Phencyclidine (PCP) blocks glutamate-activated postsynaptic currents

M. Idriss and E.X. Albuquerque*

Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, 660 W. Redwood Street, Baltimore, MD 21201, USA

Received 9 July 1985

Phencyclidine (PCP) was tested on the metathoracic tibialis muscles of *Locusta migratoria*. In physiological solution, the peak amplitude of the excitatory postsynaptic currents (EPSCs) evoked by nerve stimulation was linearly related to membrane potential between −50 and −150 mV. The decay time constant of the EPSC (τ_{EPSC}) was exponentially dependent on voltage and decreased with hyperpolarization. The membrane potential change required to produce an e-fold change in τ_{EPSC} was 315 mV. PCP (5–40 μM) produced a concentration-dependent depression of both EPSC peak amplitude and τ_{EPSC}. A slight nonlinearity in the current-voltage relationship could be discerned at high concentrations of PCP. The shortening of the decay time constant of EPSC (τ_{EPSC}) occurred without significant change in the voltage sensitivity observed under control conditions. Under all experimental conditions, the decay of the EPSCs remained a single exponential of time. Fluctuation analysis indicated that 5μM PCP shortens the lifetime of the glutamate-activated channels by 25.7 ± 3%. PCP (10–80 μM) did not induce desensitization of the glutamate receptors. These results suggest that PCP interacts with the open conformation of ion channels activated by the glutamate receptor.

Phencyclidine *Glutamate receptor* *Locust* *Neuromuscular transmission* *Excitatory postsynaptic current* *Channel blocker*

1. INTRODUCTION

The general anesthetic and hallucinogenic agent 'Angel Dust' [1-(1-phenylcyclohexyl)piperidine, phencyclidine or PCP] is a drug of abuse when used chronically or in large doses. In addition, PCP has schizophrenomimetic and convulsant properties, and triggers unpredictable, violent behavior [1,2]. A large number of studies have been devoted to PCP, and it has been demonstrated that this agent is able to affect the release of many neurotransmitters such as 5-hydroxytryptamine [3], dopamine [4], norepinephrine [5], and acetylcholine (ACh) [6]. Several studies have clearly described PCP as an anticholinergic agent [7,8]. In our laboratory, we have demonstrated that the noncompetitive action of PCP on the nicotinic ACh receptor-ion channel complex increases the affinity of the transmitter to its binding site thereby inducing activation and subsequent desensitization [6,8]. As a non-competitive antagonist of the ACh receptor, PCP was also able to produce blockade of its associated ion channel in closed [6,8] and open conformations [6]. In insects, where ACh serves as a sensory and central nervous system transmitter [9], it is well established that L-glutamate is a potent excitatory neuromuscular transmitter [10]. The peripheral location of glutamate receptors in insect muscles enabled us to perform more quantitative electrophysiological investigations. Although isolation and eventual cloning of such a receptor are not yet possible, a few drugs have been found to act on the glutamate receptor such as chlorisondamine [11], anticholinesterases [12], diltiazem [13], curare [14,15], gallamine [15], and strep-

* To whom correspondence should be addressed

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

tomycin [16]. However, studies of the glutamate receptor suffer from the relative lack of potent antagonists. This study demonstrates the effects of PCP on locust neuromuscular junctions and identifies a possible target of this agent on the glutamate receptor.

2. MATERIALS AND METHODS

Flexor and extensor metathoracic tibialis muscles of adult *Locusta migratoria* were dissected according to Hoyle [17]. The physiological solution had the following composition (mM): NaCl, 170; KCl, 10; NaH_2PO_4, 4; Na_2HPO_4, 6; $CaCl_2$, 0.8. To decrease the muscle twitch during excitatory postsynaptic current (EPSC) experiments, muscles were pretreated with glycerol (150 mM) and $MgCl_2$ (10 mM). All the preparations were pretreated with 1 μM concanavalin A for 30 min to minimize receptor desensitization [18] except in the case of experiments designed to study receptor desensitization.

