Pharmacology Biochemistry & Behavior, Vol. 16, pp. 881–895, 1982. Printed in the U.S.A.

Forebrain Serotonin and Avoidance Learning: Behavioural and Biochemical Studies on the Acute Effect of p-Chloroamphetamine on One-Way Active Avoidance Learning in the Male Rat

S. O. ÖGREN

Astra Läkemedel AB, Research and Development Laboratories, S-151 85 Södertälje, Sweden

Received 10 February 1981

ÖGREN, S. O. *Forebrain serotonin and avoidance learning: Behavioural and biochemical studies on the acute effect of p-chloroamphetamine on one-way active avoidance learning in the male rat.* PHARMAC. BIOCHEM. BEHAV. **16**(6) 881–895, 1982.—The acute effects of p-chloroamphetamine (PCA) on one-way active avoidance learning and on central monoamine concentrations were examined in the male rat. The 5-HT specificity of the acute behavioural effect of PCA was examined in several experiments. PCA (0.08–5 mg/kg IP) injected 30–60 min before testing produced a dose-related impairment of both avoidance acquisition and retention. Pretreatment with the selective serotonin (5-HT) uptake inhibitors fluoxetine and zimelidine, but not the noradrenaline (NA) uptake inhibitor desipramine, resulted in a blockade of the avoidance deficit. Degeneration of brain 5-HT neurons by a high neurotoxic dose of PCA (2×10 mg/kg IP) 7 days prior to the administration of PCA also blocked the avoidance deficit. There was also a complete blockade of the PCA-induced avoidance deficit by pretreatment with metergoline, a central 5-HT receptor blocking agent. A 2.5 mg/kg dose of PCA examined 60 min after injection produced regional changes in the 5-HT-levels preferentially in the forebrain region with significant reductions in the cerebral cortex, hippocampus and striatum while marginal effects were observed in the hypothalamus, midbrain and spinal cord. PCA failed to reduce dopamine and noradrenaline concentrations in the time- and dose-range of the avoidance deficit. Thus, the avoidance learning impairment appears to be specifically related to the acute release of endogenous 5-HT from presynaptic nerve endings possibly in the forebrain resulting in stimulation of postsynaptic 5-HT receptors. These findings indicate that 5-HT neurons in the forebrain play a role in active avoidance learning possibly by an involvement in memorial and/or retrieval processes.

p-Chloroamphetamine Active avoidance Serotonin Central monoamines

CENTRAL serotonergic (5-HT) neurones have been implicated in the physiological and biochemical sequences underlying learning and memory processes including active and passive avoidance learning in rodents [6, 10, 14, 24, 33, 44, 48, 60, 62, 65, 68]. These suggestions are partly based on results from studies which showed that depletion of brain 5-HT concentration produced either pharmacologically or by lesioning of the midbrain raphe nuclei under certain conditions can facilitate avoidance learning in the rat [10, 33, 34, 58, 59, 60, 65]. Recent studies, however, have shown a complicated relationship between 5-HT depletion and avoidance learning [34,40]. On the other hand, procedures designed to elevate brain 5-HT and hence to supposedly enhance central 5-HT activity have in some studies been shown to retard avoidance acquisition. Thus, systemic administration of the 5-HT precursor 5-hydroxytryptophan (5-HTP), which elevates central 5-HT content, has been reported to impair both active avoidance performance and retention in the rat [28,49], the acquisition of a maze-learning task [68] and the performance of schedule-controlled responses [1]. In addition, there is evidence for a role of 5-HT neurons in memorial processes: administration of 5-HTP has been reported to reverse reserpine-induced amnesia for a passive-avoidance response in mice [48]. Moreover, microinjections of 5-HT into the hippocampus immediately following acquisition of a passive avoidance task in mice, markedly impaired retention performance when the animals were tested 24 hrs later [15] and electrical stimulation of the raphe nuclei caused a passive avoidance retention deficit which appears to be due to 5-HT release [17]. However, any interpretation of the results with 5-HTP and intracerebral injections of 5-HT is difficult since the effects of both procedures may be attributable to changes in catecholamine (CA) activity and may involve non-specific central and/or peripheral mechanism. For example, intracerebral 5-HT and systemic administration of 5-HTP can alter CA neuronal activity by forming metabolites which may act as false transmitters [11], produce a decrease in central CA stores by displace-

ment of stored NA and DA [21], and in the case of 5-HTP, a dose-related suppression of ongoing behaviour is produced [1] partly due to formation of serotonin in peripheral tissues [38].

