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Doubtful Role for Phencyclidine Metabolites in PCP Enhancement of QNB Binding

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BOGGAN, W. O. AND L. D. MIDDAUGH. *Doubtful role for phencyclidine metabolites in PCP enhancement of QNB binding.* PHARMACOL BIOCHEM BEHAV **26**(4) 671–676, 1987.—*In vivo* administration of phencyclidine (PCP) has been shown to enhance the accumulation of intravenously administered ^{3}H quinuclidinyl benzilate (QNB) in certain regions of mouse brain. Since this effect can be blocked by prior administration of atropine, it has been interpreted as an enhancement by PCP of the specific binding of QNB. The present studies replicated this earlier work and determined that two major hydroxy metabolites of PCP found in rodents 1-(1-phenylcyclohexyl)4-hydroxy piperidine (4-OH-pip PCP) and 1-(1-phenyl-4-hydroxycyclohexyl)piperidine (4-OH-cyclo PCP) probably do not contribute substantially to the PCP effect on QNB binding. Though QNB accumulation in brain was increased by injection of 4-OH-cyclo PCP, the doses necessary for the effect were substantially higher than those needed for PCP. Furthermore, pretreatment of the animals with β-diethylaminoethyl diphenylpropylacetate (SKF525A), a compound known to block the metabolism of PCP in liver microsomes and thus the formation (at least in part) of these metabolites, did not attentuate the PCP effect on QNB binding.

Phencyclidine *In vivo* binding Cholinergic PCP metabolites Quinuclidinyl benzilate

THERE are numerous papers reporting the effects of phencyclidine (PCP) on brain neurotransmitters (e.g., [9,16]). Of particular interest for the studies to be reported herein are those reports of an interaction between PCP and brain cholinergic systems. Previous work by ourselves and others (e.g., [2, 4, 23]) demonstrated that PCP inhibited ^{3}H quinuclidinyl benzilate (QNB) binding to brain tissues *in vitro*. In contrast, *in vivo* injections of PCP enhanced the accumulation of systemically injected QNB into brain tissue, an effect blocked by atropine [4]. This *in vivo* effect of PCP was dose related (approximately a 10% increase in QNB binding at 2.5 mg/kg and 45% increase at 20 mg/kg). Because of the contrasting *in vitro* (inhibition) and *in vivo* (enhancement) effects of PCP on QNB binding, it was concluded that PCP *in vivo* must be acting indirectly rather than on the receptors themselves. Since drug metabolites and interactions between neurochemical systems are possible in the *in vivo* but not the *in vitro* system, we hypothesized that either or both of these factors may be accountable for the reported differences.

Studies [15, 21, 22] demonstrating that PCP metabolites influence both behavioral and biochemical systems support the metabolite hypothesis. If this hypothesis applies to the changes in QNB binding, then administration of metabolites should enhance QNB accumulation *in vivo* as noted for PCP. Furthermore, blocking the formation of the metabolites with a metabolic inhibitor should attentuate the previously observed PCP enhancement of QNB accumulation. The purpose of the present research was to test these hypotheses.

METHOD

Adult male C57BL/6 mice 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. 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 until sacrifice. Injections were either intraperitoneal (IP) for PCP, the metabolites, atropine and SKF525A or intravenous (IV) via the tail vein for the QNB. QNB was given IV in order to more closely approximate those previous studies which characterized QNB binding *in vivo* [18,25] and to follow the design of our previous research on the effect of PCP on QNB binding [4]. IV injections were 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 allow the vein to dilate, and injecting QNB. Mice were given 1.6 μg QNB/kg body wt. Doses, times and volumes of injections of the compounds are given below.