The voltage clamp circuit was similar to that in [19] with some modifications [20]. Recording and current-passing microelectrodes were filled with 3 M KCl and had resistances of 3–5 MΩ. EPSCs were evoked by stimulating the crural nerve (nerve-5), and EPSC waveforms were digitized at 3.3 kHz by a PDP 11/40 computer. The decay phase (20–80%) of the EPSC was fitted by a single exponential function (linear regression on the logarithms of the data points) from which the EPSC decay time constant was determined. EPSC fluctuations induced by bath application of monosodium L-glutamate (50 μM) and miniature EPSCs (MEPSCs) were recorded on magnetic tape by a Racal Store 4D FM tape recorder for later computer analysis. Power density spectra from L-glutamate-induced EPSC fluctuations were obtained from high-gain AC signals after filtering with a Krohn-Hite 3700 bandpass filter (1–800 Hz). Data were sampled at 2 kHz, and spectra were obtained by subtraction of the averaged baseline spectra from those during application of L-glutamate. The resulting power spectra were fitted to a single Lorentzian function using a nonlinear regression program. MEPSCs were captured by a digital oscilloscope (Gould OS4000) before being sent to the computer for analysis.

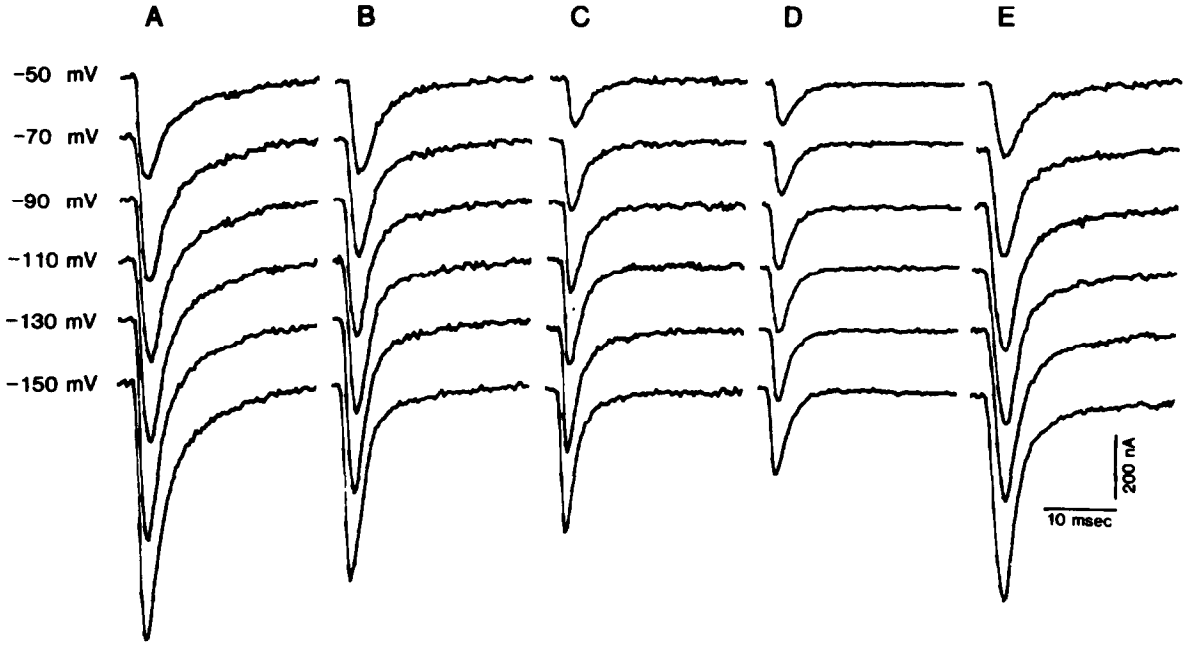


Fig.1. Effects of PCP on EPSCs. Series of EPSCs were recorded from locust flexor metathoracic tibialis muscle, at different membrane potentials, under control conditions (A), in the presence of 5 (B), 10 (C) and 40 (D) μM PCP, and after 60 min wash (E).