Another major problem with the use of 5-HTP and 5-HT is the lack of any reliable test for 5-HT specificity in vivo. The halogenated amphetamine anaogue p-chloramphetamine (PCA) offers an alternative approach since such a test can be obtained under *in vivo* conditions. There is considerable biochemical [19, 36, 50] and behavioural [36, 43, 66] evidence that PCA exerts a time dependent, biphasic action on central 5-HT neurons. A decrease in 5-HT occurs shortly following PCA injection due to rapid release of 5-HT from nerve endings concomitant with an inhibition of 5-HT reuptake [37, 52, 56]. PCA also causes a lowering of 5-hydroxyindoleacetic acid (5-HIAA) and reduction of 5-HT synthesis and turnover [19,56] probably due to inhibition of brain monoamine oxidase combined with an increase in postsynaptic 5-HT activity [52]. These acute effects of PCA precedes the long-term neurotoxic action on 5-HT neurons which develops within 24–48 hrs after injection [19,50]. PCA acutely blocks also NA reuptake [52,56], releases NA and DA [56,61] and affects NA and DA turnover [12, 56, 64]. The acute effects of PCA on catecholaminergic (CA) neurons can be dissociated from its effects on 5-HT processes by employment of specific inhibitors of monoamine reuptake. Thus, fluoxetine [19] and zimelidine [51], both specific 5-HT reuptake blockers, have been shown to inhibit the decrease in 5-HT produced by PCA [19,50]. These 5-HT uptake inhibitors also block the 5-HT dependent behaviours induced by PCA [35, 42, 66]. In contrast, the specific NA uptake inhibitor desipramine has consistently failed to block the acute 5-HT decrease and the 5-HT dependent behaviours produced by PCA [43, 44, 50]. Thus, by using specific inhibitors of the uptake mechanism in combination with acute PCA-administration, it is possible to determine the relative importance of endogenous 5-HT release and the subsequent increase in central 5-HT activity on avoidance learning.

Acute administration of PCA (5 mg/kg) was previously shown to produce a marked impairment of the retention of an unsignalled one-way active avoidance response in the rat [44]. Zimelidine (but not desipramine) pretreatment blocked this avoidance decrement. It was hypothesized that the avoidance impairment caused by PCA could be due to release of central 5-HT and increase in 5-HT availability resulting in postsynaptic 5-HT activation. The present investigation was designed to examine this hypothesis. Some specific problems were studied (1) whether or not the PCA induced effect on avoidance learning is exclusively related to an action on central 5-HT pathways and mediated via 5-HT release leading to increased stimulation of 5-HT receptors and (2) whether the effects of PCA are mediated via the ascending or descending 5-HT pathways.

GENERAL METHODS

Animals

Male Sprague-Dawley rats (AB Anticimex, Stockholm, Sweden) weighing 210–260 g (60–70 days old) were used. They were housed one to a cage under laboratory conditions (21±1°C, 40–50% relative humidity) for at least a week before the learning experiments and maintained on a 12 hr dark/light cycle, (lights on at 0700). Pellets (Astra Ewos AB) and tap water were available throughout.

Apparatus

Active avoidance learning was carried out in a modified shuttlebox apparatus consisting of two identical compartments each measuring (25×25×25 cm). The compartments were separated by a 2 mm thick black-painted wall with a semicircular gate (8×8 cm). The floor of each compartment consisted of stainless steel bars spaced 15 mm apart. Scrambled constant current shock was delivered through the grid floor (BRS SGS-003 shock generator) and served as the unconditioned stimulus (US). The avoidance apparatus was enclosed in a sound attenuating and ventilated wooden box illuminated by a shielded translucent tube placed at the top centre of the wooden box. The experimental contingencies (shock on-and offset) were controlled by solid-state programming equipment (BRS electronics).

