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Effects of Para-Chlorophenylalanine and 5-Hydroxytryptophan on Mouse Killing Behavior in Killer Rats[1]

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GIBBONS, J. L., G. A. BARR, W. H. BRIDGER AND S. F. LEIBOWITZ. *Effects of para-chlorophenylalanine and 5-hydroxytryptophan on mouse killing behavior in killer rats.* PHARMAC. BIOCHEM. BEHAV. 9(1) 91–98, 1978.—The effects of para-chlorophenylalanine (PCPA) on mouse killing behavior were examined in natural killer rats. Forty-eight hr after injection, this serotonin synthesis inhibitor, at relatively low doses of 75 and 150 mg/kg, facilitated mouse killing, as indicated by a decrease in latency to attack the mouse. This effect was revealed in a test of satiation, in which five successive mice were presented to the rat, and also in a novel cage situation. Other than the shorter latencies to attack and kill mice, the killing response was similar in topography to the natural kill. The increase in killing after PCPA injection was associated with a reliable reduction in brain serotonin and in 5-hydroxyindoleacetic acid, and the time courses of the behavioral and biochemical changes were generally similar. In contrast to PCPA, injection of the serotonin precursor 5-hydroxytryptophan (5–HTP, 100 mg/kg) reliably lengthened attack and kill latencies in killer rats. In rats pretreated with PCPA, 5–HTP not only reversed this drug's facilitation of killing, but completely blocked killing in 67% of the rats tested. These results strengthen the hypothesis that brain serotonergic neurons are involved in the inhibition of mouse killing.

PCPA Muricide Serotonin 5-HIAA 5-HTP

THERE is increasing evidence to suggest that central serotonergic systems may inhibit mouse killing behavior in rats. This hypothesis is based largely on studies using the tryptophan hydroxylase inhibitor, para-chlorophenylalanine (PCPA). Injections of PCPA induce mouse killing in nonkiller rats, and increase the proportion of killers among previously untested rats [11, 12, 23, 29, 31, 40, 41].

However, the mouse killing response after PCPA injections may differ from the natural killing response. Rather than directing bites to the head or neck region of the mouse, the PCPA-induced killer may bite indiscriminately at all areas of the mouse's body [31]. Moreover, high doses of PCPA (300 mg/kg to six injections of 700 mg/kg) are required for the increased killing to be observed. At these higher doses, PCPA appears to be acting in a nonspecific fashion, causing not only aberrant killing behavior, but also increased intraspecific fighting [45] and increased irritability [20,40].

In the above experiments, the facilitative efffects of PCPA on mouse killing were examined in nonkiller rats which were induced to kill by the drug. Since killer rats may respond differently from nonkillers to drugs and lesions [21,51], it may be helpful to study the response of killer rats to PCPA injections. In the present studies, killer rats were tested in two behavioral paradigms which allowed either an increase or a decrease in killing to be observed. Lower doses of PCPA were used since these might cause changes in ongoing behavior, while being ineffective in inducing new behaviors. The time course of PCPA's effects on mouse killing were examined in relation to changes in brain serotonin and 5-hydroxyindoleacetic acid (5–HIAA). The interaction of PCPA with the serotonin precursor 5-hydroxytryptophan (5–HTP) was also investigated. Furthermore, the specificity of PCPA's effects were assessed by the measurement of changes in irritability, food and water intake, and locomotion in the open field.

METHOD

Animals

Rats. The animals used were 157 male Long-Evans

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hooded rats, weighing 350–700 g, obtained from Blue Spruce Company (Altamont, NY). They were housed in individual cages, in a laboratory maintained at 22 ± 1°C on a 10 hr on, 14 hr off light cycle. Laboratory chow and water were available ad lib, except where otherwise specified.

Mice. Mice used as prey were adults of various strains and both sexes. They were group-housed in the same laboratory as the rats.

Drugs

PCPA ethyl ester (Aldrich Co.), was dissolved in 0.85% physiological saline, and the pH adjusted to 5.6–6.0 by the addition of a few drops of 1N NaOH. 5–Hydroxytryptophan (5–HTP; Sigma) was suspended in a 5% (w/v) solution of gum arabic (Sigma). Injections were given intraperitoneally in a volume of 1 ml. Appropriate vehicles were used in a volume of 1 ml for control animals.

Behavioral Tests

Initial mouse killing tests. After at least 8 days of adaptation to the laboratory, each rat was tested for mouse killing. A mouse was placed in the rat's home cage and the occurrence or nonoccurrence of a kill was recorded 24 hr later. Only "killers" were used in these experiments.

