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Behavioral Effects of Phencyclidine and Some of Its Metabolites in Developing Cats[1]

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LEVINE, M. S. AND S. HOWARD-BUTCHER. *Behavioral effects of phencyclidine and some of its metabolites in developing cats.* PHARMACOL BIOCHEM BEHAV **25**(2) 359–363, 1986.—Chronic treatment with low doses (1–2 mg/kg) of phencyclidine (PCP) produces marked behavioral effects in the developing kitten. These effects are age-dependent. Under 21 days the major response consists of rostrocaudal forelimb movements. After this age the major responses are ataxic locomotion and waxy rigidity. In the present experiment the behavioral effect of subcutaneous injections of higher doses of PCP, two of its metabolites, phencylclohexylamine (PCA) and an alcohol (N-(5-hydroxypentyl)-1-phenycyclohexylamine) (AL) and one PCP analog (N-N-diethylaminophenylhexylamine (NND)) were tested in kittens between 30–50 days of age. The behavior of the kittens was assessed from 10–15 min pre-injection to up to 5 hr postinjection and at 24 hr postinjection. The most intense responses were produced by PCP and NND and consisted of waxy rigidity (limbs in abnormal postures for extended time periods) with the animal completely immobile. PCA was less effective, producing only tremor and staggering followed by loss of hindlimb support. AL was the least effective producing minimal behavioral responses. These results indicate that some of the metabolites of PCP, while active, are not as potent as PCP itself. Subsequent testing indicated that behavioral tolerance to the effects of PCP, NND and PCA occurred after a single administration of these compounds.

Phencyclidine Metabolites of phencyclidine Behavior Cats Tolerance

PHENCYCLIDINE (PCP) is an arylcycloalkylamine derivative structurally and has pharmacological actions similar to the dissociative anesthetic, ketamine. It was developed as a surgical anesthetic, analgesic and for research investigations in mental disorders [6,12]. Due to the high incidence of severe central nervous system side effects its use has been discontinued. Its derivatives however, are currently in widespread use as animal tranquilizers in veterinary practice and as such may be diverted to "street" drug use.

Although the behavioral effects of PCP have been studied extensively [1] the mechanism underlying the long duration of action of PCP has not been described. One possible explanation for this long duration of action is the formation of active metabolites. While the pharmacological contribution of many of the metabolites of PCP have not been determined, the long lasting effect of phenylcyclohexylamine, one PCP metabolite, on dopamine release in the rat neostriatum has recently been described [18]. The metabolism of PCP was first described by Glazko and his colleagues [14] who reported its hydroxylation in the 4-position and the 3-position of the piperidine ring. They also noted substantial species differences in the formation of metabolites. Recently, major metabolites and analogs have been identified and synthesized in sufficient quantities to determine their actions [2,3]. It is important to determine the behavioral effects of metabolites because they are frequently biochemically active and have the potential of influencing the pharmacological profile of the parent compound [13]. Metabolites can accumulate with repeated administration and contribute to the chronic actions of PCP itself [13] and/or stimulate PCP receptor sites in the central nervous system [15, 22, 23].

In the present experiments the effects of PCP, two of its metabolites, phenylcyclohexylamine (PCA) and an alcohol (N-(5-hydroxypentyl)-1-phenylcyclohexylamine) (AL) [2,4] and one PCP analog (N-N-diethylaminophenylhexylamine) (NND) were assessed in developing kittens. There is evidence that AL is a metabolite of PCP in the cat [7,8]. PCA is a metabolite of PCP in humans and mice [11,14] although it is uncertain if it occurs in large quantities in the cat [7,8]. NND is one of the most potent synthetic analogs of PCP. This compound was used so that the behavioral effects of series of similar compounds of different potencies could be compared. The results indicated that behaviorally NND was slightly less effective than PCP but more effective than the metabolites.

