Pharmacology Biochemistry & Behavior, Vol. 17, pp. 699–702, 1982. Printed in the U.S.A.

Chronic Phencyclidine Treatment Decreases Phencyclidine and Dopamine Receptors in Rat Brain

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Received 2 April 1982

QUIRION, R., M. A. BAYORH, R. L. ZERBE AND C. B. PERT. *Chronic phencyclidine treatment decreases phencyclidine and dopamine receptors in rat brain.* PHARMAC. BIOCHEM. BEHAV. 17(4) 699–702, 1982.—Chronic phencyclidine treatment (10 mg/kg/day, SC for 14 days) significantly decreased the number of [^{3}H]phencyclidine and [^{3}H]spiperone binding sites in rat brain. [^{3}H]Dihydromorphine binding was not affected by the same treatment. An acute treatment with phencyclidine (10 mg/kg, SC) did not modify any of the binding sites under study. These results suggest that a chronic phencyclidine treatment induces a down-regulation of phencyclidine and dopamine receptors without affecting opiate receptors. These reductions in the number of phencyclidine and dopamine binding sites might be related to the development of tolerance and/or dependence to phencyclidine.

Phencyclidine Phencyclidine receptors Dopamine receptors Opiate receptors Chronic treatment Brain slices technique

PHENCYCLIDINE (1-phenylcyclohexyl piperidine hydrochloride; Angel Dust; PCP) is an anesthetic drug which has psychotomimetic properties in man [19] and produces increases in locomotor activity and stereotypic behavior in rats [9], mice [9] and monkeys [3]. These psychotomimetic effects of PCP in man have been described as resembling more closely those seen in schizophrenic psychosis than any of the psychotic states produced by several other hallucinogenic drugs [10]. Recently, the existence of specific PCP receptor in rat brain have been demonstrated [27, 28, 33, 36] and their anatomical distribution correlates well with such "schizophrenomimetic" properties [27]. Since it has been shown that continuous access to PCP may lead to tolerance [7, 8, 12, 22, 25, 26] and physical dependence [4,35] in various species, we decided to investigate the effects of chronic PCP treatment on PCP binding sites. Also, because of the possible interactions between PCP and the dopaminergic system [2, 13–15, 23] and PCP and opiate receptors [32], we studied the effects of a chronic PCP treatment on dopamine and opiate receptors.

METHOD

Male Sprague-Dawley rats weighing 250–310 g (from Zivic-Miller Laboratories, Inc., Allison Park, PA) received a daily subcutaneous injection of 10 mg/kg PCP in saline or saline alone (controls) for 14 consecutive days. Animals were sacrificed on day 15 (allowing 24 hr for drug clearance [20]), and their brains were rapidly immersed in isopentane at −40°C, mounted on cryostat chucks and olfactory bulbs (for PCP binding) and striatum (for dopamine and opiate binding) were cut into 25 μm-thick coronal sections at −14°C. These two areas were chosen because of the homogenous distribution of binding sites in each of them, all the sections are fairly the same size, and it is possible to obtain many sections (50) from one brain. Sections were thaw-mounted on gelatin-coated slides, air-dried on ice for 2 hr, and then stored at −14°C for at least 48 hr before use. Binding experiments were performed as previously described [5,27]. Briefly, for PCP, frozen olfactory bulb slide-mounted sections were preincubated for 15 min in 5.0 mM Tris-HCl, 50 mM sucrose, 20 mM NaCl, pH 7.4, at 4°C and then transferred in the same buffer without NaCl for 45 min, pH 7.4 at 4°C with various concentrations of [^{3}H]PCP (48 Ci/mmol; New England Nuclear). For the opiate binding study, striatum slide-mounted sections were incubated for 30 min in 50 mM Tris-HCl, 3 mM Mn $(OAc)_2$, pH 7.4 at 25°C with various concentrations of [^{3}H]dihydromorphine (73 Ci/mmol; Amersham, DHM). For the dopamine binding study, striatum slide-mounted sections were incubated for 30 min in 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 3 mM Mn $(OAc)_2$, 0.1% ascorbic acid, 50 nM ketanserin with pH 7.4 at 25°C with various concentrations of [^{3}H]spiperone (27.6 Ci/mmol; New England Nuclear). At the end of the incubation period, the slides were