Experimental mouse killing tests. Since the mouse killing response in an experienced killer rat may occur too rapidly for facilitation to be observed, two procedures were devised for investigating the killing response in killer rats. These procedures have the advantage of allowing either a facilitation or an inhibition of the behavior to be observed.

Satiation test. The satiation test was based on previous reports that with repeated presentations of mice to killer rats, their attack and kill latencies lengthened [2, 3, 22, 24, 36]. In the present study, five mice were successively placed in the home cage of the rat, and sniff, attack, and kill latencies were recorded. Immediately after a kill, or after 30 min if no kill occurred, the mouse was removed from the cage. Sixty sec later, the next mouse was introduced into the cage. If two successive mice were not killed within 30 min, trials were discontinued and maximal attack and kill latencies recorded for remaining trials. For all killed mice, the locations of the wounds were marked on a drawing of the mouse. The satiation score, used for some statistical analyses, was defined as the difference between the mean attack latency on the first two trials and the mean attack latency on the final two trials.

The novel cage procedure was based on a study in which rats showed longer killing latencies when placed in a cage other than the home cage [1]. In the present experiments, the rat was placed in a novel transparent Plexiglas chamber (32×32×26 cm high) with a 1.25 cm grid floor. After 5 min of adaptation, the rat was presented with a mouse. Sniff, attack, and kill latencies were recorded, and after 35 min, the rat was removed from the chamber. The mouse was removed immediately after a kill and locations of the wounds were noted.

Open field tests. The open field apparatus was a 90 cm square chamber, 32 cm high, covered by one-way glass and illuminated by two 15 W fluorescent lights. The floor was divided with masking tape into 36 squares, 15 cm on a side. The rat was introduced into one corner of the field and the number of squares crossed was recorded each minute for 10 min.

Biochemical Methods

In order to measure brain serotonin (5–HT) and 5-hydroxyindoleacetic acid (5–HIAA) levels, 29 rats were sacrificed by decapitation between 13:00–15:00 hr, during the middle of the light portion of the light-dark cycle. The brains were rapidly removed, frozen on dry ice, and stored at −50°C for a maximum of 2 weeks before analysis. 5–Hydroxytryptophan (5–HTP) and 5–HIAA in whole brain were determined spectrophotofluorometrically according to the method of Curzon and Green [10]. Recoveries of standards averaged 98% for serotonin and 71% for 5–HIAA. Values presented here have not been corrected for recovery.

Statistical Methods

In order to minimize heterogeneity of variance, the latencies to sniff, attack, and kill mice were transformed to logarithms of the form $X' = \log_{10}(X+10)$ where X was the latency in seconds [18]. Data were then analyzed by the appropriate analysis of variance (ANOVA) or by a *t* test. Error terms for the ANOVA tests were pooled when appropriate according to the procedure described by Myers [37].

Experimental Designs

PCPA and mouse killing tests. Thirty killer rats that showed increasing attack and kill latencies on a satiation test were randomly assigned to one of three groups. Animals in each group were injected with saline and tested for satiation of killing 48 hr later (pretest). Two days to 3 weeks later, they were given either saline (Group 1, 10 rats), 75 mg/kg PCPA (Group 2, 10 rats), or 150 mg/kg PCPA (Group 3, 10 rats), and tested once again for mouse killing 48 hr after injection (post-test).

In order to examine the time course of PCPA's effects on mouse killing behavior, 18 killer rats (N=18) were tested using the novel cage procedure. A crossover design was used, with nine animals injected with PCPA (150 mg/kg) and nine with saline. All were tested 48 hr later for mouse killing in the novel cage. After 14–16 days, the injections were reversed and the rats tested 48 hr later in the novel cage.

Interaction of 5–HTP and PCPA in their effects on mouse killing behavior. The drug 5–HTP, which restores brain serotonin after PCPA injections by bypassing the hydroxylation step [21,31], was studied for its effects on mouse killing behavior and its interaction with PCPA in mouse killing behavior. Thirty-seven killer rats that showed increasing attack and kill latencies on a satiation pretest were randomly divided into three groups. Rats of Group 1 (n=12) were injected with PCPA (150 mg/kg) and 48 hr later with the 5–HTP vehicle, and then tested 30 min later on a satiation mouse killing test. Rats of Group 2 (N=12) were injected with saline and 48 hr later with 5–HTP (100 mg/kg), and then tested 30 min later on a satiation mouse killing test. Rats of Group 3 (N=13) were injected with both PCPA (150 mg/kg) 48 hr before testing and 5–HTP (100 mg/kg) 30 min before testing on a satiation killing test.

PCPA and ingestive behaviors. For 11 killer rats, daily food and water intake and body weights were measured for 1–4 days before and for 16 days after injection of PCPA (150 mg/kg). Food intake was recorded as the difference in weight of the food pellets from one day to the next with the amount spilled on the trays measured and taken into account.