The developing cat was chosen to determine the effec-

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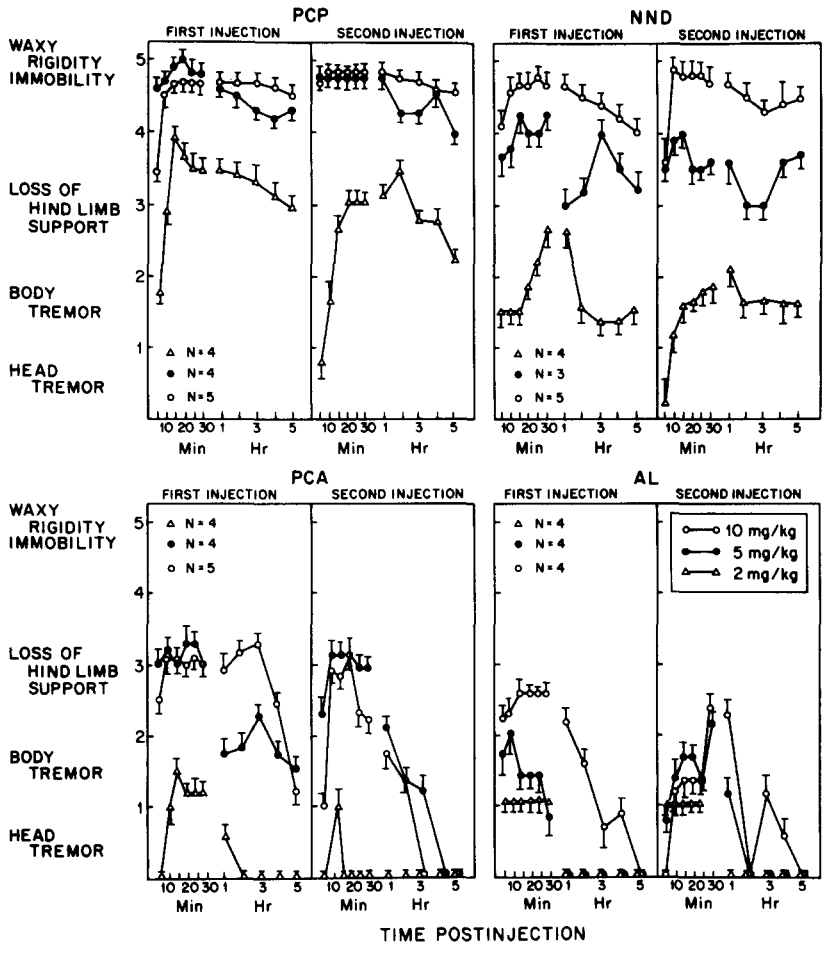

FIG. 1. Comparison of effects of different doses of each compound for two injections spaced one week apart. Each point represents the average rating score of all kittens tested at that time postinjection. Ranges at each point are standard errors of the mean. Note scale change on abscissa.

tiveness of these compounds for several reasons. Our previous findings [9] indicate that the kitten seems to be particularly sensitive to PCP. Many of the behavioral effects of this drug are seen at much lower dosages in the cat than in the rodent. In the cat treatment with low doses of PCP (1–2 mg/kg IP) produced motor disturbances that were age-related. In animals under 3 weeks of age the major response consisted of rostrocaudal forelimb movements. After this age the major drug-induced responses were ataxic locomotion (consisting of staggering, swaying, and falling during walking) and waxy rigidity. Since a portion of this research is directed toward determining the effects of these compounds during early postnatal periods, the longer period of postnatal development in the cat compared to the rodent allows more time for chronic treatment to produce long-lasting effects.

METHOD

A total of 50 (28 males and 22 females) (28–38 days of age at the time of the first injection) kittens taken from 16 litters born in the Mental Retardation Research Center cat breeding colony at UCLA were used in these experiments. Our previous developmental study indicated that the behavioral effects of PCP did not vary significantly in this age range [9]. Animals were divided into 12 groups. Each group was balanced to have approximately the same number of males and females. Age ranges in each group were also similar. No gender differences in the effects of the tested compounds were observed and data from males and females were pooled in each group. Three dosages (2, 5 and 10 mg/kg) of PCP, PCA, AL and NND were administered subcutaneously behind the neck to the kittens. This route allows immediate absorption into the vascular system and produces rapid behavioral effects. In order to avoid the effects of multiple doses of the same compound or interactions among different compounds, each animal received only one dosage level of a single compound. Number of kittens in each group is shown in Fig. 1.