Tissue Treatment

At the time of sacrifice both blood and brain were collected. The blood (200 μl) was collected in heparinized capil-

TABLE 1

DOSE EFFECTS OF 4-OH-PIP PCP ON QNB ACCUMULATION

		Brain Region†‡§				
Dose*	N	Cere	Hyp	Hip	Cor	Str
0	5	1.90 ± 0.08	2.16 ± 0.10	2.40 ± 0.14	2.25 ± 0.12	2.37 ± 0.06
4	5	1.90 ± 0.12	2.24 ± 0.18	2.38 ± 0.14	2.35 ± 0.10	2.35 ± 0.12
8	5	1.88 ± 0.16	2.23 ± 0.12	2.38 ± 0.08	2.41 ± 0.08	2.64 ± 0.09
17	5	1.76 ± 0.12	2.15 ± 0.10	2.17 ± 0.12	2.25 ± 0.16	2.54 ± 0.10
34	5	1.89 ± 0.12	2.19 ± 0.12	2.48 ± 0.16	2.43 ± 0.16	2.64 ± 0.10

*4-OH-pip PCP (mg/kg) was given IP 30 min before IV administration of QNB (1.6 μg/kg). Animals were sacrificed 60 min after QNB.

†Values given as mean (pmol/mg tissue) ±S.E.

‡There was a significant ($p<0.05$) difference across region at each dose.

§There was no difference between doses within a region.

TABLE 2

TIME EFFECTS OF 4-OH-PIP PCP ON QNB ACCUMULATION

		Brain Region†‡				
Time*	N	Cere	Hyp	Hip	Cor	Str
0	5	2.44 ± 0.08	2.97 ± 0.16	3.17 ± 0.11	3.66 ± 0.11	3.00 ± 0.12
15	5	2.51 ± 0.08	2.86 ± 0.12	3.25 ± 0.16	3.48 ± 0.27	3.20 ± 0.34
30	5	2.64 ± 0.25	2.98 ± 0.14	3.24 ± 0.13	3.45 ± 0.33	3.21 ± 0.23
60	5	2.33 ± 0.18	2.82 ± 0.20	3.06 ± 0.25	3.66 ± 0.18	3.03 ± 0.26

*Time is given in minutes. 4-OH-pip PCP (17 mg/kg) was given IP in a lactic acid solution before IV administration of QNB (1.6 μg/kg). Zero (0) time reflects lactic acid only. Animals were sacrificed 60 min after QNB.

†Values given as mean (pmol/mg tissue) ±S.E.

‡There was a significant ($p<0.05$) region effect, but no time or time × region interaction.

lary tubes and then spun in a hematocrit centrifuge for 5 minutes. At the end of that time, duplicate 10 μl aliquots of plasma were taken, added to counting vials and treated as described for brain tissue below.

After removal, the brain was placed on a cold glass plate. The hypothalamus, hippocampus, striatum, a portion of the cortex lying along the midline, and the cerebellum were rapidly dissected [12]. The tissue was weighed and placed into glass counting vials. Unisol (1/2 ml Isolab) was added and the vials capped and allowed to sit at room temperature overnight. The next day one ml of methanol was added and the sample lightly shaken. Thirty min later, Unisol Compliment (10 ml) was added. The samples were shaken and then counted for two min in a Beckman LS350 or Tri Carb Liquid Scintillation Counter.

Drugs

Phencyclidine HCl (PCP), 1-(1-phenylcyclo-hexyl)-4-hydroxy piperidine (4-OH-pip PCP), and 1-(1-phenyl-4-hydroxycyclohexyl) piperidine (4-OH-cyclo PCP) were graciously supplied by the National Institute on Drug Abuse. Atropine sulfate was purchased from Sigma Chemical Company. ^{3}H quinuclidinyl benzilate (30–60 Ci/mmol) was purchased from New England Nuclear. β-Diethylaminoethyl diphenylpropylacetate (SKF525A) was obtained from Smith, Kline and French.

Statistical Analysis

Data were analyzed using analyses of variance (ANOVAs) with the type dependent upon the experiment. F values associated with probabilites of 0.05 or less were considered to be statistically 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 [24].

EXPERIMENT 1

The purpose of this experiment was to determine whether or not 4-OH-pip PCP or 4-OH-cyclo PCP would enhance QNB accumulation in brain. Both the dose response and the time course were determined.

Design

The dose response experiments were two factor designs with measures across dose and brain region. 4-OH-pip PCP or 4-OH-cyclo PCP in lactic acid (3 parts 8.5% lactic acid, 2 parts 1 M NaOH) was injected IP at a volume of 0.1 ml/10 g.