PCPA and irritability. For 15 rats used for the PCPA novel cage testing, described above, irritability to rough and

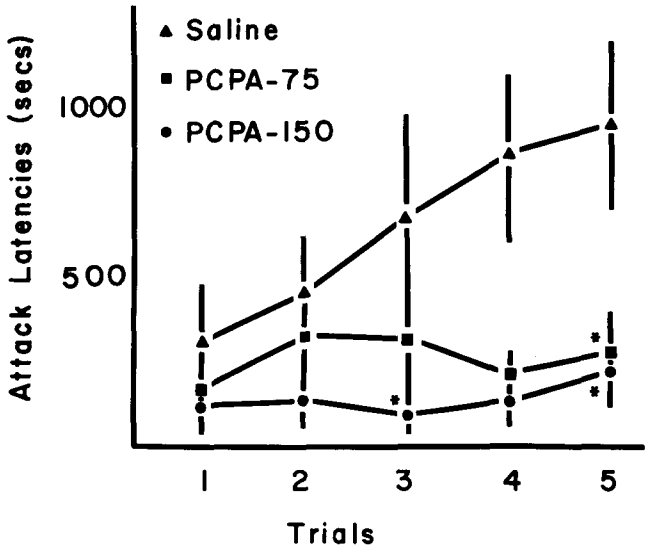

FIG. 1. Attack latencies ± SEM on the five mouse presentation trials of the posttest. (PCPA–75 is PCPA 75 mg/kg, and PCPA–150 is PCPA 150 mg/kg. *Signifies significantly different from the pretest value, $p<0.05$.)

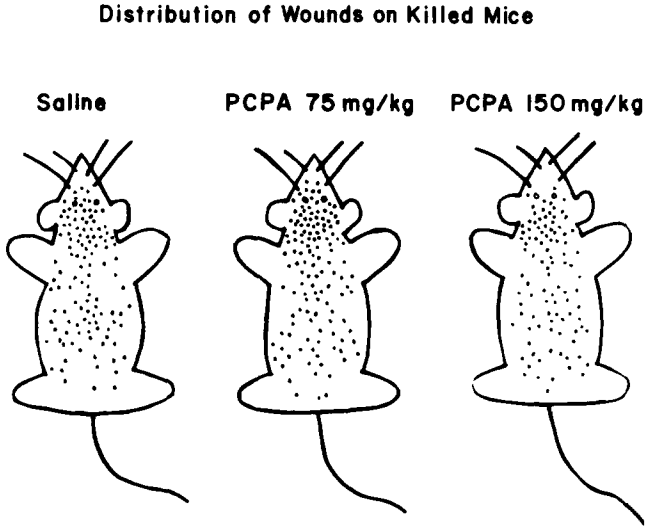

FIG. 2. Locations of wounds on all mice killed during the posttest trials. The total number of mice killed by each group were: saline, 36 mice; PCPA 75 mg/kg, 50 mice; and PCPA 150 mg/kg, 45 mice.

normal handling was studied. While being transferred to the novel cage, the number of squeals, bites, and the amount of struggling (none, moderate, or extreme) were noted. In addition, after the novel cage test, biting and squealing responses to rougher handling were noted. Stimuli included a probe placed in the mouth, turning the rat over on its back, and approaching from the front with a gloved hand.

PCPA and activity. Locomotion in an open field was measured in killer rats (N=10) injected with PCPA (150 mg/kg) or saline, 48 hr before testing. Each animal received both treatments in a counterbalanced order, with 14–16 days intervening between tests.

Brain 5-HT and 5-HIAA levels after PCPA injection. Twenty-nine rats (approximately half killers and half nonkillers) were sacrificed and their brains analyzed for 5-HT and 5-HIAA. Seven rats injected with 150 mg/kg PCPA, five rats injected with 75 mg/kg PCPA, and six rats injected with saline were sacrificed 48 hr after injection. Six additional rats were sacrificed 8 days after injection of PCPA (150 mg/kg) and five rats 14 days after PCPA (150 mg/kg).

RESULTS

PCPA and mouse killing behavior. PCPA significantly facilitated mouse killing in killer rats, by decreasing the latencies to attack and kill mice, particularly on the later trials of the satiation test. These results are presented in Fig. 1.

There were no significant differences in the pretest latencies to sniff, attack, and kill the mice among the three groups. Moreover, the saline-saline control group (Group 1) showed no changes in sniff, attack, or kill latencies from pretest to posttest. Over the five trials of mouse presentations, the attack and kill latencies increased as expected, $F(4,81)=9.75$, $p<0.001$ and $F(4,81)=9.93$, $p<0.001$, respectively.