3. RESULTS

3.1. *Effects of PCP on the peak amplitude and rise time of nerve-evoked excitatory postsynaptic currents*

EPSCs were recorded from surface fibers of glycerol-treated locust flexor metathoracic tibialis muscles under control conditions and in the presence of PCP (fig.1). In physiological solution, the peak amplitude was linearly related to membrane potential between +50 and −140 mV (fig.2). Superfusion of PCP (5–40 μM) for 30 min produced a concentration-dependent depression of the peak amplitude of the EPSC. The dependence of the EPSC amplitude on voltage showed a slight nonlinearity at hyperpolarized potentials. Indeed, at 40 μM PCP, a small upward curvature in the current-voltage relationship could be discerned (fig.2). These effects of PCP were reversed after washing the preparation for 60 min, the EPSC amplitude and the characteristics of the *I/V* relationship being recovered to those observed under control conditions.

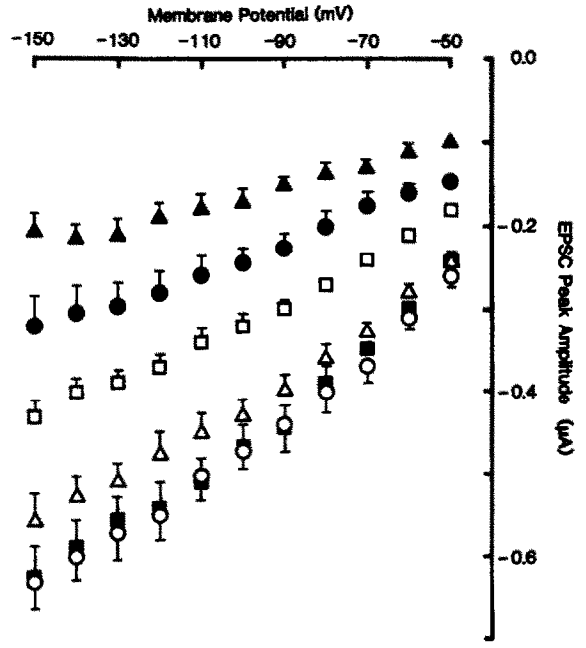


Fig.2. Current-voltage relationship of EPSCs recorded under control conditions (○), in the presence of 5 (△), 10 (□), 20 (●), and 40 (▲) μM PCP and after 60 min wash (■). Each point represents the mean ± SE of 9–21 records from 5–11 different fibers.

In addition, PCP did not show a time-dependent effect on the EPSC amplitude in glutamate receptors, i.e., there was no 'hysteresis loop' in the *I/V* relationship (fig.2). Further, there was no desensitizing effect on glutamatergic transmission in the presence of PCP (10–80 μM), since stimulation of the crural nerve at frequencies of 10–100 Hz did not cause any significant decrement in peak EPSC amplitude during the train of evoked currents (fig.3). These findings indicate some dissimilarities between the effects of PCP on the glutamate-mediated neuromuscular transmission and those observed on the nicotinic synapses. As reported in [6], on the end-plate currents elicited on the frog neuromuscular junction, PCP produces a marked voltage- and time-dependent depression of the peak amplitude with a clear upward curvature at hyperpolarized potentials and hysteresis loop in the *I/V* relationship.

The alterations of the EPSC induced by PCP also involved a shortening of the rise time, at concentrations $\geqslant 10\,\mu$M. For example, the EPSCs recorded at −80 mV after 30 min exposure to PCP (20 μM) showed a rise time shortened from 2.0 ± 0.09 (n = 18) to 1.5 ± 0.08 ms (n = 12).