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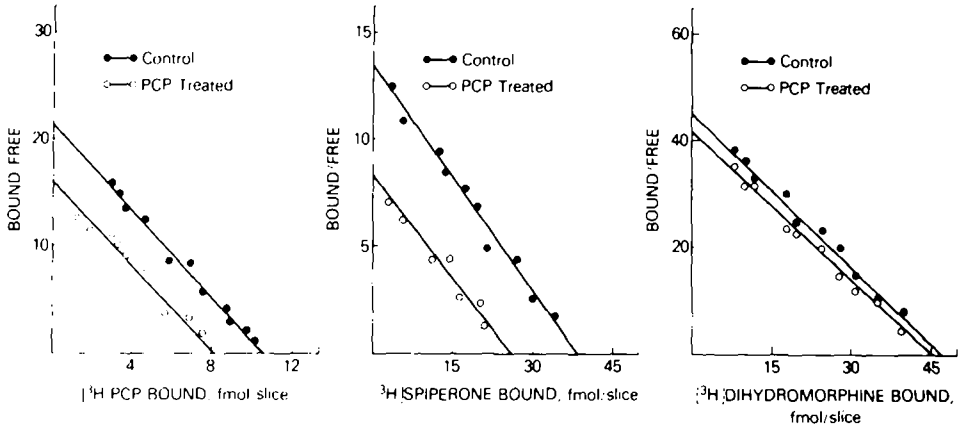

FIG. 1. Scatchard plot of specific binding data for [^{3}H]PCP, [^{3}H]spiperone and [^{3}H]dihydromorphine binding to brain slide-mounted sections from PCP treated (○) and control (●) rats. Slide-mounted sections were incubated with various concentrations of [^{3}H]PCP, [^{3}H]spiperone or [^{3}H]dihydromorphine. Non-specific binding in the presence of 0.1 mM PCP, 10 μM (+)butaclamol or 1.0 μM etorphine has been subtracted from all experimental points. Values are the mean of three determinations (each in triplicate) and varied less than 20%.

washed in cold buffer as described for [^{3}H]PCP [27] and [^{3}H]DHM [5]. For [^{3}H]spiperone, the slides were transferred through six rinses (1 min in each) of cold incubation buffer and then dipped in and out of distilled water to remove ions. Under these conditions, specific [^{3}H]spiperone binding represents 70–75% of total binding (Quirion and Pert, in preparation). Binding of [^{3}H]PCP, [^{3}H]DHM and [^{3}H]spiperone to the tissue slice were quantitated by assaying the tissue-bearing slide fragment in 10 ml of Aquassure scintillation cocktail (New England Nuclear). Specific binding was calculated as the difference in counts bound in presence and absence of 0.1 mM PCP for [^{3}H]PCP binding, 1.0 μM etorphine for [^{3}H]DHM binding and 1.0 μM (+)-butaclamol for [^{3}H]spiperone binding. Scatchard analysis of the binding data was used for calculation of receptor concentration (B_{max}) and dissociation constant (K_D). The means of the calculated values (B_{max} and K_D) for control (n=4) and treated (N=4) rats were compared for significant differences by the Student's *t*-test. For the acute experiment, rats were injected SC with 10 mg/kg PCP in saline or saline alone (controls) and killed 1 hr after the treatment and their brains were processed as described above.

RESULTS AND DISCUSSION

Chronic PCP treatment caused a significant decrease in the number (B_{max}) of [^{3}H]PCP and [^{3}H]spiperone binding sites in rat brain slices (Fig. 1). For [^{3}H]PCP, we observed a 33% decrease in the number of sites and for [^{3}H]spiperone, the B_{max} is 31% lower in treated than in control rats (Table 1). No changes in the affinity (K_D) of the receptors for [^{3}H]PCP and [^{3}H]spiperone were observed (Table 1, Fig. 1). Also, [^{3}H]DHM binding was not affected (B_{max} and K_D) by a chronic PCP treatment (Table 1, Fig. 1). However, an acute PCP treatment did not modify K_D and/or B_{max} of any of the binding sites under study: [^{3}H]PCP: K_D 42±6 nM, B_{max} 12.0±0.8 fmole/slice; [^{3}H]spiperone: K_D 0.6±0.1 nM, B_{max} 38.4±2.0 fmole/slice; [^{3}H]DHM: K_D 1.1±0.2 nM, B_{max} 46.0±3.4 fmole/slice. These results indicated that the changes observed after chronic treatment are probably not related to the presence of PCP or its metabolites on receptors when performing binding assays.

