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Parametric Studies on Phencyclidine Enhancement of ^{3}H Quinuclidinyl Benzilate Accumulation In Vivo

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BOGGAN, W. O., K. FAUGHT, S. BOYD AND L. D. MIDDAUGH. *Parametric studies on phencyclidine enhancement of ^{3}H quinuclidinyl benzilate accumulation in vivo.* PHARMACOL BIOCHEM BEHAV **30**(1) 31–35, 1988.—The purpose of these experiments was to define the temporal parameters involved in the phencyclidine (PCP) enhancement of ^{3}H quinuclidinyl benzilate (QNB) accumulation in mouse brain. PCP enhanced QNB accumulation in brain if given intraperitoneally (IP) 1 and 4, but not 16 hours before intravenous (IV) administration of QNB. This effect was found in hypothalamus, striatum, cortex and hippocampus, but not cerebellum. PCP given after QNB did not alter QNB accumulation. The PCP enhancement persisted for at least 72 hours after QNB administration. These results confirm previous studies demonstrating that PCP must be present prior to QNB administration to enhance the accumulation and show that the effect persists for an extended period of time.

Quinuclidinyl benzilate (QNB) Phencyclidine (PCP) Muscarinic receptors Mice

THE interaction of phencyclidine (PCP) with various neurochemical systems has been the focus of many investigations [9, 14, 20]. Of particular relevance to the current research are those studies on the effects of PCP on cholinergic systems [2, 5, 21]. For example, previous studies by our laboratory and others have demonstrated that PCP inhibits ^{3}H quinuclidinyl benzilate (QNB) binding in vitro. In sharp contrast, we have found that PCP increases the binding of QNB in both rat and mouse brain when given prior to intravenous administration of QNB [5–7]. These increases are seen in hippocampus, hypothalamus, cortex, brain stem and striatum, but not cerebellum. Further, this effect seems to be on specific binding since it is blocked by prior administration of the muscarinic antagonist atropine. Since no changes have been found in cerebellar or plasma concentration of QNB as a result of PCP administration, the PCP effect does not appear to be due to the transport of PCP into brain from the blood.

Since all our previous experiments have utilized a single temporal sequence in studying this PCP effect, questions about the time parameters of PCP administration in relation to QNB injection and the duration of the PCP effect on QNB accumulation have not been answered. The purpose of the present research is to more clearly define the parameters involved in the PCP enhancement of QNB binding in vivo.

GENERAL METHOD

Adult male C57BL/6 mice from Charles River Laboratories (Wilmington, MA) 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. During that time they had ad lib access to Wayne Rodent Blox 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 with appropriate compounds and returned to their home cages. PCP was always given intraperitoneally (IP). QNB was given intravenously (IV) and was accomplished by placing the animal in a restraining cage which allowed the tail to protrude, dipping the tail in warm water for a few seconds to dilate the vein, and injecting QNB. Mice were always given 1.6 μg QNB/kg body weight. The volume of PCP and QNB injection was 0.1 ml/10 g. The vehicle for both PCP and QNB was 0.9% saline. Doses and times of injection are given below.

Tissue Treatment

At the time of sacrifice, both blood and brain were collected. The blood (200 μl) was collected in heparinized capillary tubes and spun in a hematocrit centrifuge for 5 min. Duplicate 10 μl aliquots of plasma were then taken, added to counting vials and treated as described for the brain below.

After removal of the brain, the cerebellum, hypothalamus, hippocampus, striatum and a portion of the cortex lying along the midline, were rapidly dissected over ice [11]. After dissection, the tissue was weighed and immediately placed

TABLE 1

PERSISTENT EFFECTS OF PCP ON ACCUMULATION OF QNB IN BRAIN TISSUE

		Brain Region*†				
Group	Time‡	Cere	Hyp§	Cor§	Str§	Plasma
Sal	4 hr	0.97 ± 0.05	1.22 ± 0.04	1.33 ± 0.06	1.35 ± 0.06	3.01 ± 0.09
PCP¶	4 hr	1.00 ± 0.05	1.48 ± 0.12	1.74 ± 0.10	1.85 ± 0.11	3.27 ± 0.16
Sal	72 hr	0.16 ± 0.00	0.85 ± 0.01	1.31 ± 0.04	1.17 ± 0.06	1.35 ± 0.07
PCP¶	72 hr	0.18 ± 0.01	1.08 ± 0.12	1.82 ± 0.17	1.77 ± 0.22	1.24 + 0.05

*Values given as fmole QNB/mg tissue ±S.E.

†Mice were injected with PCP (20 mg/kg, IP) 60 minutes before QNB (1.6 μg/kg, IV).

‡Time of sacrifice after QNB.

§All values for this tissue were significantly greater than corresponding cerebellum value.