3.2. *The action of PCP on decay of the EPSCs*

In physiological solution, τ_{EPSC} was exponentially dependent on voltage and decreased with hyperpolarization. An e-fold change in τ_{EPSC} was produced by about 315 mV change in membrane potential. The effect of PCP (5–50 μM) on the decay of EPSCs recorded at the postjunctional region of the glutamatergic synapse was analyzed, and the concentration-dependent effect on τ_{EPSC} at membrane potentials varying from −50 to −150 mV is illustrated in fig.4. At all the concentrations of PCP tested, the decay of the EPSCs remained a single exponential function of time (fig.4). According to the scheme presented in section 4, the adequate fit of EPSC decay to a single exponential function of time indicates that drug dissociation governed by the rate constant k_{-3} was too slow to contribute to the EPSC. Therefore, in the presence of PCP, the observed rate constant of the EPSC decay ($1/\tau_{EPSC}$) is the sum of two rate constants, k_{-2} for EPSC decay in the absence of drug and k_3 for PCP binding, and is linearly related to PCP concentration, according to the following expression: $1/\tau_{EPSC} = k_{-2} + [D]k_3$. Fig.5

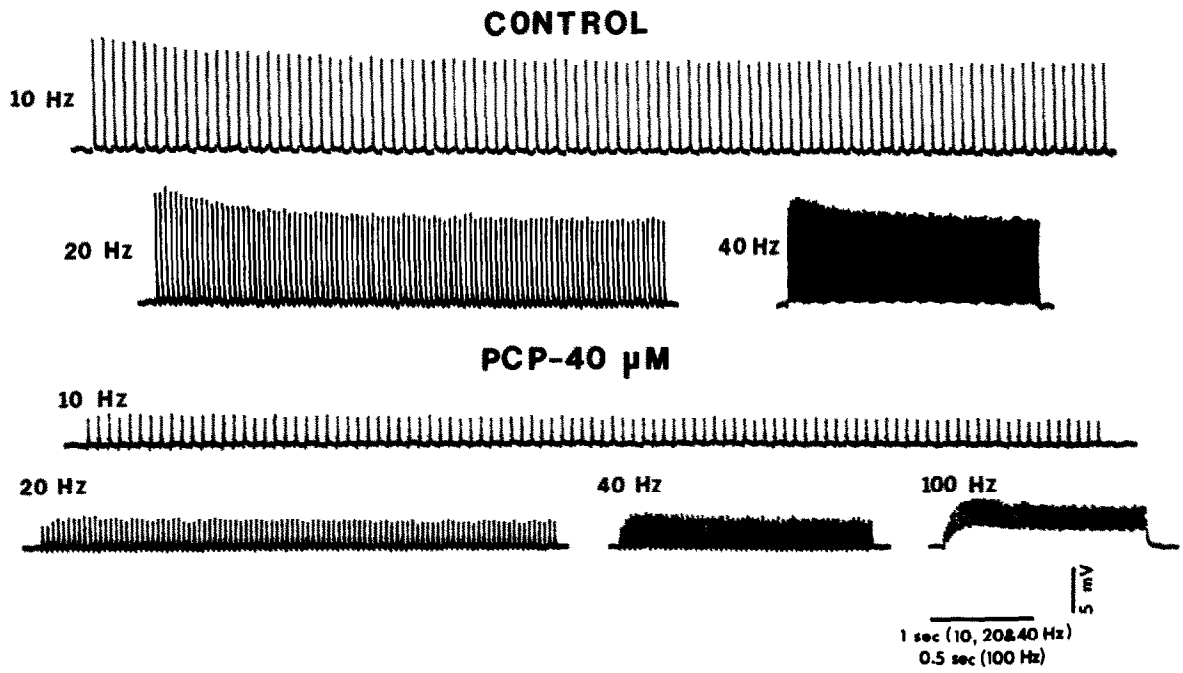
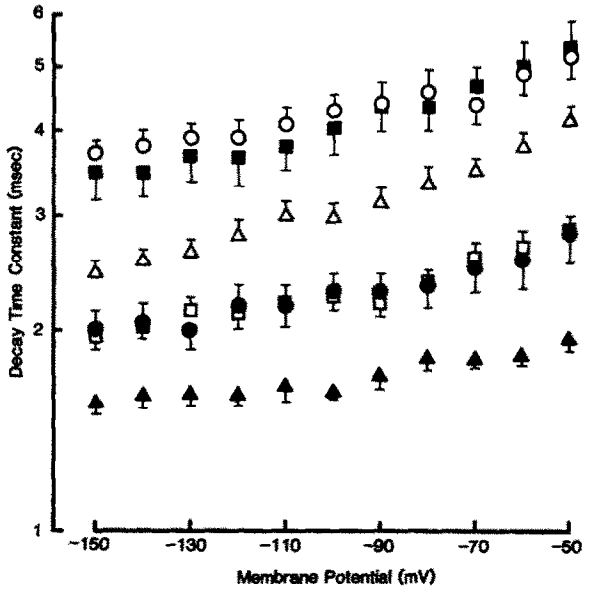