In contrast to the control group, both the groups injected with PCPA showed decreased attack latencies on the third, fourth, and fifth trials of the posttest. This was indicated by a significant interaction between the test session (saline or PCPA) and the trial (five mouse presentations), for 75 mg/kg PCPA, $F(4,72)=3.34$, $p<0.025$, for 150 mg/kg PCPA, $F(4,72)=2.73$, $p<0.05$. An analysis of this interaction (test of simple main effects, [18]) showed that the attack latencies increased over trials on the saline pretest, $F(4,81)=5.28$, $p<0.001$ (Group 2), $F(4,81)=4.14$, $p<0.05$ (Group 3), while on the PCPA posttest, latencies did not increase over trials, $F(4,81)=0.20$, N.S. (Group 2), $F(4,81)=0.38$ N.S. (Group 3). Similar results were obtained for the kill latencies which were highly correlated with attack latencies (r=.95 to .99), although there was only a trend toward an interaction between the test and the trial, $F(4,36)=2.49$, $p<0.10$ (Group 2) and $F(4,72)=2.21$, $p<0.10$ (Group 3). There were no significant differences among sniff latencies.

The increased mouse killing response to PCPA treatment appeared to be unrelated to weight loss after injection. During the 48 hr between injection and testing, the 150 mg/kg PCPA group lost 12.5 g (SEM=4.9) while the 75 mg/kg group gained 1.4 g (SEM=2.9). For the 150 mg/kg group, the correlation between weight loss and the response to PCPA (as measured by the change in satiation scores) was −.20 and was not significant.

Other than the shorter attack latencies on later trials of the satiation test, the killer rats facilitated by PCPA showed no differences from untreated rats in their killing responses. The locations of the wounds on the bodies of the mice killed by PCPA treated rats were also similar to untreated controls (see Fig. 2). The mean number of wounds on the head, neck, and back during the pretest were compared to the mean number of wounds in those areas on the posttest. There were no significant differences for any group for any area of the body except that the animals injected with PCPA (75 mg/kg) on their posttest inflicted more wounds to the head than on the saline pretest, $t(8)=3.67$, $p<0.01$.

Injections of PCPA also reduced brain serotonin and 5-HIAA (Table 1). PCPA (150 mg/kg) reduced 5-HT to 38% of saline-injected control values, $t(8)=6.57$, $p<0.001$, and 5-HIAA to 34% of controls, $t(11)=5.67$, $p<0.001$. The lower

TABLE 1

WHOLE BRAIN 5-HT AND 5-HIAA LEVELS 48 HR AFTER PCPA INJECTION

	5-HT	5-HIAA
Saline Controls	611 ± 48 (5)	276 ± 32 (6)
PCPA (75 mg/kg)	533 ± 40 (5)	165 ± 53 (5)
PCPA (150 mg/kg)	235 ± 31 (5)*	93 ± 11 (7)*

Entries represent mean ng/gm brain ± SEM
Numbers in parentheses represent the N for each group.
*Significantly different from saline controls, $p<0.001$.

dose of PCPA (75 mg/kg) led to lower levels of brain 5–HT (87% of controls) and 5–HIAA (61% of controls) although these were not significantly different from controls. Killer and nonkiller rats did not appear to differ significantly in their whole brain levels of 5–HT and 5–HIAA, nor in their biochemical response to PCPA injection.

An analysis of the time course of PCPA's facilitation of mouse killing, presented in Fig. 3, demonstrated that the behavioral and biochemical effects of PCPA followed a similar time course, with some recovery at 8 and 14 days after administration.

Attack latencies showed significant differences over test days (pretest, 2, 8, or 14 days), $F(3,27)=3.94$, $p<0.05$, when analyzed by a two-variable ANOVA (test days and trials). Comparisons of the test days to each other by a Newman-Keuls test [18] showed that attack latencies were significantly shorter two days after injection with PCPA than on the pretest but that there were no other significant differences among test days. There was also a significant interaction between test days and trial, $F(12,108)=1.85$, $p<0.05$. Tests of simple main effects showed that this interaction was due to a significant satiation effect on the pretest, $F(4,17)=4.51$, $p<0.01$, but not on Days 2, 8, or 14 after injection. Kill latencies showed results similar to attack latencies. There were no significant differences among sniff latencies.