¶Except for cerebellum and plasma, tissue values were significantly greater than corresponding saline group.

into glass counting vials. Unisol (1/2 ml, Isolab) was added to the vials which were then capped and allowed to sit at room temperature overnight. The next day, one ml of methanol was added to the vials, the sample lightly shaken and allowed to sit for 30 minutes. An aliquot of Unisol Compliment (10 ml) was then added to each vial. Samples were thoroughly mixed and their radioactivity measured for 2 minutes each in a Packard Liquid Scintillation Counter.

TLC Determination of ^{3}H QNB 72 Hours After Injection

In order to determine the extent to which metabolites of the ^{3}H QNB contributed to the radioactivity found in brain tissue after intravenous injection of the label, ^{3}H QNB (1.6 μg/kg) was injected into the tail vein of C57BL/6 mice (N=8) 15 minutes after treatment with 20 mg/kg PCP-HCl or its vehicle (0.9% saline). The animals were sacrificed 72 hours later. Hippocampus, cortex and cerebellum tissue samples were dissected over ice, frozen and stored at −80°C until analyzed.

On the day of analysis, the tissue samples were allowed to thaw at room temperature. ^{3}H QNB was extracted using a solvent extraction procedure previously described by Yamamura, Kuhar and Snyder [24]. The tissue was homogenized in 0.2% acetic acid in 96% ethanol and centrifuged. An aliquot of the supernatant was evaporated and then reconstituted in absolute ethanol. Duplicate 10 μl aliquots of reconstituted supernatant or authentic ^{3}H QNB were spotted on G250 Silica Gel Chromatography plates and placed in a chromatography tank containing 4:1:1 N-butanol:acetic acid:H_2O. After 3 hr, the plates were removed from the tank and allowed to air dry. One centimeter scrapings of the silica gel were placed in liquid scintillation vials containing 3 ml of Scintiverse E (Fisher Scientific). Samples were counted for 1 minute in a Packard Model 4350 Tri Carb Beta Counter at 69% efficiency. The radioactivity from the tissue extract samples migrated as a single peak identical to that of the authentic ^{3}H QNB and accounted for approximately 88±2% of the total. The remainder was distributed evenly along the rest of the plate suggesting streaking rather than metabolites. These results indicate that the radioactivity found in the brain 72 hours after IV administration of QNB was essentially unchanged QNB. Therefore, it is unlikely that metabolites confound the persistence of enhanced QNB accumulation seen after PCP.

Drugs

Phencyclidine (PCP) was graciously supplied by the National Institute on Drug Abuse. ^{3}H Quinuclidinyl benzilate (30–60 Ci/mmol) was purchased from New England Nuclear.

Statistical Analysis

Data were analyzed using analyses of variance (ANOVA) with the type dependent upon the experiment. F-values associated with probabilities of 0.05 or less were considered to be significant. Subsequent post hoc analysis was performed utilizing Dunnett's t statistic after one-way ANOVAs or analysis of the simple main effects after two-way ANOVAs according to Winer [23].

EXPERIMENT 1

The purpose of this experiment was to determine whether the enhanced accumulation of QNB after PCP persisted over an extended period of time.

Method

Mice were injected with saline or PCP (20 mg/kg) one hour before QNB administration. This dose of PCP, though admittedly high, was chosen to assure a substantial effect and to allow us to see a decline in the PCP effect if one should occur. The animals were sacrificed either 4 or 72 hours after the QNB injection. At the time of sacrifice, the blood and brain were taken and treated as described above.

Results

The data indicate that PCP enhanced QNB accumulation in all brain regions except cerebellum and that this effect persisted for at least 72 hours after QNB administration (Table 1). In confirmation of previous studies, the striatum and cortex manifest the highest increase followed by hippocampus (data not shown) and hypothalamus. Though the concentration of QNB in cerebellum declined substantially between 4 and 72 hours after QNB administration, that in hippocampus, cortex and striatum remained relatively stable. The concentrations of QNB in the hypothalamus declined at a rate intermediate to that of cerebellum and the other areas.

TABLE 2

EFFECT OF PCP GIVEN VARIOUS TIMES BEFORE QNB ON QNB ACCUMULATION IN BRAIN TISSUE

Time (hr)	N	Brain Region*† Cere	Hyp‡	Cor‡§	Str‡§	Plasma
Sal	5	0.32 ± 0.02	1.36 ± 0.06	1.88 ± 0.07	1.78 ± 0.11	2.00 ± 0.1
1	5	0.33 ± 0.01	1.64 ± 0.12¶	2.87 ± 0.19¶	2.86 ± 0.19¶	2.07 ± 0.2
4	4	0.35 ± 0.02	1.56 ± 0.05¶	2.26 ± 0.10¶	2.04 ± 0.12¶	2.10 ± 0.2
16	5	0.32 ± 0.01	1.26 ± 0.04	1.84 ± 0.05	1.72 ± 0.09	1.91 ± 0.2

*Values given as fmole QNB/mg tissue ± S.E.

†Mice were injected IP with PCP (20 mg/kg) and then IV with QNB (1.6 μg/kg) and sacrificed 32 hours later.

‡All values for this tissue were significantly greater than corresponding cerebellum value.