TABLE 3

DOSE EFFECTS OF 4-OH-CYCLO PCP ON QNB ACCUMULATION

		Brain Region†‡§				
Dose*	N	Cere	Hyp	Hip	Cor	Str
0	5	1.77 ± 0.04	2.10 ± 0.09	2.13 ± 0.17	2.05 ± 0.08	2.13 ± 0.08
14	5	2.02 ± 0.12	2.33 ± 0.13	2.38 ± 0.16	2.45 ± 0.18	2.62 ± 0.17
28	5	1.96 ± 0.09	2.43 ± 0.14	2.54 ± 0.16	2.54 ± 0.16	2.79 ± 0.17
56	5	2.00 ± 0.08	2.64 ± 0.12	2.59 ± 0.20	2.77 ± 0.16	3.24 ± 0.20
112	5	1.82 ± 0.18	2.53 ± 0.34	2.71 ± 0.38	2.77 ± 0.46	2.94 ± 0.38

*Dose given in mg/kg. 4-OH-cyclo was injected 30 min before IV administration of QNB (1.6 μg/kg). Animals were sacrificed 60 min after the QNB.

†Initial two-way ANOVA demonstrated that there was an overall region effect and dose by region interaction.

‡One-way ANOVA revealed a significant ($p<0.05$) regional effect at each dose. Additional post-hoc analyses of means demonstrated that significant ($p<0.05$) regional differences as compared to cerebellum included striatum at 14 mg/kg, hippocampus, cortex and striatum at 28 mg/kg and all four regions at 56 and 112 mg/kg.

§One-way ANOVA within region across doses revealed a significant ($p<0.05$) dose effect in striatum. Further post-hoc analyses of means showed that there were no dose effects in cerebellum or hypothalamus, however the hippocampus at 112 mg/kg, the cortex at 56 and 112 mg/kg, and the striatum at 28, 56 and 112 mg/kg had significantly greater QNB accumulations ($p<0.05$) than the corresponding control tissues.

TABLE 4

TIME EFFECTS OF 4-OH-CYCLO PCP ON QNB ACCUMULATION

		Brain Region†‡				
Time*	N	Cere	Hyp	Hip	Cor	Str
0	5	1.91 ± 0.09	2.21 ± 0.11	2.34 ± 0.15	2.38 ± 0.17	2.37 ± 0.10
15	5	2.27 ± 0.13	2.90 ± 0.20	3.13 ± 0.21	3.17 ± 0.19	3.33 ± 0.20
30	10	2.02 ± 0.05	2.55 ± 0.07	2.60 ± 0.09	2.67 ± 0.09	2.94 ± 0.04
60	5	2.15 ± 0.08	2.59 ± 0.11	2.70 ± 0.04	2.82 ± 0.11	2.99 ± 0.07

*Time is given in minutes. 4-OH-cycle PCP (56 mg/kg) was given IP in a lactic acid solution before IV administration of QNB (1.6 μg/kg). Zero (0) time reflects lactic acid only. Animals were sacrificed 60 min after QNB.

†Values given as mean (pmol/mg tissue) ±S.E.

‡There was a significant ($p<0.05$) time and region effect, but no time × region interaction.

The doses for 4-OH-pip PCP were 0, 4, 8, 17 or 34 mg/kg body weight. The doses for 4-OH-cyclo PCP were 0, 14, 28, 56 or 112 mg/kg. Zero dose animals received the lactic acid vehicle only. The doses used in this experiment were chosen on the basis of relative potency (PCP to metabolite) data obtained from several sources [1, 10, 15, 22]. The time of 30 min was chosen because our previous studies had demonstrated that PCP was effective for at least 4 hr, therefore it was felt that if the metabolites were salient, their effect would take place within this time frame.

In the time course studies, mice were randomly assigned to various groups. 4-OH-pip PCP (17 mg/kg) or 4-OH-cyclo PCP (56 mg/kg) was given IP in the lactic acid vehicle, as above, at a volume of 0.1 ml/10 g body weight, 15, 30 or 60 min before QNB. Control groups of mice received the lactic acid vehicle at these same times. These control mice were considered a 0 time group. The animals were sacrificed 60 min after QNB. At the time of sacrifice the blood and brain were taken and treated as described above.