Fig.3. Effect of repetitive stimulation of the crural nerve on the peak amplitude of excitatory postsynaptic potentials recorded from flexor muscle of locust under control conditions and in the presence of 40 μM PCP. The membrane potential was -30 ± 2 mV.

illustrates the linearity of the relationship between the reciprocal of τ_{EPSC} and PCP concentrations. Additionally, the similar voltage dependence of



τ_{EPSC} observed in the absence and in the presence of all concentrations of PCP tested, suggested that voltage has little effect on the rate constant, k_3, of PCP binding. All these effects of PCP were reversible since 60 min washing resulted in recovery of the characteristics of τ_{EPSC} seen under control conditions.

3.3. *Effect of PCP on the peak amplitude and decay time constant of the miniature excitatory postsynaptic currents (MEPSCs)*

Fig.6 shows typical MEPSCs recorded under control conditions and 30 min after exposure of locust muscle to 10 μM PCP. In physiological solution, MEPSC peak amplitude and time cons-

Fig.4. Voltage- and concentration-dependent effect of PCP on the decay time constant of the EPSC. EPSCs were recorded under control conditions (○), in the presence of 5 (△), 10 (□), 20 (●), and 40 (▲) μM PCP and after 60 min wash (■). Each plotted point represents the mean ± SE of 9–21 records from 5–11 different fibers.

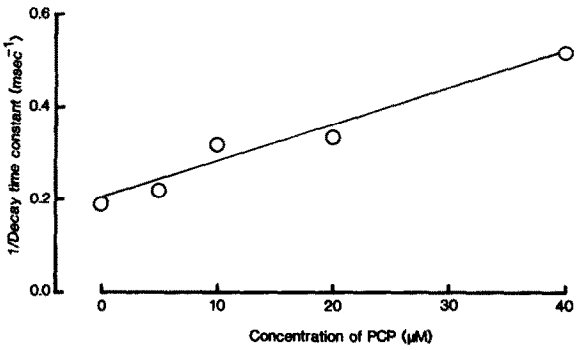


Fig.5. Relationship between the reciprocal of EPSC decay time constant and PCP concentration. The membrane potential was −50 mV.

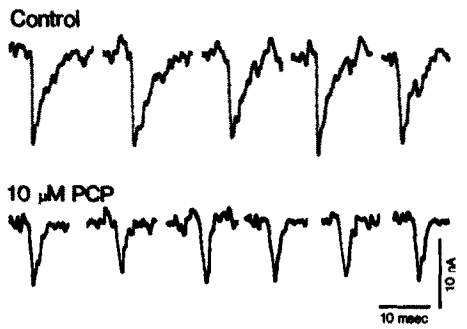


Fig.6. Typical MEPSCs recorded from extensor muscle of locust under control conditions and in the presence of 10 μM PCP. All the MEPSCs were recorded from the same fiber clamped at −45 mV.

tant of decay (τ_{MEPSC}) were voltage sensitive in a manner similar to that of EPSCs. The analysis of MEPSCs recorded (from the same fiber clamped at −50 mV) under control conditions (n = 47) and after 30 min exposure to 10 μM PCP (n = 43) showed a decrease in peak amplitude from 11.3 ± 2.0 to 6.9 ± 1.3 nA and a shortening of τ_{MEPSC} from 2.2 ± 0.7 to 0.9 ± 0.5 ms.

