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BRIEF COMMUNICATION

Effect of Phencyclidine and two Monohydroxy Metabolites on ^{3}H QNB Binding *In Vivo* in Rats

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BOGGAN, W. O., A. J. STRINGER, K. FAUGHT AND L. D. MIDDAUGH. *Effect of phencyclidine and two monohydroxy metabolites on ^{3}H QNB binding* in vivo *in rats*. PHARMACOL BIOCHEM BEHAV 26(4) 847–849, 1987.—The purpose of these investigations was to determine if phencyclidine (PCP) and/or two of its major monohydroxy metabolites [1-(1-phenylcyclohexyl)4-hydroxy piperidine (4-OH-pip PCP) and 1-(1-phenyl-4-hydroxycyclohexyl) piperidine (4-OH-cyclo PCP)] influence ^{3}H quinuclidinyl benzilate (QNB) binding in rat brain after *in vivo* administration. PCP and 4-OH-pip PCP but not 4-OH-cyclo PCP enhanced QNB binding. The effect was blocked by atropine. Since the dose of 4-OH-pip PCP necessary to alter QNB binding in rat brain is substantially higher than that required for PCP, this metabolite probably does not play a major role in the PCP effect on QNB binding in rat brain.

Phencyclidine *In Vivo* binding Cholinergic PCP metabolites Quinuclidinyl benzilate

PREVIOUS studies in our laboratory have demonstrated that PCP can enhance the accumulation of QNB in mouse brain if both are given *in vivo* [1]. Since atropine will block this action, the effect has been attributed to an enhancement of specific binding of QNB. A study on the role of two major PCP metabolites [2,5] in this effect in mouse (submitted) determined that 4-OH-cyclo PCP, but not 4-OH-pip PCP, would also enhance QNB binding, but only at much higher doses than needed for PCP.

Since the majority of research concerning the biochemical and behavioral effects of PCP on the cholinergic system has been performed in rats [2,5], the present research was designed to extend our *in vivo* binding studies in mice to rats.

EXPERIMENT 1

The purpose of the first experiment was to determine if PCP, 4-OH-pip PCP, or 4-OH-cyclo PCP would enhance QNB accumulation in rat brain.

Adult male Sprague Dawley rats (150–250 g) from Charles Rivers Laboratories were used in these studies. The animals were housed 4–5 per cage in our colony rooms (72±2°F, 7:00 a.m.–7:00 p.m. light-dark cycle) for at least one week prior to use and had ad lib access to Wayne Rat Chow and water.

On the day of testing, the animals were moved to the chemistry laboratories, weighed, and assigned to treatment groups. At appropriate times, the animals were injected and returned to their home cages until sacrifice. The doses used were PCP (2.5, 5.0, and 10 mg/kg), 4-OH-pip PCP (3.0 and 17 mg/kg), and 4-OH-cyclo PCP (5.6 and 56 mg/kg). The metabolite doses were chosen on the basis of behavioral data [7] showing that the higher doses were effective in altering the performance of rats on an avoidance task whereas the lower doses were ineffective. Drugs or saline were injected IP 15 min prior to QNB (150 μCi/kg, IV 30–60 Ci/mmol, New England Nuclear). The animals were sacrificed 60 min after the QNB. These routes of injection were chosen in order to more closely approximate previous studies [1, 6, 7].

Tissue Treatment

At the time of sacrifice, both blood and brain were collected. The blood (200 μl) was collected in heparinized capillary tubes and after centrifugation, duplicate 10 μl aliquots of plasma were taken, added to counting vials and treated as described for brain tissue.

The brain was dissected on a cold glass plate into the hypothalamus, hippocampus, striatum, a portion of the cortex lying along the midline, the corpora quadrigemina plus underlying brainstem and the cerebellum [3]. The tissue was weighed and placed into glass counting vials containing Unisol (1 ml Isolab) and allowed to sit at room temperature overnight. One ml of methanol was then added and followed

TABLE 1

EFFECT OF PCP, 4-OH-PIP PCP, OR 4-OH-CYCLO PCP ON ^{3}H QNB ACCUMULATION IN VARIOUS REGIONS OF RAT BRAIN

		Brain Region*				
Drug	Dose (mg/kg)	Cere	Hippo	Cortex	Hypo	Stria
PCP	0.0	100	100	100	100	100
	2.5	103	119	138†	116†	134†
	5.0	108	122†	175†	121†	186†
	10.0	104	150†	171†	120†	186†
4-OH-pip PCP	0.0	100	100	100	100	100
	3.0	88	94	93	95	98
	17.0	102	118	125†	110	138†
4-OH-cyclo PCP	0.0	100	100	100	100	100
	5.6	87	100	95	99	104
	56.0	87	110	114	111	117

*Data presented as percent control.

†Indicates p at least <0.05 as compared to control.

PCP, 4-OH-pip PCP, and 4-OH-cyclo PCP were given IP in saline (PCP) or a lactic acid solution (metabolites) 15 min before injection of ^{3}H QNB (150 μCi/kg, IV). The animals were sacrificed 1 hr after the ^{3}H QNB.