The time course of PCPA's effects on brain 5–HT and 5–HIAA paralleled to some extent the behavioral effects of the drug. By 8 and 14 days after administration of PCPA, both 5–HT and 5–HIAA levels were significantly higher than at two days after injection, for 5–HT at two days, $t(9)=5.17$, $p<0.001$ at 14 days, $t(8)=9.55$, $p<0.001$, for 5–HIAA at 8 days, $t(10)=3.37$, $p<0.01$, at 14 days, $t(10)=3.63$, $p<0.01$. At 8 and 14 days after injections of PCPA, 5–HT was at 94% and 98% of control levels, and did not differ significantly from controls. 5–Hydroxyindoleacetic acid, however, was at 62% of control levels at 8 and 14 days after administration of PCPA, and was significantly lower than controls, $t(9)=2.58$, $p<0.05$, and $t(9)=2.76$, $p<0.05$.

These results show that there is a generally similar time course between the effects of PCPA on brain serotonin and 5–HIAA and its effects on mouse killing behavior. At two days after PCPA injection, when mouse killing was facilitated, both brain 5–HT and 5–HIAA were significantly reduced. At 14 days after administration, 5–HT and 5–HIAA had significantly recovered; mouse killing was no longer facilitated and did not differ significantly from pretest levels.

When tested using the novel cage procedure, PCPA also facilitated mouse killing by significantly shortening attack

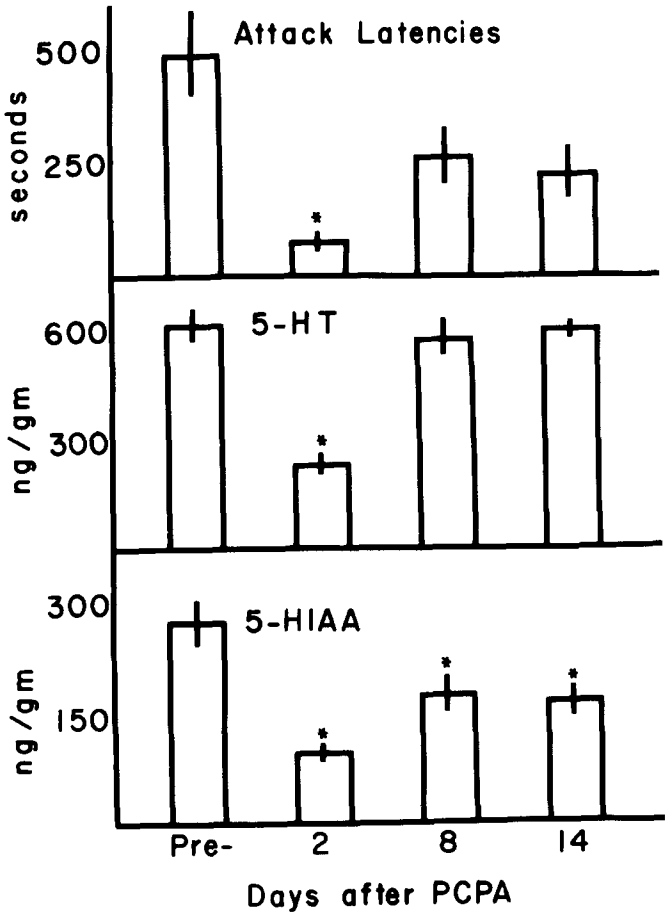

FIG. 3. Time course of PCPA's effects on mouse killing, brain 5–HT, and brain 5–HIAA. Values presented are mean latencies or levels ± SEM. Latencies have been summed over the five trials of mouse presentations. The 5–HT and 5–HIAA values pre- and at two days after PCPA are from Table 1. *Signifies significantly from saline or pretest control values, $p<0.05$.

latencies, $F(1,16)=5.30$, $p<0.05$. Kill latencies were also shorter after PCPA injections, although this difference did not quite reach statistical significance, $F(1,16)=3.90$, $p<0.10$. The latencies to sniff the mouse were not affected by PCPA.

Interaction of 5–HTP and PCPA in their effects on mouse killing behavior. Injections of 5–HTP, the serotonin precursor, inhibited mouse killing behavior by increasing attack and kill latencies. The inhibition by 5–HTP occurred both in rats pretreated with saline and to a somewhat greater extent in rats pretreated with PCPA. These results are presented in Fig. 4.