3.4. *The action of PCP on glutamate-induced EPSC fluctuations*

The effect of PCP (5 μM) on single channel

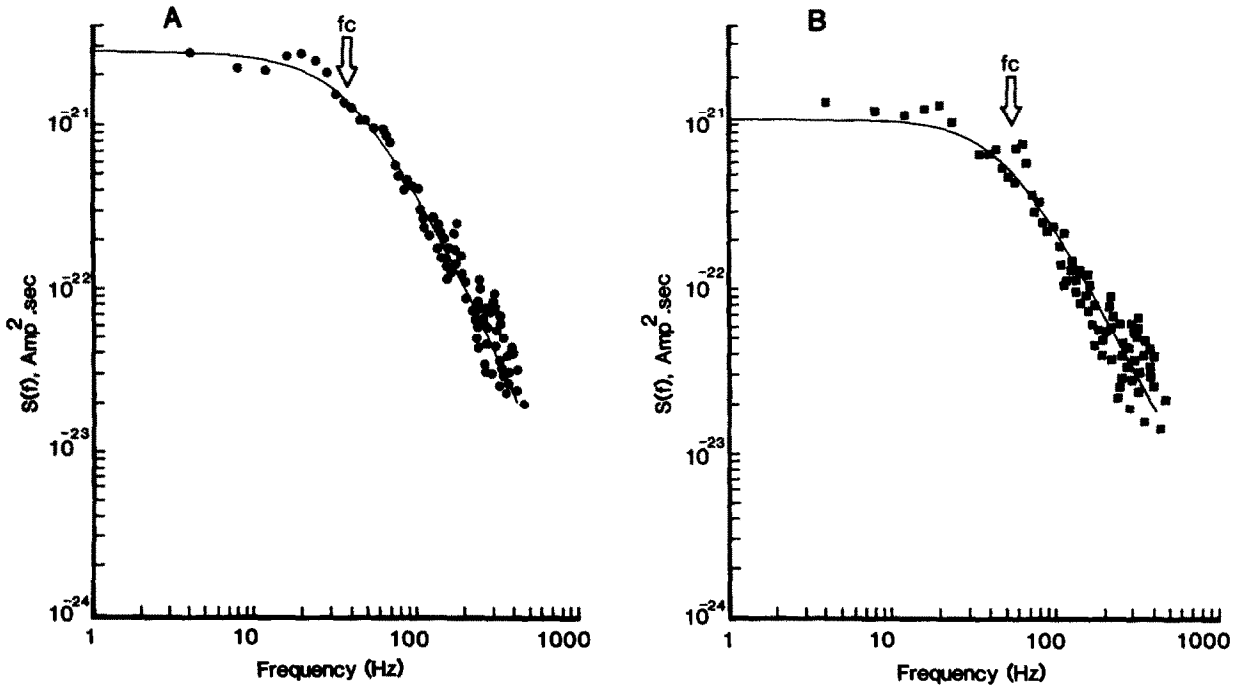


Fig.7. Effect of PCP on glutamate-induced EPSC fluctuations in locust muscle. Power density spectra were produced by bath application of 50 μM monosodium L-glutamate before (A) and after 30 min superfusion of 5 μM PCP (B). Spectral analysis provided a τ_1 of 4.13 ms under control conditions, and 3.03 ms in the presence of 5 μM PCP. These spectra were obtained from a same fiber clamped at −50 mV.

lifetime was determined from fluctuation analysis on voltage-clamped surface fibers of the extensor metathoracic tibialis muscles of the locust. Previous studies have shown that the channel lifetime of the glutamate receptor is dependent upon the glutamate concentrations [21]. Under such conditions, we used a single concentration of glutamate applied to the bath to generate control values for channel lifetime and subsequently exposed the preparation to a combination of glutamate and PCP (5 μM). Power density spectra obtained from the same fiber clamped at -50 mV showed that PCP decreased the channel lifetime from 4.1 ms in control condition to 3.0 ms (fig.7). All the experiments were done at room temperature.

