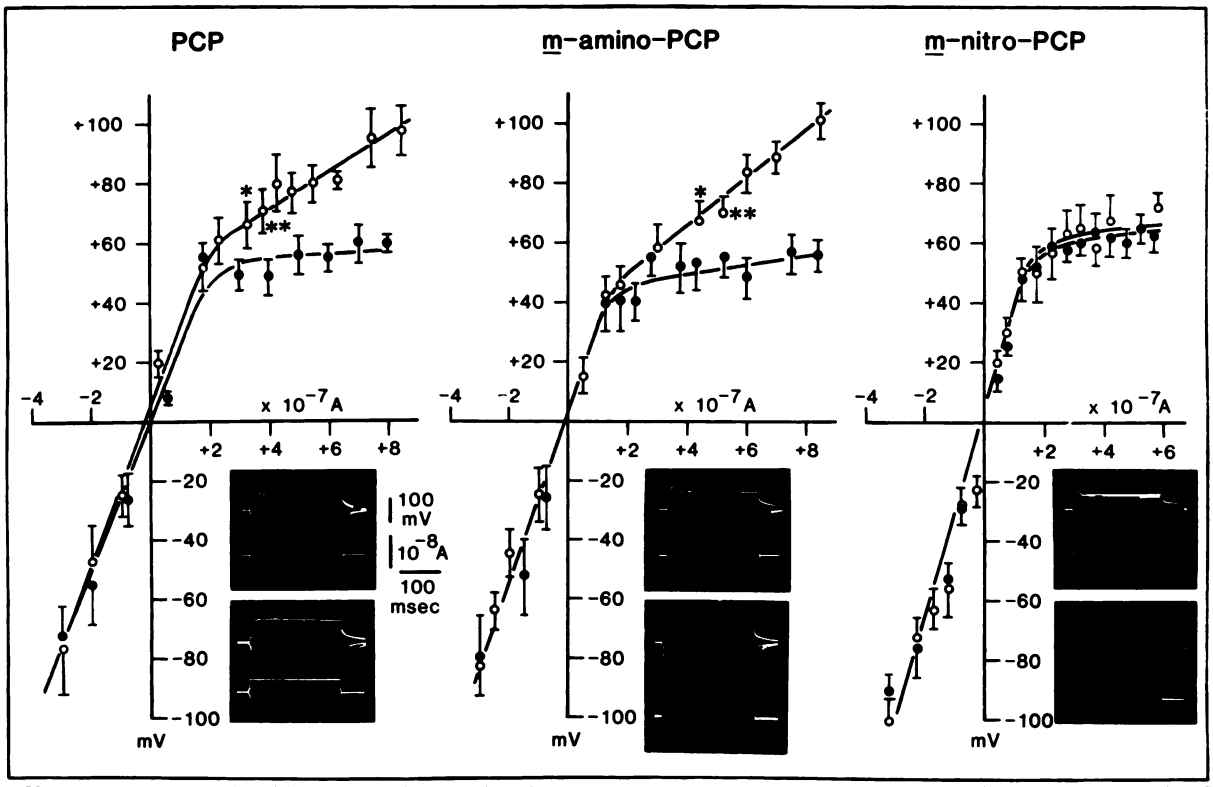

FIG. 7. *Voltage-current relationship recorded from surface fibers of frog sartorius muscle in the presence of 10 μM PCP, m-amino-PCP, and m-nitro-PCP*

The muscles were continually exposed to tetrodotoxin (1 μM) to block sodium conductance both before (control; ●) and during exposure to the compounds (○) (see Materials and Methods). *Insets* show typical records from control fibers (*upper*) and drug-treated fibers (*lower*) after 45–60 min of exposure. Each point represents the mean (± standard deviation) of 3–8 determinations from 7–10 fibers for control and 10–15 fibers during drug. Each muscle served as its own control. *Single asterisks* indicate a significant difference between control and drug-treated values ($p < 0.02$). *Double asterisks* indicate $p < 0.001$ at the point indicated and when greater currents were applied.