For attack latencies, a three-way ANOVA (two within-subjects variables and one between-subjects variable) showed a significant interaction between group and test (pretest vs. posttest), $F(2,34)=14.73$, $p<0.001$. Consistent with the previous results, those rats injected with PCPA plus a vehicle (Group 1) had shorter attack latencies on the posttest following PCPA treatment than on the pretest before PCPA treatment, $F(1,34)=7.62$, $p<0.01$. In contrast, rats injected with 5–HTP plus the saline vehicle (Group 2) had longer attack latencies on the posttest, as compared to their pretest attack latencies, $F(1,34)=10.7$, $p<0.01$. Finally, rats injected

TABLE 2

EFFECTS OF PCPA ON LATENCIES TO ATTACK AND KILL MICE IN A NOVEL CAGE

	N	Saline	PCPA (150 mg/kg)	p
Attack	18	862 ± 172	533 ± 163	<0.05
Kill	18	918 ± 178	624 ± 176	<0.10

Entries represent mean seconds ± SEM.

with both PCPA and 5–HTP (Group 3) also had longer attack latencies on the posttest than on the pretest $F(1,34)=19.88$, $p<0.001$. Although the three groups did not differ on their baseline pretest attack latencies, $F(2,34)=1.58$, N.S., they differed significantly on their posttest latencies, $F(2,34)=37.53$, $p<0.001$. Both groups which received 5–HTP, either with or without PCPA had significantly longer latencies than the PCPA alone group (Neuman-Keuls test). These two groups did not differ significantly from each other although there was a tendency for longer attack latencies in Group 3, the group treated with both PCPA and 5–HTP. However, when the complete blocking of mouse killing was examined, rats injected with PCPA were more sensitive to 5–HTP than those not treated with PCPA. A χ^2 analysis of those rats killing at least one mouse vs. those not killing on the posttest was done for the group injected with 5–HTP alone (Group 2), and the group injected with both PCPA and 5–HTP (Group 1). Three of 13 rats of Group 2 failed to kill a mouse on the posttest and eight of 12 rats of Group 1 failed to kill, $\chi^2(1)=4.81$, $p<0.05$.

Kill latencies showed a pattern of results similar to attack latencies. There were no significant differences among sniff latencies.

PCPA, ingestive behaviors, and weight. Both food and water intake were decreased by PCPA injections, and gradually recovered. Food intake which prior to injection was 30 ± 1 g/day decreased to 13 ± 2 g on the day after injection. By 12 days after injection of PCPA, intake had recovered to 31 ± 1 g/day. Water intake, which was at 43 ± 2 ml/day before injection, decreased to 29 ± 3 ml on the day after injection. Water intake rapidly recovered to 45 ± 6 ml by the fourth day after injection. Body weight also decreased acutely after injection, to a mean loss of 21 g (3% of original body weight) on the 4th day after PCPA. By ten days after injection, body weight had started to increase again.

PCPA and irritability. Rats injected with PCPA did not appear to be any more irritable than when treated with saline. There were no bites to either the glove or the probe during any test with PCPA or with saline. The number of squeals was not significantly different after PCPA injection than after saline injection (mean ± SEM=2.3 ± 0.9 for PCPA and 1.7 ± 0.7 for saline).

PCPA and activity. Spontaneous locomotor activity was also decreased by low doses of PCPA. Rats showed 30% fewer crossings in the open field after 150 mg/kg PCPA injection than after saline injection, $F(1,161)=14.20$, $p<0.001$.

DISCUSSION

The primary finding of these experiments is that relatively low doses of the serotonin synthesis inhibitor PCPA can facilitate mouse killing behavior in killer rats. The 5–HT precursor 5–HTP inhibits mouse killing in these rats and also reverses the effects of PCPA. The time course of PCPA's effects generally parallels its effects on brain 5–HT and 5–HIAA.

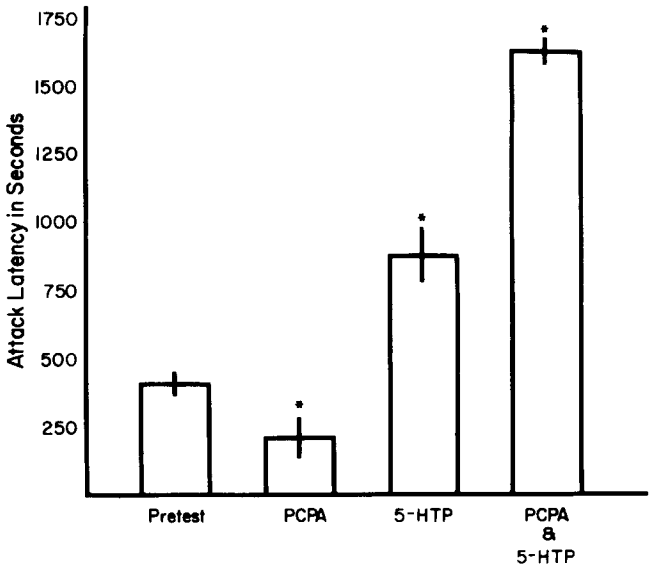

FIG. 4. Interaction of PCPA and 5–HTP in their effects on mouse killing behavior. Values presented are mean latencies ± SEM for all five trials of the posttest. *Signifies significantly different from pretest control values, $p<0.05$.