Results

None of the doses of 4-OH-pip PCP tested altered QNB accumulation in the brain (Table 1). There was a significant regional effect and a significant dose × region interaction. Subsequent ANOVA revealed a significant effect across region at each dose. For example, in the zero dose condition, hippocampus, cortex and striatum all had a greater accumulation of QNB than did the cerebellum.

Analyses of the data revealed that there was no effect of 4-OH-pip PCP over time and no time × region interaction (Table 2). There was a significant regional difference in QNB accumulation. Subsequent ANOVA and post-hoc tests showed that hippocampus, cortex and striatum all had a significantly higher accumulation of QNB than did the cerebellum. No effect of 4-OH-pip PCP was seen in the blood in either the dose or the time response experiments.

Analyses of the 4-OH-cyclo PCP dose response data (Table 3) indicated that there was a significant effect of re-

TABLE 5

EFFECTS OF ATROPINE ON THE 4-OH-CYCLO PCP ENHANCEMENT OF QNB ACCUMULATION

Group*	N	Region†‡§¶ Cere	Hyp	Hip	Cor	Str
S+L	5	1.69 ± 0.06	2.07 ± 0.11	2.09 ± 0.10	2.08 ± 0.10	2.00 ± 0.08
S+C	5	1.87 ± 0.06	2.33 ± 0.10	2.42 ± 0.12	2.40 ± 0.08	2.62 ± 0.08
A+L	5	0.39 ± 0.02	0.61 ± 0.04	0.89 ± 0.05	0.80 ± 0.04	1.00 ± 0.10
A+C	5	0.36 ± 0.02	0.56 ± 0.02	0.85 ± 0.02	0.76 ± 0.02	0.92 ± 0.03

*Saline (S) or atropine (A) was given IP 15 min before an IP injection of a lactic acid solution (L) or 4-OH-cyclo PCP (56 mg/kg, C) given 30 min before QNB (1.6 μg/kg, IV). The animals were sacrificed 60 min later.

†Data are presented as mean (pmol/mg tissue) ±S.E.

‡Three-way ANOVA revealed that overall there were significant ($p<0.05$) drug and region effects as well as significant interactions of the three factors.

§One-way ANOVA of data within each group across region showed a significant ($p<0.001$) regional difference in each group. Analysis of dose within each region across groups showed significant ($p<0.001$) group differences.

¶Post-hoc analyses comparison of means failed to show any significant difference between A+L vs. A+C within any region.

gion and a dose by region interaction. Subsequent ANOVA on data within each dose demonstrated regional differences in QNB accumulation. Post-hoc comparison of other brain parts to cerebellum showed significant regional differences for striatum at 14 mg/kg, hippocampus, cortex and striatum at 28 mg/kg, and all four regions at 56 and 112 mg/kg. ANOVA on data within each region across dose showed a significant increase in QNB accumulation only in the striatum. However, post-hoc comparison of means from other brain parts to cerebellum showed significant increases in QNB accumulation in the hippocampus at 112 mg/kg, cortex at 56 and 112 mg/kg and striatum at 28, 56 and 112 mg/kg.

Analyses of the 4-OH-cyclo PCP time course data showed a significant time and region effect (Table 4), but no interaction.

EXPERIMENT 2

The purpose of this experiment was to determine whether or not the enhanced QNB accumulation after 4-OH-cyclo PCP was related to specific or nonspecific binding.

Design

The mice were assigned to one of the four groups: saline-lactic acid solution, saline-4-OH-cyclo PCP, atropine-lactic acid solution or atropine-4-OH-cyclo PCP. Saline or atropine (30 mg/kg) was given 15 min before 4-OH-cyclo PCP (56 mg/kg) or vehicle. The animals were then injected with QNB 30 min after the 4-OH-cyclo PCP and sacrificed 60 min after the QNB. The volume of injections was 0.1 ml/10 g body weight. The dose of atropine was chosen in an attempt to block specific binding to muscarinic receptors [25], since only low concentrations of atropine are found in the mouse brain after injection [1] and to allow comparison of these data to that published previously by us [4].