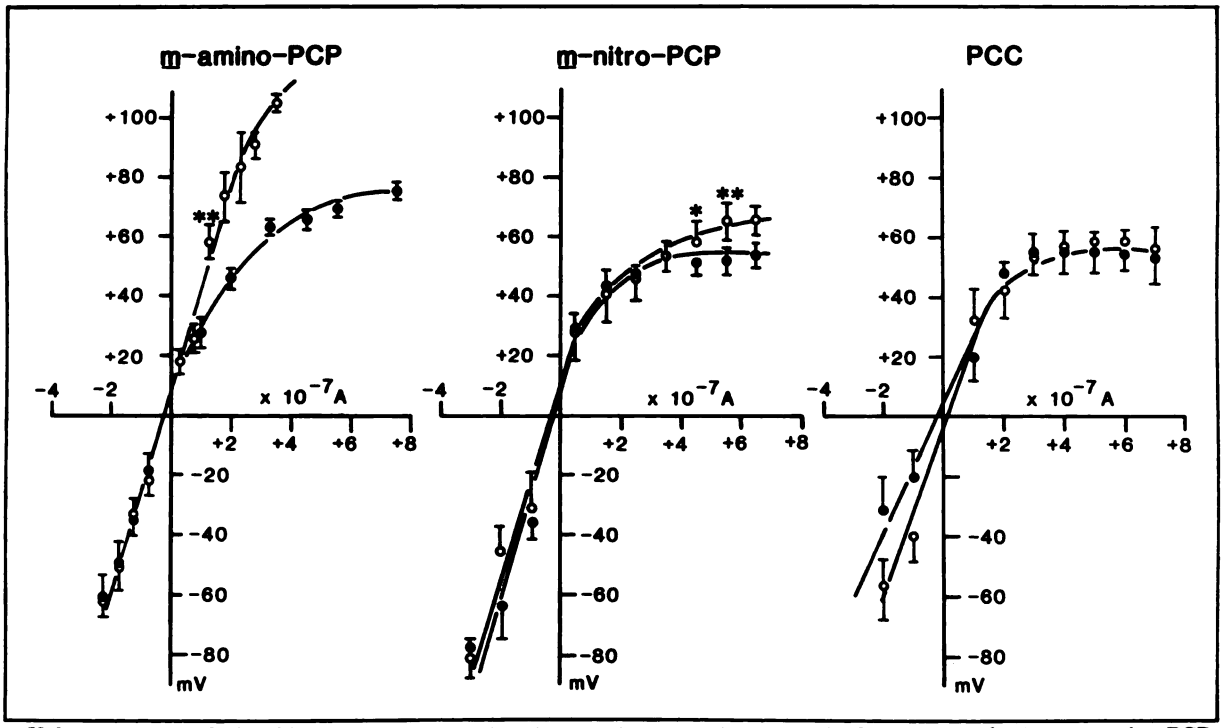

FIG. 8. *Voltage-current relationship recorded from surface fibers of frog sartorius muscle in the presence of 100 μM m-amino-PCP, m-nitro-PCP, and PCC*

The muscles were exposed to tetrodotoxin as in Fig. 7. Each point represents the mean (± standard deviation) of 5–10 determinations from 15–20 fibers of 4–5 muscles. *m*-Amino-PCP blocked delayed rectification completely, whereas *m*-nitro-PCP was only partially effective and PCC was totally ineffective. *Asterisks* indicate $p < 0.02$ (*) or p 0.001 (**). ●, Control; ○, drug.