TABLE 2

EFFECT OF PCP AND/OR ATROPINE ON ^{3}H QNB ACCUMULATION IN VARIOUS REGIONS OF THE MOUSE BRAIN

	Treatment Group*			
Region	Sal + Sal	Sal + PCP	Atr + Sal†	Atr + PCP†
Cerebellum	63.6 ± 6.3	78.6 ± 3.5	17.1 ± 0.98	16.8 ± 0.64
Hippocampus	77.5 ± 3.6	122.4 ± 4.3†	42.6 ± 2.7	37.7 ± 2.0
Cortex	108.8 ± 7.9	237.1 ± 14†	45.1 ± 3.1	38.4 ± 1.7
Hypothalamus	83.8 ± 4.8	120.4 ± 4.3†	29.7 ± 1.6	27.0 ± 0.55
Striatum	78.3 ± 2.5	147.3 ± 7.4†	50.3 ± 6.3	37.3 ± 3.9

*Values are given as CPM/mg tissue ± S.E.

†Denotes p at least <0.05 as compared to Sal + Sal. Each atropine group was significantly less than the control Sal + Sal group for the same region. No Atr + Sal group was different from the Atr + PCP group for the same region.

Saline or atropine (30 mg/kg, IP) was given 15 min before saline or PCP (5.0 mg/kg, IP) which was given 15 min before ^{3}H QNB (150 μCi/kg, IV). The animals were sacrificed 1 hr after the ^{3}H QNB.

thirty minutes later by Unisol Compliment (10 ml). After mixing, the samples were counted for two minutes in a Beckman LS350 or Tri Carb Liquid Scintilation Counter.

Drugs

Phencyclidine HCl (PCP), 1-(1-phenylcyclohexyl)-4-hydroxy piperidine (4-OH-pip PCP), and 1-(1-phenyl-4-hydroxycyclohexyl) piperidine (4-OH-cyclo PCP) were supplied by the National Institute on Drug Abuse.

Statistical Analysis

PCP and PCP-atropine data were analyzed using analyses of variance (AOVs). Post hoc analysis was performed utilizing Dunnett's t statistic after one-way AOVs or analysis of the simple main effects after two-way AOVs according to Winer [8]. Data from the metabolite experiments were analyzed using AOVs while controlling for differences between replicate experiments [4]. In both cases, F values associated with probabilities of 0.05 or less were considered to be statistically significant.

RESULTS

PCP enhanced QNB accumulation in rat brain and the magnitude of the effect was brain region and dose dependent (Table 1). No changes were manifest in cerebellum at any dose. The greatest effects were seen in the striatum followed by the cortex, hippocampus, and hypothalamus. PCP did not

alter the amount of QNB found in the plasma (data not shown). The highest dose of 4-OH-pip PCP significantly enhanced QNB accumulation in cortex ($p<0.004$) and striatum ($p<0.02$), but not cerebellum ($p<0.67$), hippocampus ($p<0.06$), or hypothalamus ($p<0.08$). 4-OH-cyclo PCP did not have a statistically significant effect in any brain area.

EXPERIMENT 2

The purpose of the second experiment was to determine whether or not the increase in QNB in brain after PCP could be blocked by atropine, a specific muscarinic antagonist.

Rats were injected with saline or atropine (30 mg/kg, Sigma Chemical Co.) followed 15 min later by PCP (5 mg/kg) or saline. QNB was injected 15 min after the second treatment. Sacrifice was 60 min after the QNB. The tissues were treated as described above.

RESULTS

Analysis of the data revealed (Table 2) that, as in Experiment 1, PCP increased QNB concentration in all brain regions except cerebellum as compared to controls. Atropine pretreatment substantially reduced the amount of QNB accumulated. The amount of reduction by atropine was such that there were no differences between the atropine-saline group and the atropine-PCP group.

DISCUSSION

This study shows that with respect to PCP, the effects on QNB binding seen in rats are qualitatively similar to previously published mouse data [1]. PCP increased QNB accumulation in all brain areas tested except cerebellum. Further, no changes in plasma concentrations were observed. Because of these cerebellar and plasma findings, enhanced transport of QNB into brain is apparently not responsible for the observed increase in QNB binding. Rather, since pretreatment with atropine blocked the observed increase, it appears that the PCP effect is on specific binding.

The influence of the 4-OH-pip PCP and 4-OH-cyclo PCP on QNB binding in rat appear to differ from that observed in mice. In the present study, the high dose of 4-OH-pip PCP (17 mg/kg) significantly increased QNB accumulation in striatum and cortex with a strong trend towards increases in hippocampus and hypothalmus. We have no such effect in mouse, even at twice the dose. Because this dose of 4-OH-pip PCP is at least 3 times larger than that needed for PCP to enhance QNB binding, the role of this metabolite in the PCP effect seems doubtful. Differential transport of the compounds to the brain cannot be ruled out, however. No effects on QNB accumulation were seen in the rat after 4-OH-cyclo PCP, however, an enhanced accumulation was observed in all brain areas in the mouse at a similar dose. It appears that, with respect to *in vivo* QNB accumulation, 4-OH-pip PCP is more active in the rat while 4-OH-cyclo PCP is more active in the mouse. Whether differences in transport processes or other possible indirect mechanisms account for these species differences remains speculative.

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