4. DISCUSSION

Several electrophysiological and biochemical studies have demonstrated the interaction of PCP with the ionic channels associated with nicotinic receptors at the neuromuscular junction and in *Torpedo* or *Electrophorus* electric organ [6,8]. Thus, PCP and perhydrohistrionicotoxin (H_{12}-HTX) in their tritiated forms have been used extensively as important tools for identification of mechanisms underlying noncompetitive antagonisms at the nicotinic synapses and the active sites on the ionic channel [22]. The present study reveals that PCP blocks the glutamate-mediated neuromuscular transmission of the locusts. PCP at concentrations similar to those which block the neuromuscular transmission of the nicotinic synapses produces a depression of the peak amplitude and marked shortening of the decay time constants of EPSC and MEPSC recorded at the junctional region of the locust glutamatergic synapse. As revealed by the analysis of the glutamate-induced EPSC fluctuations, PCP decreased channel lifetime and did not change channel conductance. These findings suggest that the hallucinogenic PCP can block the glutamate receptor by interfering with mechanisms involved in the control of ionic permeability leading to an action which resembles that of an ionic channel blocker. Therefore, most of the effects of PCP on the glutamate receptor appear to fit the sequential model [23] for open channel blockade, with a kinetic process similar to that used for the nicotinic receptor [22,24]:

$$n\mathrm{Glu} + \mathrm{R_r} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{Glu}_n\mathrm{R_c} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}}$$

$$\mathrm{Glu}_n\mathrm{R_o} \underset{\mathrm{D}}{\overset{\mathrm{D}\quad k_3}{\rightleftharpoons}} \mathrm{Glu}_n\mathrm{R_bD}$$

where nGlu represents n agonist (glutamate) molecules, R_r is the resting state of the glutamergic channel complex, Glu_nR_c is the agonist-bound (closed) state, and Glu_nR_o the state of the channel in open conformation. Glu_nR_bD is the nonconducting state in the presence of the drug D. The rate constants are as indicated.

The following experimental observations are consistent with this model: a shortening of the mean channel open time with no change of the conductance of the elementary events (fig.7); an acceleration of the EPSC decays (figs 1 and 4), since τ_{EPSC} is a reflection of the lifetime of the open ion channels; a single exponential decay which suggested that the rate constant for PCP unbinding (k_{-3}) is negligible; and linear plots of $1/\tau_{EPSC}$ vs PCP concentration (fig.5) with slope k_3 and intercept k_{-2}. A comparison, however, with its actions on the postsynaptic nicotinic receptor-ion channel complex [6] shows that PCP produces a marked voltage-dependent acceleration of the decay of endplate currents due to an opposing voltage dependence of these two rate constants, k_{-2} and k_3. The fact that the voltage sensitivity of τ_{EPSC}, observed under control conditions, remained unchanged in the presence of PCP suggested little influence of voltage on k_3, thus suggesting a very external location of PCP binding site on the postsynaptic glutamate receptor at the locust neuromuscular junction.

The present results suggested that in contrast to PCP's actions on the ACh-mediated nicotinic synapses where this agent interacts with both open and closed conformations of the nicotinic receptor, on the glutamate receptor this agent acts as a noncompetitive antagonist, primarily blocking the open ionic channels. Therefore PCP may be a useful tool for the biochemical characterization and possible isolation of the glutamate receptor of the locust. If, to some extent, the glutamate recep-

tors involved in the neuromuscular transmission of the locust are similar to those located in central nervous system of different species (e.g. vertebrate brain), one could assume the implication of glutamergic synapses in the clinical actions and symptomatology induced by PCP.

In summary, our studies show a noncompetitive blockade of the glutamate receptor produced by PCP through an interaction with the open conformation of the ion channels activated by this receptor. In these synapses PCP apparently does not interact with the receptor recognition sites. Thus, these findings provide evidence of PCP actions at the glutamate-mediated neuromuscular junction of the locust and reveal some similarities between the ion channels of the nicotinic and glutamergic synapses.