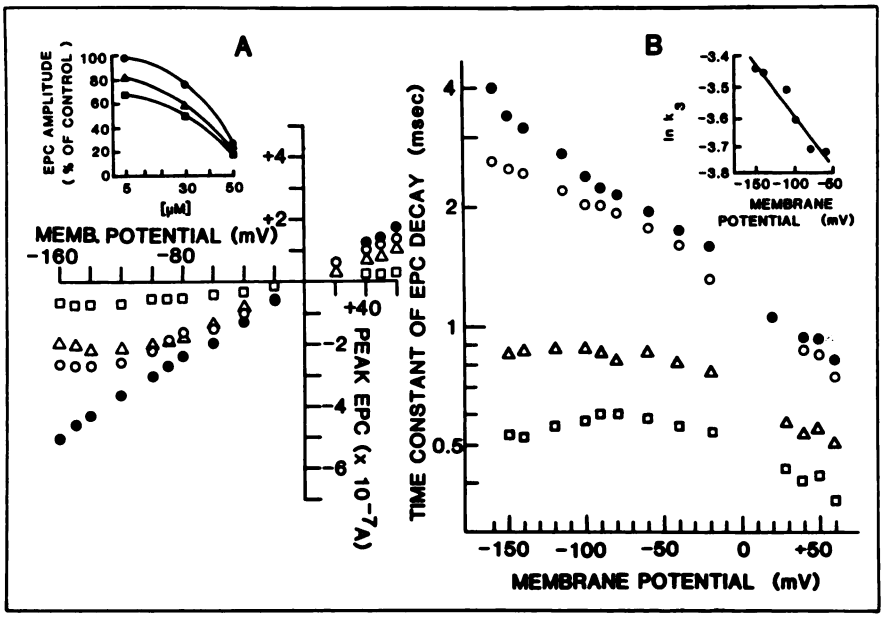

FIG. 9. *Effect of m-nitro-PCP on the current-voltage relationship of the EPC (A) and on the relationship between the time constant of EPC decay and membrane potential (B)*

Each point represents the mean values from 8–14 end plates from 3–5 muscles. ●, Control; *m*-nitro-PCP: ○, 5 μM; △, 30 μM; □, 50 μM. The *inset* in A shows the concentration-response curves indicating the voltage-dependent action of *m*-nitro-PCP on peak EPC amplitude as a percentage of control at −150 (■), −40 (▲), and +50 mV (●). The *inset* in B shows the relationship between ln k_3, the second-order rate constant for drug binding obtained from the slope of the relationship $1/\tau$ versus the concentration of drug, and membrane potential (see text for further details). The standard errors were too small to be shown.

The marked voltage-dependent action of *m*-nitro-PCP on the peak EPC amplitude is demonstrated by the curve in Fig. 9A (*inset*), where EPC amplitudes at −150, −40, and +50 mV are plotted against the concentration of *m*-nitro-PCP (5, 30, and 50 μM). The curves show that the agent is more effective at −150 than at −40 or +50 mV, which suggests that it reacts with a site which affects the voltage sensitivity of the EPC. Thus, at 30 μM, *m*-nitro-PCP reduced the EPC amplitude to 50% at −150 mV but to only 62 and 84% of control at −40 and +50 mV, respectively. In contrast to PCP (4), *m*-nitro-PCP did not produce a time-dependent depression of EPC amplitude. The onset of nonlinearity in the current-voltage relationship was shifted to more depolarized membrane potentials with increasing concentrations of *m*-nitro-PCP. However, this onset of nonlinearity was less marked than the one produced by PCP. In the presence of 5 μM *m*-nitro-PCP, the nonlinearity began at −100 mV, whereas at concentrations of 50 μM the nonlinear segment began at −60 mV. Similarly, another PCP derivative, *m*-amino-PCP (30 μM) significantly decreased the peak amplitude of the EPC and induced a small curvature of the current-voltage relationship at negative membrane potentials (Fig. 10). In fact, at −90 mV the peak amplitude of the EPC was decreased to 26% of control. *m*-Amino-PCP (30 μM) had only a small voltage-dependent effect on the current-voltage relationship, but affected τ_{EPC} in a rather similar manner. Like *m*-nitro-PCP, the minimal concentration of *m*-amino-PCP which altered EPC amplitude was 5 μM.

Concentrations of PCC between 10 and 70 μM depressed the peak EPC amplitude (Fig. 11A). At −90 mV, the peak amplitude of the EPC was decreased to 50 and 15% of control at concentrations of 30 and 70 μM, respectively. None of these compounds altered the equilibrium potential for the EPC.