Results

An ANOVA indicated significant differences due to the three main factors 4-OH-cyclo PCP, atropine, region, as well as significant interaction of these factors. Analyses within group-across region as well as within region-across group showed significant effects. Post-hoc analyses found 4-OH-cyclo PCP to significantly enhance QNB accumulation in all brain areas. Atropine significantly decreased QNB accumulation in all areas. No differences were found, however, in comparisons of the atropine plus lactate group with the atropine plus 4-OH-cyclo PCP group demonstrating that atropine blocked the 4-OH-cyclo PCP enhancement of QNB accumulation (Table 5). Atropine significantly elevated the amount of QNB in the plasma, however in contrast to Experiment 1, 4-OH-cyclo PCP did not affect the plasma QNB concentration.

EXPERIMENT 3

The purpose of this experiment was to determine whether or not blockade of PCP metabolism in the liver would alter the effect of PCP on QNB accumulation. Since PCP is metabolized at least in part by the liver [13] and 4-OH-cyclo PCP is one of the resultant metabolites, then blockade of the formation of this metabolite should diminish the PCP-induced increase in QNB accumulation, if in fact 4-OH-cyclo PCP is important. In order to examine this possibility, SKF525A, a compound which blocks drug metabolizing enzymes in liver microsomes [3,7] was used. This compound has been shown to inhibit the biotransformation of PCP *in vitro* in liver microsomal preparations [5, 6, 8, 14, 17] and also *in vivo* [8].

Design

The experimental design was factorial which resulted in four groups: saline plus saline, saline plus PCP, SKF525A plus saline and SKF525A plus PCP. The mice were randomly assigned to one of these four groups with five animals per group. Saline or SKF525A (25 mg/kg) was given 30 min before saline or PCP (10 mg/kg). QNB was injected 15 min later and the animals were sacrificed 60 min after that. The volume of all injections was 0.1 ml/10 g body weight.

Results

Analyses of the data revealed that there were significant

TABLE 6

EFFECTS OF SKF525A ON PCP ENHANCEMENT OF QNB ACCUMULATION

Group*	N	Region†‡§ Cere	Hyp	Hip	Cor	Str
S+S	5	1.13 ± 0.08	1.32 ± 0.08	1.38 ± 0.08	1.39 ± 0.08	1.32 ± 0.10
S+P	5	1.12 ± 0.10	1.41 ± 0.12	1.70 ± 0.17	1.79 ± 0.17	1.78 ± 0.17
SKF+S	5	1.54 ± 0.08	1.81 ± 0.12	1.92 ± 0.09	1.94 ± 0.07	1.89 ± 0.08
SKF+P	5	1.49 ± 0.06	2.05 ± 0.10	2.45 ± 0.12	2.41 ± 0.15	2.54 ± 0.09

*Saline (S) or SKF525A (SKF, 25 mg/kg) was given IP 30 min before S or PCP (10 mg/kg, IP) which was given 15 min before IV administration of QNB (1.6 μg/kg). Animals were sacrificed 60 min after QNB.

†Data presented as mean (pmol/mg tissue) ± S.E.

‡Three-way ANOVA revealed that there were significant ($p<0.05$) PCP, region and SKF effects as well as PCP × region and SKF × region interactions.

§One-way ANOVA demonstrated that the effects within each group across region were significant ($p<0.05$) as were within region across group comparisons.

PCP, region and SKF525A effects as well as PCP × region and SKF525A × region interactions (Table 6). Further ANOVAs demonstrated that the effects within each group-across region were significant as were the within region-across group effects (Table 6). In comparison to controls, SKF525A elevated QNB accumulation in all brain areas and PCP enhanced QNB accumulation in hippocampus, cortex and striatum. The effects of SKF525A plus PCP on QNB accumulation was not different from that expected if the effects of SKF525A and PCP individually were added together. SKF525A, but not PCP, decreased the concentration of QNB in the plasma.

GENERAL DISCUSSION

Previous studies of *in vivo* binding of QNB by autoradiographic and quantitative radioisotopic techniques [18,25] have suggested the usefulness of this approach. It allows studying the effects of compounds in a system undisturbed by membrane disruption, providing for the presence of normally distributed metabolites of the drug of interest, and allowing for interactive effects of the various pertinent systems. For example, both morphine and amphetamine, but not other compounds (diazepam, phenobarbital, meperidine, naloxone), appear to increase QNB binding to muscarinic receptors after *in vivo* administration via indirect mechanisms [20]. We previously reported [4], and have confirmed herein, that PCP also increases QNB binding when given *in vivo*. This enhancement does not appear to be due to enhanced transport of QNB into brain because no changes in plasma or cerebellar QNB concentrations were observed. Rather, the PCP effect appears to be on specific binding since prior administration of atropine is able to block the enhancement [4]. Because these data are in contrast to the inhibition by PCP on QNB binding *in vitro* [2, 4, 23] we conclude that PCP must be acting indirectly to produce these effects, though differences between physiological and test tube conditions with respect to ionic concentrations, pH, etc., cannot readily be excluded.