ACKNOWLEDGEMENTS

We are most grateful to Dr G.R. Wyatt for his generous provision of the locusts. We would also like to thank Ms Mabel A. Zelle for computer programming and assistance in the analysis of the data. We are indebted to Dr Y. Aracava for her most helpful criticism of this manuscript. This study was supported by NIDA grant no.DA02804.

REFERENCES

[1] Johnson, K.M. (1971) in: PCP Abuse: An Appraisal (Petersen, R. and Stillman, C. eds) pp.42–52, NIDA Res. Monogr. no.21, National Institute of Drug Abuse, Rockville, MD.
[2] Snyder, S.H. (1980) Nature 285, 355–356.
[3] Vincent, J.P., Vignon, J., Kastalovski, B. and Lazdunski, M. (1981) in: PCP (Phencyclidine): Historical and Current Perspectives (Domino, E.F. ed.) pp.83–103, NPP Books.
[4] Garey, R.E. and Heath, R.G. (1976) Life Sci. 18, 1105–1110.
[5] Marwaha, J. (1982) Biol. Psychiatr. 17, 155–198.
[6] Albuquerque, E.X., Tsai, M.-C., Aronstam, R.S., Eldefrawi, A.T. and Eldefrawi, M.E. (1980) Mol. Pharmacol. 18, 167–178.
[7] Albuquerque, E.X., Aguayo, L.G., Warnick, J.E., Weinstein, H., Glick, S.D., Maayani, S., Ickowicz, R.K. and Blaustein, M.P. (1981) Proc. Natl. Acad. Sci. USA 78, 7792–7796.
[8] Albuquerque, E.X., Warnick, J.E., Aguayo, L.G., Ickowicz, R.K., Blaustein, M.P., Maayani, S. and Weinstein, H. (1983) in: Phencyclidine and Related Arylcyclohexylamines: Present and Future Applications (Kamenka, J.M. et al. eds) pp.579–594, NPP Books.
[9] Corteggiani, E. and Serfaty, A. (1939) CR Soc. Biol. (Paris) 131, 1124–1126.
[10] Usherwood, P.N.R. and Grundfest, H. (1965) J. Neurophysiol. 28, 497–518.
[11] Idriss, M.H., Filbin, M.T., Eldefrawi, A.T., Eldefrawi, M.E. and Albuquerque, E.X. (1984) Fed. Proc. 43, 342.
[12] Idriss, M. and Albuquerque, E.X. (1985) Biophys. J. 47, 259a.
[13] Ishida, M. and Shinozaki, H. (1980) J. Physiol. 298, 301–319.
[14] Yamamoto, D. and Washio, H. (1979) Nature 281, 372–373.
[15] Cull-Candy, S.G. and Miledi, R. (1983) Proc. R. Soc. Lond. B 218, 111–118.
[16] Usherwood, P.N.R. (1981) in: Glutamate as a Neurotransmitter (DiChiara, G. and Gessa, G.L. eds) pp.183–193, Raven, New York.
[17] Hoyle, G. (1955) Proc. R. Soc. Lond. B 143, 281–292.
[18] Mathers, D.A. and Usherwood, P.N.R. (1976) Nature 259, 409–411.
[19] Takeuchi, A. and Takeuchi, N. (1959) J. Neurophysiol. 22, 395–411.
[20] Kuba, K., Albuquerque, E.X., Daly, J. and Barnard, E.A. (1974) J. Pharmacol. Exp. Ther. 189, 499–512.
[21] Gration, K.A.F., Lambert, J.J., Ramsey, R.L. and Usherwood, P.N.R. (1981) Nature 291, 423–425.
[22] Spivak, C.E. and Albuquerque, E.X. (1982) in: Progress in Cholinergic Biology: Model Cholinergic Synapses (Hanin, I. and Goldberg, A. eds) pp.323–357, Raven, New York.
[23] Andersen, C.R., Cull-Candy, S.G. and Miledi, R. (1978) J. Physiol. 282, 219–242.
[24] Adler, M., Albuquerque, E.X. and Lebeda, F.J. (1978) Mol. Pharmacol. 14, 514–529.