Effects on τ_{EPC}. The relationship between τ_{EPC} and membrane potential was examined under control conditions and in the presence of *m*-nitro-PCP, *m*-amino-PCP, and PCC and compared with that of PCP. The time constant, τ_{EPC}, which characterizes the exponential decay of the EPC in normal muscle, varies with the membrane potential according to the relationship:

$$\tau = \tau_{(0)} \exp^{V/H}$$

where H is a constant which indicates the voltage sensitivity of the decay phase (13–15). In other words, τ_{EPC} becomes progressively longer as the membrane potential is made more negative (Fig. 9B). Figure 9B shows τ_{EPC} determined from a series of end plates plotted semilogarithmically against membrane potential before and after treatment of frog sartorius muscle with three concentrations of *m*-nitro-PCP. The minimal effective concentration for altering τ_{EPC} was 5 μM. Little alteration occurred between +60 and −60 mV, but there was greater shortening of τ_{EPC} at potentials more negative than −100 mV (Fig. 9B). Higher concentrations (30–50 μM) caused a marked acceleration of the decay phase of the EPC at hyperpolarized potentials such that τ_{EPC} at −150 mV was reduced to 26% and 16% of control, respectively, by 30 and 50 μM *m*-nitro-PCP. At concentrations of 30 μM or higher, τ_{EPC} was virtually independent of the membrane potential (Fig. 9B). The alteration in the voltage sensitivity of τ_{EPC} is demonstrated by the change in the slopes of the relationship between log of τ_{EPC} versus membrane potential (Fig. 9B). The effects of *m*-amino-PCP on τ_{EPC} were nearly identical with those of *m*-nitro-PCP. Thus, at −150 mV τ_{EPC} in the presence of 30 μM *m*-amino-PCP

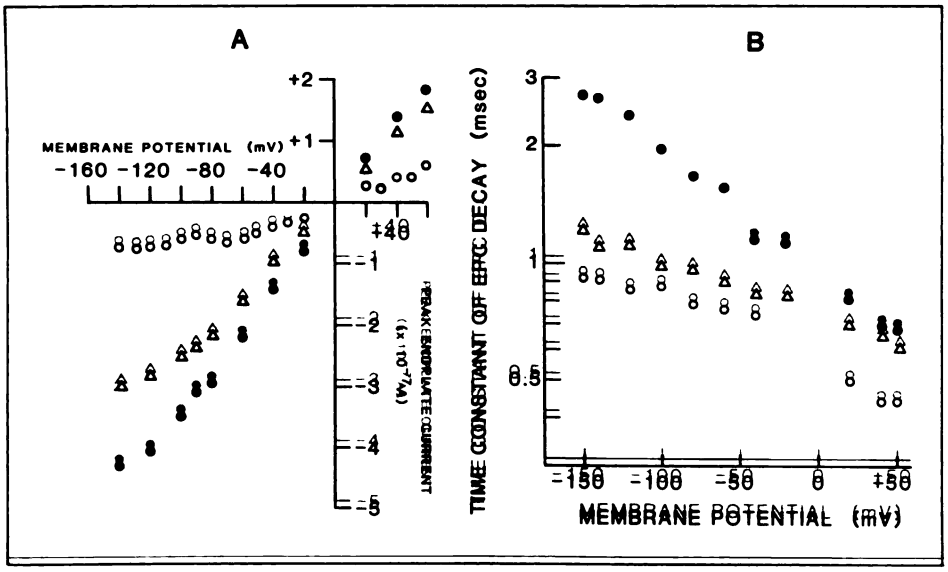

FIG. 10. *Effect of m-amino-PCP on the current-voltage relationships of the EPC (A) and on the relationship between the time constant of EPC decay and membrane potential (B)*

Each symbol represents the mean of results with 7–14 fibers obtained from 4 or more muscles. ●, Control; *m*-amino-PCP: △, 10 μM; ○, 30 μM.

decreased significantly to 34% of control ($p \leq 0.001$) (Fig. 10B).

However, PCC did not cause any significant alteration of τ_{EPC} recorded at membrane potentials between +50 and −150 mV at all of the concentrations studied (Fig. 11B). Indeed, this relationship remained linear with slopes of −0.0067 msec/mV under control conditions and −0.0062, −0.0057, and −0.0078 msec/mV at 30, 50, and 70 μM PCC, respectively (Fig. 11B).