Consistent throughout these studies was the finding of regional differences in QNB accumulation in that the cerebellum consistently had significantly less QNB than did the hippocampus, cortex and striatum as would be expected for an area with few cholinergic receptors. The hypothalamus had values intermediate to those of the cerebellum and other regions. These findings are consistent with other reports from rats [25] with respect to the contrast between cerebellum and other regions.

Our present work was to determine whether two major PCP metabolites found in rodents might contribute to the PCP effect on QNB. At the times and dosage tested 4-OH-pip PCP produced no effect on QNB accumulation in plasma or in any brain area tested.

In contrast to the 4-OH-pip PCP, 4-OH-cyclo PCP increased QNB accumulation in brain when given 30 minutes before the QNB. These effects were dose and region dependent. No significant changes were seen in cerebellum or hypothalamus (though a trend toward an increase was seen) while significant increases were seen in the remaining areas tested. At 28 mg/kg, only the striatum showed the 4-OH-cyclo PCP induced effect. Whether this regional difference reflects an ability of the drug to more readily penetrate the striatum, an interaction of the metabolite with endogenous compounds mediating the QNB effect which are in higher concentration in the striatum than the other areas, or a reflection of QNB receptor differences between regions is not known.

The enhancement of QNB accumulation in brain by 4-OH-cyclo PCP was blocked by prior administration of atropine suggesting that the action of this metabolite was on specific binding. This effect is similar to that found with PCP [4].

A difficulty for these studies on the *in vivo* effects of peripherally administered metabolites is that they may not reach the brain hence any effects observed may be a result of peripheral influences on the brain. Though there is little doubt that peripheral administration of PCP results in the accumulation of measurable quantities of monohydroxylated metabolites in the rodent brain [19], there appear to be no studies which directly measure these metabolites after their own administration. There is, however, indirect evidence indicating that biologically significant quantities of metabolite do accumulate in brain after peripheral administration since increases in the locomotor activity of rats [10], induction of convulsions in dogs [10], increases in avoidance response rate in rats [22], and EEG patterns in monkeys [22]

have been observed after administration of these metabolites. Though these measures could be influenced by peripheral effects of the drugs, their action in inhibiting ^{3}H-dopamine uptake into rat brain striatal tissue *in vitro* [15] could not.

In a further attempt to partition any possible effects of metabolites from that of PCP, animals were treated with a microsomal enzyme inhibitor SKF525A which has been shown to block the metabolism of PCP and enhance its effects. Though SKF525A by itself enhanced the accumulation of QNB in brain, it did not appear to alter the increase in QNB in the brain produced by PCP, i.e., the effect of SKF525A plus PCP did not appear greater than the effect of SKF525A alone added to the effect of PCP alone. As an assurance that SKF525A was producing the desired effect of enzyme inhibition, other groups of animals treated in a manner similar to those above were observed over a 90 minute period. SKF525A substantially prolonged the gross behavioral (e.g., activity, stereotyped behavior) effects of PCP. This indicates that at the doses tested, liver microsomal derived PCP metabolites did not contribute to the effects on QNB.

It is recognized that the two metabolites tested in these studies do not reflect the total of PCP derived metabolic products in mice [6, 13, 14], however, to test them all is beyond the scope of this work. Though doubtful, it is possible that there may be some contribution of 4-OH-cyclo PCP to the PCP effect. However, the dose of 4-OH-cyclo PCP necessary to produce significant changes in QNB accumulation was much greater than that needed for PCP. Furthermore, similar effects of PCP on QNB accumulation have been found with doses of PCP at least one fourth of those used in these studies [4]. Given these facts, it appears probable that PCP is producing its effects on QNB accumulation via other systems.

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