Our results show that a chronic PCP treatment induced a decrease in number of [^{3}H]PCP binding sites in rat brain. Many evidences suggest that there is an inverse relationship between the numbers of receptors and the degree of receptor occupancy by agonists [24,31]. PCP receptors also appear to be similarly regulated. This down-regulation of the number of PCP sites induced by chronic PCP treatment may explain the development of tolerance [7, 8, 12, 22, 25, 26] and dependence [4,35] to this drug observed in various species. However, changes in drug distribution, uptake or degradation are also likely to be involved in the development of tolerance and dependence to PCP.

The diminution in the number of [^{3}H]spiperone binding sites, an antagonist of the D-2 dopamine receptor [17,18] after chronic PCP treatment is interesting, especially in regard to the "schizophrenic-like" effects induced by PCP. Very recently, Robertson and Paterson [29] obtained similar results in rat brain membrane preparations. Since a direct interaction of PCP on dopamine binding sites is unlikely [16] the decrease in D-2 binding sites in rat striatum might be related to the various effects of PCP on the dopaminergic system [2, 13–15, 22]. Since it has been shown that PCP can induce the release of dopamine in various preparations [1, 2, 11, 14], it is possible that some PCP binding sites might be located on dopaminergic terminals. Activation of these binding sites can stimulate the release of dopamine which in a chronic situation would induce a down-regulation of the number of D-2 dopamine binding sites. Another explanation might be that PCP can block the uptake of dopamine [11, 14, 29], thus inducing a decrease in the number of binding sites because of the higher than normal concentration of dopamine in the synaptic cleft. In any case, more experiments are needed to precise the mechanism of action involved in this peculiar effect.

The absence of effect of a chronic PCP treatment on opiate binding sites indicates that there is little cross-reactivity between opiates and PCP. It has already been

TABLE 1

EFFECTS OF CHRONIC PCP TREATMENT ON PCP, OPIATE AND DOPAMINE BINDING PARAMETERS IN RAT BRAIN SLICES

	K_D (nM)		B_{max} (fmol/slice)	
Ligand	Control	Treated	Control	Treated
[^{3}H]PCP	44 ± 5*	52 ± 5	11.4 ± 0.9	7.6 ± 0.6†
[^{3}H]Spiperone	0.54 ± 0.05	0.62 ± 0.04	38.0 ± 2.1	26.0 ± 1.9‡
[^{3}H]DHM	1.3 ± 0.2	1.5 ± 0.2	47.2 ± 3.7	45.1 ± 3.6

*Values represent mean ± SEM of 3 experiments, each in triplicate.
†$p<0.01$.
‡$p<0.005$.

shown that very high concentrations of PCP are necessary to inhibit opiate binding *in vitro* [32]. The only class of opiates which possess strong interaction with PCP-like drugs are benzomorphans [27, 30, 37], which have peculiar "σ-opiate effects." Recently, Balster and co-workers [6] have shown that only the (+) isomers of these drugs appeared to interact with PCP. It is also very possible that a chronic PCP treatment might affect other classes of receptors. Already, Ward and Trevor [34] reported recently that chronic PCP treatment decreased muscarinic cholinergic receptors in rat brain.

In summary, we have shown that chronic PCP treatment decreases the number of [^{3}H]PCP and [^{3}H]spiperone binding sites in rat brain. These results reveal the plasticity of the PCP binding site and suggest that this site is relevant to the pharmacological effects of PCP.

ACKNOWLEDGEMENT

Dr. Remi Quirion is a Fellow of the Medical Research Council of Canada.

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