The voltage-dependent effect of *m*-nitro-PCP is consistent with an action of the drug on the ionic channel. Assuming that the probability for a single-charged cationic species to bind at a site within the channel is determined by a Boltzmann equation and that the activated conducting species of the channel, AR^*, can decay to either of two nonconducting states by a mechanism similar to that described by the sequential model (16),

$$AR \rightleftharpoons AR^* \overset{b}{\rightleftharpoons} AR^*D$$

then τ_{EPC} (17) is described by the expression:

$$\tau \equiv (a + [D]k_3)^{-1}$$

Here k_3, the second-order rate constant for drug binding, is the slope of the plot of the inverse τ_{EPC} versus concentration of the drug. The fraction of the membrane potential sensed by externally applied *m*-nitro-PCP can affect the rate constant as follows (17, 18):

$$k_3 \equiv K_0 \exp \frac{(-ne\delta V)}{kT}$$

where K_0 is the rate constant at 0 mV, ne is the charge of

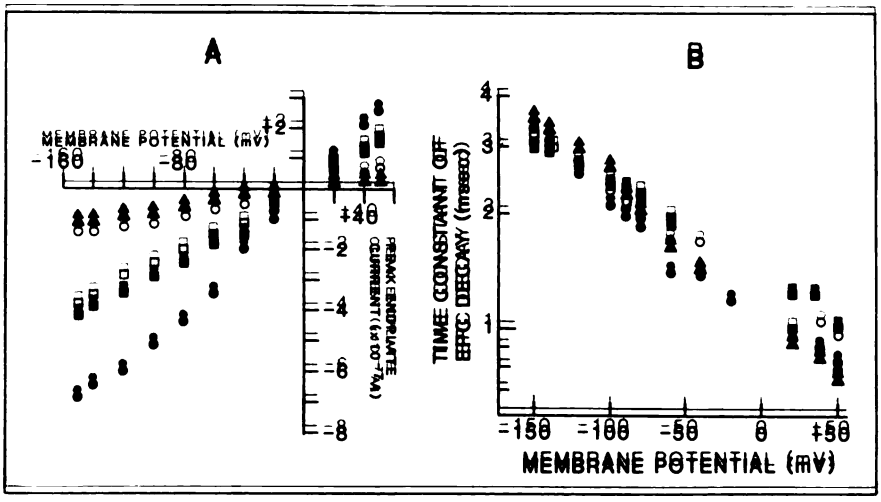

FIG. 11. *Effect of PCC on the current-voltage relationships of the EPC and on the relationship between the time constant of EPC decay and membrane potential (B)*

Each symbol represents the mean of results with 6–13 fibers from 4 or more muscles. ●, Control; PCC: ■, 10 μM; □, 30 μM; ○, 50 μM; ▲, 70 μM.

the agent, V is membrane potential, k is the Boltzmann constant, and T is absolute temperature. The value of δ can be obtained from the slope of a plot of ln k_3 versus membrane potential. This analysis showed that *m*-nitro-PCP senses 9% of the membrane potential at its limiting energy barrier (binding site) (Fig. 9).

Effects on behavior. The effects of the PCP analogues on the spatial alternation performance (7, 11) are illustrated in Table 2. As shown earlier (7, 11), the effect of PCP on the error ratio is monotonic, i.e., the ratio increases with dose. Aside from ketamine and the PCP derivatives, the only other agents which to date have been found to mimic the effect of PCP on the error ratio are the classical antimuscarinic anticholinergics scopolamine and atropine, as well as quinuclidinyl benzilate. Other drugs were tested and shown to be without effect on the error ratio. These include (7) haloperidol, chlorpromazine, (+)-amphetamine, apomorphine, lysergic acid diethylamide, morphine, naloxone, mecamylamine, nicotine, pilocarpine, oxotremorine, physostigmine, and arecoline. Methscopolamine, a peripheral antimuscarinic agent which does not distribute significantly into the brain, has no effect.

TABLE 2

Effects of drugs on spatial alternation performance

All drugs were administered i.p. 15 min prior to testing. Drug treatments were spaced 3–4 days apart. Saline (control) was repeated at 2–3-week intervals. N = five to six per drug dose. All standard deviations were within 15% of the means.