In order to determine if behavioral tolerance occurred within each dosage, each animal received a second injection of the same compound (same dosage) one week after the first injection. Animals were 35–45 days of age during this second test. An additional group of 5 kittens (2 males and 3 females 29–32 days of age during the first test) received a single dose of the vehicle (saline, volume equivalent to 2 mg/kg PCP) for

tolerance control. One week later they received 2 mg/kg PCP.

Before receiving an injection each animal was placed in a sound-attenuating isolation chamber and observed for 15 min. Behavioral ratings were always at zero during this observation period when assessed with the rating scale described below. This observaton period was followed by drug administration. After the injection, the behavior of each animal was assessed and rated on a six-point scale for up to 5 hr postinjection. Animals were then either returned to their home cage or, if still under the effects of the drugs, kept in a temperature-controlled environment until they recovered. Behavior of all animals was assessed and rated 24 hr postinjection as well. For the first 45 min postinjection behavioral assessments were made every 5 min. From 1–5 hr postinjection assessments were made every 15 min. The rating scale used was as follows: 0—no effect, 1—head tremor, 2—head and body tremor, 3—loss of hindlimb support, 4—loss of forelimb and hindlimb support, but capable of locomotion by crawling, 5—animal immobile, waxy rigidity. Segments of behavior at fixed time periods postinjection were videotaped for subsequent reanalysis. These segments were independently rated by two raters, blind to the compound and dose administered. Interrater reliability coefficients exceeded 0.95. This rating scale has been developed to discriminate among the behavioral effects of different dosages of PCP administered to either developing kittens or adult cats [9,10]. It is capable of discriminating interactions among other compounds that potentiate or attenuate the behavioral effects of PCP in both developing and adult cats [16].

These procedures were repeated for each animal one week following the first injection. Each animal received the same treatment in the second test as in the first. Comparison of intensity and duration of action of each compound for the two tests determined if tolerance had occurred.

In order to perform statistical assessments of differences among the groups receiving each compound, data from each animal were first averaged across two time periods, 1–30 min and 1–5 hr postinjection. Data from all animals in each group were then averaged for each time period. Differences among the group means were analyzed with the unweighted-means solution for a two-way analysis of variance since group sizes were unequal ([21], p. 374). Individual comparisons among cells used appropriate error terms for computing F or t values ([21], pp. 377–378). In order to simplify the statistical reporting, only probability values are indicated in the text. Details of values of statistical tests for each comparison are available from the authors upon request. For statistical assessment of the effects of tolerance, the behavioral effects of each substance were tested separately with a two-way analysis of variance for repeated measures [21]. Again data for each animal were averaged across the two time periods (1–30 min and 1–5 hr postinjection) and then for all animals in each group.

RESULTS

All compounds produced behavioral alterations at each dose tested. The intensity and the duration of the effects were both dose-dependent and a function of the compound. Figure 1 illustrates the effects of administration of the four compounds at each dose for the entire 5 hr observation period. At each time point postinjection, ratings of behavior of all animals receiving the same compounds and dosages were averaged. Rating scores were averaged over each 15 min epoch for 1–5 hr postinjection. After 30 min behavioral effects did not change markedly over short time periods. Ratings were thus averaged over each 1 hr period for each animal.

The most intense and persistent responses were produced by PCP at all doses. At 5 and 10 mg/kg PCP induced immobility and waxy rigidity (limbs in abnormal postures for extended time periods) for the full 5 hr observation period. These effects developed rapidly within the first 5–10 min postinjection. At 2 mg/kg the effects of PCP were less marked and occurred more slowly. All animals lost hindlimb support but some were capable of locomotion by crawling. The behavioral effects lasted for the full 5 hr observation period. NND, the PCP analog, at each dose produced slightly less intense behavioral responses than PCP but the types of responses produced were similar. At 10 mg/kg waxy rigidity and immobility occurred in virtually all animals. Decreases in both the intensity and duration of the responses occurred at both 5 and 10 mg/kg. The major behavior produced was loss of limb support and this effect decreased in intensity over the 5 hr observation period. At 2 mg/kg NND produced body tremor which was followed by loss of hindlimb support. This effect peaked at about 1 hr postinjection and then decreased in intensity. The two metabolites of PCP, PCA and AL, were clearly less potent than either PCP or NND. PCA at doses of 5 and 10 mg/kg produced loss of hindlimb support. This response began to subside 3–5 hr postinjection. At 2 mg/kg the maximum response (body tremor) was slow to develop and lasted less than 2 hr. AL was the least effective compound. Maximum responses at 5 and 10 mg/kg consisted of loss of hindlimb support and body tremor. At 2 mg/kg only body tremor occurred. The duraton of the effect of this substance was short even at the highest doses administered. At 10 mg/kg the behavioral effects decreased from 1 to 5 hr. At 2 and 5 mg/kg no effects were observed after 1 hr postinjection.