Previous experiments with PCPA have shown this drug to be effective in eliciting mouse killing in rats which normally do not exhibit killing behavior [11, 12, 22, 28, 30, 39, 44]. This effect required PCPA doses ranging from 300 mg/kg to six injections of 700 mg/kg, and the induced killing behavior in these nonkiller rats appeared abnormal in that the rats bit indiscriminately over the mouse's body, instead of predominantly in the neck region as is normally seen in killer rats. A primary objective of the present study was to examine PCPA at lower doses, in testing conditions which might allow one to detect more subtle changes in killing behavior. These testing procedures, which have previously been employed in only a few studies [2, 3, 15], used killer rats as subjects and measurements of latencies to attack and kill mice as indicators of drug-induced changes in killing behavior. One of the techniques employed was the satiation technique. This procedure involved the presentation of five successive mice to a rat in his home cage, a paradigm which caused the rats' attack and kill latencies to gradually increase with each trial of mouse presentations. The second technique, the novel cage test, involved placing the killer rat in an unfamiliar cage, which also increases his attack and kill latencies. Consistent with previous studies which showed PCPA to induce mouse killing in nonkiller rats, both of these testing paradigms were effective in revealing a facilitation of killing effect, as indicated by reduced latencies to attack and kill mice. In contrast to the PCPA killing induction experiments, however, this facilitation effect was observed at doses as low as 75 mg/kg. Furthermore, the killing response exhibited by the PCPA-injected rats appeared similar to the natural kill, both in topography as well as in the location of the wounds on killed mice. Moreover, nonspecific irritability, sometimes

observed at high PCPA doses, did not accompany the observed facilitation of killing behavior.

Thus, the satiation technique appears to be a useful tool in permitting one to examine drug changes in killer rats, changes which may be more subtle and actually qualitatively different from those exhibited by nonkiller rats [21,51]. It should be noted, however, that satiation of mouse killing may be related to habituation processes, and PCPA has been shown to decrease habituation to a variety of stimuli [9,41]. It is unlikely, however, that PCPA's effect on mouse killing, namely a decrease in satiation, is due predominantly to decreased habituation to the mouse stimulus. In an investigation of the parameters of satiation of mouse killing, Potegal and colleagues [43] found that satiation of killing differed from the habituation process in that killing did not show dishabituation when other strong stimuli were introduced. Satiation also differed from habituation in that with repeated testing over days, the satiation did not occur more quickly [43]. Secondly, in the present studies, the novel cage test served as a useful control for the possibility of PCPA's effect being mediated through decreased habituation. Novelty in the environment is known to disrupt killing and thus increase the rat's latency to respond. If PCPA were decreasing habituation to the stimuli of the novel cage, one would expect to observe an increase in attack and kill latencies. A more specific facilitatory effect of PCPA on mouse killing, however, would be expected to produce the opposite effect, a decrease in response latencies. This latter effect was observed in the present experiment.

In general, the effects of PCPA on killing behavior were accompanied by predictable changes in 5–HT and 5–HIAA. Both 5–HT and its metabolite were significantly reduced at 48 hr after injection when mouse killing was increased, and by 8 and 14 days after drug administration, mouse killing and brain 5–HT were once again at control levels. Brain 5–HIAA showed significant recovery at 8 and 14 days, although it still remained significantly lower than the control values. One other discrepancy between the behavioral and the biochemical data was detected at the lower dose of PCPA (75 mg/kg). This dose significantly facilitated killing behavior while only showing a tendency towards reduced 5–HT and 5–HIAA. This discrepancy might be attributed to several factors, one being that whole brain analyses were conducted, thus masking regional differences. Another possibility is that the levels of 5–HT and 5–HIAA do not reflect functional activity of the serotonergic system and that changes in turnover of 5–HT might be more closely linked to behavioral changes.

The primary effect of PCPA on brain chemistry, and the presumed basis for its behavioral effects, is believed to be its inhibition of tryptophan hydroxylase and consequent reduction of brain serotonin [20]. However, PCPA has also been shown to alter brain catecholamines, and this effect may possibly play a role in modifying the behavior under investigation. The action of PCPA on brain catecholamines (CA), however, appears to have a shorter time course than its effect on brain 5–HT, and also to require higher doses for a reliable change to be observed [4, 20, 28, 42, 50, 53]. At a dose of 150 mg/kg PCPA, the highest dose used in the present study, no change in catecholamine levels was found by 26 hr after injection. Furthermore, only at higher doses of 400 mg/kg PCPA was a significant decrease in norepinephrine apparent as long as 48 hr after injection [33].