Drug	Dose	Mean response/reward
	mg/kg	
Saline	—	1.15
PCP	0.25	1.18
	0.5	1.20[a]
	1.0	1.27[a]
	2.5	1.79[a]
	5.0	2.87[a]
m-Nitro-PCP	1.0	1.14
	2.5	1.15
	10.0	1.15
	20.0	1.25[a]
m-Amino-PCP	1.0	1.24[a]
	1.75	1.97[a]
	2.50	3.14[a]
PCC	5.0	1.16
	10.0	1.16
	20.0	1.18
Ketamine	5.0	1.26[a]
	10.0	1.64[a]
	20.0	2.81[a]
Scopolamine	0.25	1.23[a]
	0.5	1.81[a]
	1.0	2.04[a]
	10.0	2.07[a]
Atropine	10.0	1.46[a]
	20.0	2.01[a]
Quinuclidinyl benzilate	1.0	1.45[a]
	5.0	1.98[a]

[a] Significantly different from closest saline data ($p \leq 0.05$ by paired Student's t-test).

DISCUSSION

PCP and *m*-amino-PCP potentiated the directly elicited muscle twitch, prolonged the action potential, and blocked delayed rectification and the ionic channel of the ACh receptor as revealed by changes in peak EPC amplitude and τ_{EPC}. In contrast, *m*-nitro-PCP potentiated the muscle twitch only to a small extent and prolonged the action potential only slightly. PCC did not potentiate the muscle twitch and had an even smaller effect on the half-decay time of the action potential than *m*-nitro-PCP. Moreover, delayed rectification was not affected by PCC, and *m*-nitro-PCP caused only a partial blockade at concentrations 4–5 times higher than that required of *m*-amino-PCP. Both PCC and *m*-nitro-PCP depressed peak EPC amplitude, but only *m*-nitro-PCP shortened τ_{EPC}. In the behavioral tests only PCP and *m*-amino-PCP caused marked changes in alteration performance, whereas *m*-nitro-PCP and PCC did not cause significant impairment of this performance at relevant doses (see Table 2).

Earlier reports have suggested that doses of PCP which stimulate the central nervous system of rats also produce extensive damage to the skeletal muscle fibers in the limbs of these animals when they are placed in restraining cages (19). This finding was previously attributed to large, intermittent increases in muscle tension developed under those conditions (20). In view of the present findings, it could well be explained by the marked alteration of the electrical properties of the muscle membrane elicited by PCP and its behaviorally active analogues. The effects of *m*-amino-PCP on potassium conductance were reflected in the marked potentiation of the direct muscle twitch which results from a significant prolongation of the muscle action potential (Table 1; Fig. 6) and blockade of delayed rectification (Figs. 7 and 8). These effects are similar to that produced by histrionicotoxin (21). Unlike the active *m*-amino-PCP, the behaviorally inactive *m*-nitro-PCP and PCC had a weaker action on the falling phase of the action potential; *m*-nitro-PCP but not PCC blocked delayed rectification to a small extent at the highest concentration examined. It is significant to the hypothesis that behavioral effects of PCP are related to action potential prolongation by potassium channel blockade that ketamine, a congener of PCP which is much less active as a psychotomimetic (Table 2), manifests no apparent effect on the potassium channel of electrically excitable membranes. Yet ketamine also prolongs the action potential in muscle, although apparently by an alternative mechanism, i.e., by blocking delayed sodium inactivation (22). Ketamine is only about 20% as active as PCP and 10% as active as *m*-amino-PCP in behavioral tests (Table 2). The low psychotomimetic activity of atropine (about 10 times the dose of PCP and *m*-amino-PCP was required) revealed in spatial alternation tasks also fits with the hypothesis that such activity is due to an effect of the drug on potassium conductance, for atropine was previously shown to prolong the action potential in skeletal muscle by blocking potassium conductance (23) at concentrations at least 3 log-dose units greater than those affecting binding of drugs to muscarinic receptors. This would explain the difference be-

tween the rank order of potency for muscarinic blockade and for PCP-like behavioral effects.