Only PCP and NND at 5 and 10 mg/kg produced behavioral effects 24 hr postinjection. Animals displayed tremor, staggering and ataxic locomotion. Behavior was not rated systematically for all animals between 5 and 24 hr postinjection. However, at 9–12 hr postinjection most animals that were not returned to the home cage were observed for 5–10 min. Animals that received PCP and NND at 5 and 10 mg/kg showed altered behavior. Animals receiving PCP at 10 mg/kg displayed loss of hindlimb support (average rating score=3.2) while those receiving 5 mg/kg displayed only body tremor (average rating score=1.8). Animals receiving 10 mg/kg NND displayed body tremor but only rarely lost hindlimb support (average rating score=2.7) while those receiving 5 mg/kg displayed only head tremor (average rating score=0.8). These results indicate that the effects of NND may decrease faster than those of PCP.

The results of the statistical analyses indicated that PCA and AL produced significantly less intense responses than either PCP or NND ($p<0.05$). NND at 2 mg/kg produced a significantly less intense response than PCP at both time periods and from 1–5 hr postinjection at 5 mg/kg ($p<0.05$). At 5 and 10 mg/kg for each time period PCA produced significantly more intense responses than AL ($p<0.05$). At 2 mg/kg there were no significant differences between the intensity scores for these two compounds.

Behavioral tolerance occurred to the effects of most compounds at the lowest dosage administered (Fig. 1). No tolerance occurred when PCP at either 5 or 10 mg/kg was

administered. However, at 2 mg/kg the effects of the second injection began with a longer time course and peaked slightly later and at a lower rating score. The difference between rating scores for the first 30 min for the two tests one week apart was statistically significant ($p<0.05$). A similar result occurred when NND was administered. There were no differences between the effects produced by either 5 mg/kg or 10 mg/kg for the two injections. However, when 2 mg/kg was administered one week later the behavioral effects occurred more slowly over the first 30 min after the second injection ($p<0.05$) and remained significantly lower ($p<0.05$) over the 1–5 hr postinjection period.

When PCA was administered the second time, behavioral tolerance occurred at both 5 and 10 mg/kg for 1–5 hr postinjection and for 2 mg/kg for the first 30 min postinjection. Differences among average rating scores for each of these dosages and time periods were statistically significant ($p<0.05$). There was no behavioral tolerance to the effect of AL at any dosage administered.

Although not shown in Fig. 1, data from the first dose vehicle-control group that received 2 mg/kg PCP one week after a saline injection were similar to data obtained from the animals receiving 2 mg/kg PCP first. Average rating score for the first 30 min was 3.46 ± 0.53 (mean±S.E.) for vehicle-control group after 2 mg/kg PCP (compared to 3.25 ± 0.47 for 2 mg/kg PCP first injection) and was 3.67 ± 0.61 for 1–5 hr postinjection (compared to 3.35 ± 0.21 for 2 mg/kg PCP, first injection). When compared statistically (two-way analysis of variance), there were no significant differences among the groups indicating that placing the animals in the observation chamber or performing injections of saline vehicle do not produce behavioral tolerance.

DISCUSSION

Two major findings emerged from these experiments. First, metabolites of PCP are behaviorally active. They produce similar but less intense and shorter duration behavioral effects than those produced by equal doses of PCP. NND, a PCP analog, produces similar though slightly less intense effects than PCP. Second, behavioral tolerance to the effects of one injection of PCP, NND, and PCA occur in the developing cat. These effects are most prominent with low dosages of the compounds. No behavioral tolerance to the effects of AL was observed.