This evidence, supported by the biochemical analyses of the present study, suggests that PCPA's effect on brain serotonin is key to its facilitatory effect on killing behavior. Consistent with this hypothesis are the findings of lesion studies, which have shown that electrolytic or neurochemical destruction of the serotonergic cell bodies in dorsal and median raphe nuclei induces killing behavior in nonkiller rats [7, 16, 39, 52]. In the present study, furthermore, the serotonin precursor 5–HTP was found to inhibit mouse killing in killer rats, as well as to block the killing of rats injected with PCPA. Since 5–HTP restores brain 5–HT levels after PCPA injection [21,31], its reversal of PCPA's behavioral effect provides further evidence that the increased killing after PCPA alone is due to its inhibition of tryptophan hydroxylase and 5–HT synthesis. Of particular note is the finding that animals pretreated with PCPA show significantly greater responsiveness to 5–HTP (in terms of killing inhibition) than do animals treated with the PCPA vehicle. Although the development of supersensitivity after temporary or permanent disruption of nerve function is well established for the CA systems [44,49] only a few studies have demonstrated supersensitivity in 5–HT systems [19, 47, 48]. In these latter studies, the serotonin neurotoxin 5,6-dihydroxytryptamine was generally employed to reveal the phenomenon [47,48]. In one study, however, PCPA was used and found to be ineffective [47]. This study, which administered 400 mg/kg of PCPA every three days for a total of 24 days and studied the complex behavioral syndrome including tremor and rigidity occurring after 5–HTP injection, has marked experimental differences from the present study which may very likely account for the contrasting results.

Although the above evidence has tentatively linked central serotonergic systems to the inhibition of mouse killing behavior, the precise nature of this link, in terms of its primacy or specificity, requires closer scrutiny. In the present experiment, PCPA was found to alter several behaviors besides mouse killing. Consistent with previous reports [6, 27, 50], this drug produced a decrease in food and water intake as well as a decrease in open field locomotion. In view of the close relationship which exists between general activity, hunger motivation, and aggression, the possibility that PCPA's effect on mouse killing behavior is perhaps secondary to its additional effects on locomotion and food intake needs to be seriously considered. For example, the involvement of serotonergic systems in producing sleep has been well documented [35]. On the basis of this evidence, one might suggest that PCPA could be facilitating mouse killing simply by decreasing sleep and consequently increasing irritability. Similarly, 5–HTP could be inhibiting mouse killing by producing sleep or decreasing locomotor activity [34,35]. Although possible, it seems unlikely that the facilitation of killing by PCPA is an indirect consequence of its effect on sleep or irritability, since a general increased reactivity and irritability were not evident in the present experiment with low PCPA doses. Nor was a change observed in the rat's latency to sniff the mouse, either in the satiation tests or in the novel cage.

With regard to the relationship which exists between feeding and killing behavior, rats will eat the mice they kill, and food deprivation has been shown to increase the percentage of rats killing mice [26,30]. In the present studies, PCPA might have acted to increase hunger motivation and thus indirectly increase killing behavior. Rats in the present study did reduce their food intake after injections of PCPA (150 mg/kg) and lost an average of 12 g body weight between injection and testing. However, as described in a recent study which employed the same experimental paradigms as were used here, even greater restriction of food intake over a

four-day period was not found to facilitate mouse killing [15]. Moreover, there was no significant correlation in the present study between weight loss and increased killing after PCPA. These results are consistent with Malick's parametric study [26] of the effects of food deprivation on mouse killing, in which he found no significant increase in killing with one week or less of food restriction. Nevertheless, central administration of PCPA has been shown to increase feeding [8], and it is still possible that an increase of hunger motivation, not reflected in a change in body weight, was responsible for the increased mouse killing.

In addition to facilitating mouse killing behavior in rats, PCPA has been shown to enhance brain stimulated affective attack in cats [17], and to increase intraspecies fighting in rats [45]. Moreover, brain serotonergic functioning has been linked to other behaviors such as male and female sexual behavior [32], locomotor activity [13, 46, 52, 53], feeding behavior [5], pain sensitivity [30], and sleep [35]. From these findings, it is clear that brain serotonin has a modulatory influence over many behavioral systems. Whether this influence is mediated by different components of the serotonergic system or by one component affecting all behaviors will need to be investigated. Projections from the dorsal and median raphe nuclei ascend to different forebrain structures, and a recent study has shown that selective lesions of these nuclei have different effects on locomotor activity and perseveration on a learning test [14]. Similar investigations of discrete brain areas will need to be conducted in studying the relationship of specific parts of the serotonergic pathways to mouse killing behavior.

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