The close correlation between the ability of PCP and its analogues to block potassium permeability and their effect on behavior is also supported by the dose-response relationship in behavioral tests. Assuming that the volume of distribution of PCP and *m*-amino-PCP in a rat is 60% of body weight, then a behaviorally active dose of 1–5 mg/kg (Table 2) is equivalent to about 30 μM. These values are comparable to the concentration (10 μM) at which both active drugs, i.e., PCP and *m*-amino PCP, block delayed rectification (Fig. 7). On the other hand, the lowest dose of *m*-nitro-PCP needed to exert a behavioral effect was 20 mg/kg, the equivalent of 115 μM, and PCC (20 mg/kg or about 146 μM) had no behavioral effect. This is in excellent agreement with the concentration of *m*-nitro-PCP that did block delayed rectification to a small extent, i.e., 100 μM. As described above, PCC had no effect even at this high concentration.

Several combinations of drugs have also been tested in the behavioral paradigm described here. Positive findings obtained so far include partial antagonism of the effect of PCP on spatial alternation by physostigmine, pilocarpine, oxotremorine, and arecoline (7). Analyzed together, all of these data indicate that impairment of spatial alternation performance by PCP, although characteristic of muscarinic agents, cannot be explained solely by their action as muscarinic receptor blockers. Indeed, the rank order of potency of the drugs to elicit the behavioral effects differs from that established in muscarinic systems. Thus, results in Table 2 show that *m*-amino-PCP is more potent than either *m*-nitro-PCP or PCP itself, and this correlates well with their affinities for sites labeled in brain homogenates by [^{3}H]atropine (K_d = 4.4, 22, and 110 μM for *m*-amino-PCP, PCP and *m*-nitro-PCP, respectively). However, the derivative of PCP in which the piperidine is replaced by a morpholino group is practically inactive in the behavioral test but is a potent muscarinic blocker. Similarly, the rank order of potency for atropine, scopolamine, and quinuclidinyl benzilate, which are nearly equipotent to PCP in the behavioral test, is not commensurate with the very high affinities of these drugs for muscarinic binding sites in brain homogenates, for which these drugs exhibit an affinity that is 3–4 orders of magnitude higher than that of PCP (10).

In contrast to these congruent dose relationships, we found that the effects of the compounds on the ionic channels of the nicotinic ACh receptor start at concentrations of 5–10 μM. However, this action does not appear to be related to the behavioral activity because *m*-nitro-PCP has low behavioral activity and PCC totally lacks an effect on spatial alternation, whereas both drugs affect peak EPC amplitude in a manner similar to PCP and *m*-amino-PCP. It is also noteworthy that the potency ranking in spatial alternation performance paradigm agrees very well with the rank orders of potency in behavioral studies of PCP derivatives by Shannon (24) and Kalir *et al.* (25) in which different paradigms were used.

Further support for our hypothesis that the decrease in potassium conductance is involved in eliciting the behavioral effect of PCP and *m*-amino-PCP is also provided by their effect on presynaptic membranes (26). Thus, PCP and *m*-amino-PCP significantly increased the quantal release of ACh from the nerve terminal in frog sartorius muscles at concentrations as low as 0.1 μM, but *m*-nitro-PCP (0.8 μM) and PCC (1.6 μM) were ineffective. In addition, PCP and *m*-amino-PCP significantly decreased potassium-stimulated ^{86}Rb efflux from brain synaptosomes; *m*-nitro-PCP and PCC were practically inactive (26). This finding further supports the idea that the discriminant action of the behaviorally active agents is potassium conductance.

The details of the central mechanism involved in PCP action have yet to be elucidated, but it appears clear that PCP-induced changes in electrical excitability of membranes have to be directly considered. Most studies of the effects of PCP on neurotransmitter levels and receptors until now have indicated only that serotonin, dopamine, norepinephrine and epinephrine, ACh, and γ-aminobutyric acid may somehow be involved in the mediation of the behavioral effects produced by PCP. It is important to stress that the changes produced by PCP in the electrical excitability of membranes apparently do modify significantly the release of neurotransmitters as well as their postsynaptic effects. This could explain why previous studies of the direct effects of PCP on neurotransmitter levels and receptors *in vitro* have failed to produce good correlations with behavioral activity (27).

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