The results of the present experiments demonstrate that at least two metabolites of PCP are behaviorally active in that they produce similar although less intense responses than PCP itself or NND, a PCP analog. In a recent experiment [17,18] it was also shown that metabolites of PCP had pharmacological activity in rats. It is possible that some of the longer-lasting effects of PCP might be due to the action of metabolites that form in the organism as the PCP is degraded. Brain levels of some of these metabolites are quite high for periods up to 3 weeks after adminstration of PCP in the rat [12]. There are reasons why behavioral actions of the metabolites might be less intense than those of the parent compound. Metabolites are more water soluble than the parent compound and could distribute in less quantity in the brain [2,4]. They might be acting on a different neural region than the parent compound. Differential action of the compounds might also relate to route of administration.

A series of recent studies has described the metabolism of PCP in humans and several species of animals [3–5, 7, 8, 11, 14]. Extensive metabolism occurs within 1 hr of PCP administration [11]. Approximately 31% of urinary excretion products of PCP after low acute doses in humans, were composed of the hydroxylated metabolites [3] while 16% were unmetabolized PCP. PCA has been identified as a significant metabolite in the urine of the rat, mouse [5] and rabbit [8]. These *in vivo* studies indicate that two major metabolic pathways involving the cycloalkyl and the piperidine rings exist. While hydroxylation appears to be the primary pathway, the overall excretion of PCP indicates that a substantial amount of the metabolized drug was not accountable by these substances. Therefore, additional pathways should exist, some of which may be pharmacologically important and may account for the long duration of action of PCP. The presence of PCA in urine as the efflux of PCP indicates that the degradation of the piperidine ring has occurred and our behavioral studies demonstrate that PCA is pharmacologically active.

Recent reports have indicated the presence of highly specific PCP receptors in the brain [15, 20, 22, 23]. Such receptors are of highest density in cortex and hippocampus, intermediate density in striatum, cerebellum and hypothalamus and low density in brain stem and spinal cord [15,22]. In addition, the potencies of a number of PCP analogs in displacing [^{3}H]PCP binding *in vivo* have been found to correlate highly with their potencies in behavioral tests [20,22]. Although there is little information available, it is possible that metabolites, as well as analogs of PCP, are capable of stimulating PCP receptors and producing behavioral actions. Metabolites are known to accumulate with repeated use and may either nonspecifically occupy or stimulate PCP receptor sites.

The behavioral effects of all compounds tested in this study reflect a basic disturbance in motor activity. Moderate to low intensity behavioral alterations consisted of tremor, ataxia, and staggering. More marked effects consisted of loss of all limb support with eventual cataleptic-like behavior. Although finer aspects of sensory disturbances were not assessed, animals were usually responsive to auditory, visual and somatosensory stimulation except when immobile and displaying waxy rigidity. They were, however, responsive to strong nociceptive stimuli during this latter phase. Intense sensory stimulation usually elicited vocalization and gross body movement.

There was little difference between the effects of 5 and 10 mg/kg of both PCP and NND. This probably represents both a ceiling effect of the rating scale and the fact that observations were not systematically made between 5 and 24 hr postinjection. Some of the animals that displayed behavioral effects 5 hr postinjection were observed about 9–12 hr postinjection. These observations indicated that animals receiving NND recovered more rapidly than those receiving PCP.

In the present study relatively high doses of PCP were used compared to those reported in previous experiments on developing cats [9]. Such doses were necessary in order to make comparisons with the effects of the metabolites because at doses less than 2 mg/kg the behavioral effects of metabolites were not notable. In research presently underway the effects of lower doses of the compounds are being assessed. In addition, more sensitive measures such as disruption of ongoing operant behavior (e.g., lever-pressing or discrimination performance) are being utilized to determine the effectiveness of these substances.

One dose of either PCP, NND or PCA produced behavioral tolerance. Tolerance was not observed to administra-

tion of AL. Usually tolerance occurs to high dosages of compounds [19]. It was, therefore, surprising that tolerance effects were most often observed at the lowest dosage. This result might relate to the ceiling effects of our rating scale at the higher dosage. For example, if systematic observations were continued beyond 5 hr postinjection the behavioral effects of the higher dosages might have decreased more rapidly after the second injection.

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