§All values for this tissue were significantly greater than corresponding hypothalamus value.

¶Significantly greater than Sal or 16 hours.

TABLE 3

EFFECTS OF PCP GIVEN AFTER QNB ON THE ACCUMULATION OF QNB IN BRAIN TISSUE*

Group	n	Brain Region† Cere	Hyp‡	Cor‡	Str‡	Plasma
QNB-Sal	6	1.75 ± 0.07	2.18 ± 0.07	2.29 ± 0.10	2.35 ± 0.15	5.34 ± 0.18
QNB-PCP	7	1.74 ± 0.05	2.23 ± 0.07	2.31 ± 0.07	2.40 ± 0.05	5.17 ± 0.24

*Values given as fmol QNB ± S.E.

†QNB (1.6 μg/kg, IV) was given one hour prior to Sal or PCP (20 mg/kg). The animals were sacrificed 15 minutes after the second treatment.

‡All values for this tissue were significantly greater than corresponding cerebellum value.

EXPERIMENT 2

The purpose of this experiment was to examine the duration of the interval between PCP injection and QNB administration in which PCP would remain effective in altering the accumulation of QNB in brain.

Method

Saline or PCP (20 mg/kg) was injected 1, 4 or 16 hours prior to QNB. The animals were sacrificed 32 hours after QNB. Upon sacrifice, blood and brain were taken and treated as described above.

Results

PCP was found to enhance QNB accumulation in hypothalamus, hippocampus (data not shown), cortex and striatum when given 1 or 4 hours before QNB, but not 16 hours before (Table 2). Again, the greatest effect was seen in cortex and striatum followed by hippocampus (data not shown) and hypothalamus. The magnitude of the increase appeared greatest after the 1 hour pretreatment. The effect was region and tissue specific in that increases were seen in all areas except cerebellum and plasma.

EXPERIMENT 3

The purpose of this experiment was to determine whether PCP would alter QNB accumulation if given after QNB.

Method

Mice were given QNB (as above) followed 60 minutes later by either saline or PCP (20 mg/kg). They were sacrificed 15 minutes after the PCP. Blood and brain were taken and treated as previously described.

Results

No differences in QNB accumulation were seen in plasma or brain between saline and PCP treated animals in any brain region (Table 3). A significant regional difference in QNB accumulation was found as before.

DISCUSSION

The results of these experiments confirm and extend those previously published by us [6,7] showing an enhancement of QNB accumulation in brain by PCP. Our previous studies indicate that the enhancement was an increase in specific binding since prior administration of atropine (a muscarinic antagonist) which blocks specific binding eliminated the PCP effect. Though the current work did not examine the effect of atropine, the assumption was made that the enhanced QNB accumulation after PCP seen in these studies was also an enhancement in specific binding.

The current studies demonstrate that the mechanisms responsible for the enhancement of QNB accumulation are operative in some brain regions for as long as four hr, but not for 16 hours after PCP administration. The mechanisms do

not appear operative in cerebellum at all. Whether this enhancement is dependent upon the presence of PCP is not known, however, since PCP has been shown to inhibit QNB binding in vitro [2, 5, 21], the presence of PCP at muscarinic receptors does not seem to be necessary and in fact is probably detrimental to the enhancement. What is known from these studies is that PCP must be present at some time prior to the in vivo administration of QNB for accumulation to occur since administration of PCP after QNB had no effect.

The enhancement of QNB accumulation by PCP parallels the persistence of QNB in the brain tissue. However, it does not appear to parallel the tissue levels of PCP which, though persistent in lipids, do decline appreciably in brain and plasma within several hours after administration of the drug [17]. Whatever the modification which produces the enhanced QNB accumulation, our data indicate the enhancement to be relatively stable, for at least 72 hours. Whether additional treatments can "undo" the PCP effect is not known.

The mechanism by which PCP induces the enhanced QNB binding is not known. Recent studies have demonstrated that two major hydroxy PCP metabolites (1-(1-phenylcyclo-hexyl)4-hydroxy piperidine and 1-(1-phenyl-4-hydroxycyclohexyl) piperidine) apparently do not account for the ability of PCP to enhance QNB binding in mice and rats [6,7]. Whether the interactions of PCP with other neurotransmitter systems [8, 10, 12, 13, 15, 18] or ion channels [1, 3, 4] are influential remains to be determined. It is of interest that several catecholamines, apomorphine (a dopamine agonist), and amphetamine (a known catecholamine releaser) all have been shown to enhance the binding of QNB [19]. Whether PCP is producing its effects via its action on catecholamine systems is not known. In addition, such questions as: do the changes in QNB accumulation after PCP occur in tissue other than the brain?; are the changes specific to M_1 or M_2 cholinergic receptors [22]?; is this in vivo enhancement reflective in a change in K_d and/or B_{max}?; and are these effects of PCP medicated via sigma or PCP receptor systems for which PCP has a high affinity [21, 25, 26]? should be answered.

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