Acquisition and Retention Test Procedure

One-way, unsignalled active avoidance conducted by the same operator was studied in all experiments during the light phase (0900–1700). The testing procedure was the same in all experiments. The rats were transported in their home cage to the experimental room at least 30 min prior to the first acquisition trial. At the start of first trial the rat was taken from its home cage and allowed to explore both compartments for a period of 3 min. Following exploration the rat was placed in the "shock" compartment and shock (nominal 0.065 W) was administered if the rats did not cross to the opposite (safe) compartment within 5 sec. Continuous shock (US) was delivered during a period of 5 sec. If escaping to the non shock compartment, the rats remained in the safe compartment for a 20 sec interval and were thereupon placed in a holding cage similar with the home cage for a period of 10 sec prior to the start of the next trial. A response occurring within the 5 sec interval before shock or during the shock (US) terminated shock presentation. If the rats failed to escape within the 5 sec shock interval they were immediately placed in the safe compartment and thereupon placed in their home cages following the same procedure as for escape. The intertrial interval was constant at 40 sec. Intertrial responses were not punished. The acquisition trials continued until a criterion of 9 out of 10 consecutive avoidance responses were obtained. The maximal number of trials were in most experiments set at 30 (cut-off point). The following parameters were recorded manually: response latencies, the time interval between the placement of the animal in the shock compartment and its crossing over to the safe compartment; avoidance responses, a response before delivery of US (shock); escape responses, a response during shock (US); and response failures, a failure to perform an escape response. The following response parameters were calculated: trials to first avoidance; trials to criterion (9 out of 10 consecutive avoidance responses); number of shocks to criterion and number of response failures.

Retention testing was conducted according to the same procedure with the same parameters as in the acquisition trials with the exception that a constant number of 10 trials was run. The rats were trained to a criterion of 9 out of 10 consecutive avoidance responses. The trained rats were randomly distributed into groups and injected with saline or the test compounds. The testing of retention performance was carried out 60 min after drug injection. The number of avoidance responses out of 10 trials was used as a measure of retention.

The rats were observed throughout each experiment and scored for reactivity to shock (flinching, jumping, vocalization), locomotor activity, immobility and freezing. Freezing was defined as a failure to escape the shock due to induction of an immobility posture (crouching, bar-gripping) [7]. The onset and extent of the "serotonergic syndrome" caused by PCA and consisting of reciprocal forepaw treading, head-twitches, head weaving, tremor and hindlimb abduction (see [27,47]) was noted for each rat at the time of testing.

Monoamine Assays

The rats were sacrificed by decapitation at various times after PCA injections (see results). Each brain was quickly removed, dissected on a cold petri dish on a tray of dry ice and stored at −70°C about 7–14 days until measurements of monoamine concentrations. The following brain regions were dissected out: cerebral cortex (including the entorhinal cortex), the hippocampus (excluding septum), striatum (including nucleus caudate and the putamen), the hypothalamic region (including the preoptic area), midbrain (including mesencephalon with the exception of superior colliculi, pons and medulla oblongata) and spinal cord (excluding medulla oblongata). Tissue weights will be reported in a separate paper [41]. The brain regions were homogenized (Ultra turrax™) in 10 ml icecold 0.4 N perchloric acid containing 0.1 ml (5%) sodium metabisulphite and 0.2 ml (10%) EDTA. The homogenization, extraction, purification and isolation of the amines was performed according to the ion exchange chromatography procedure described by Atack and Magnusson [4] with minor modifications. The homogenates were centrifuged for 10 min (14.000 × g). After filtration of the supernatant, the tissue extracts were applied to a strongly acidic cation-exchange column (Dowex 50 W, X-4, 200–400 mesh). Following eluation the amines were assayed by flourimetry using an Aminco-Bowman spectrofluorometer. A trihydroxyindole assay for used for NA [4], a dihydroxyindole assay for DA [29] and an OPT-condensate assay for 5-HT [4]. For all determinations internal and external blank solutions were analysed. Recovery (in percent±S.E.M.) from this procedure averaged; NA=90±5, DA=100±5 and 5-HT=79± (n=5). The results were not corrected for recovery. Each determination refers to the result from one animal.

Compounds

All injections were administered intraperitoneally (IP) into the left abdomen. Control animals received 0.9% NaCl (saline 5 ml/kg). In all the experiments the doses refer to the respective salt of each compound. dl-p-Chloroamphetamine hydrochloride (PCA) (Sigma Chemical) was dissolved in 0.9% NaCl in distilled water. Desipramine hydrochloride (Ciba Geigy), fluoxetine hydrochloride (Lilly 100 140; Eli Lilly) and zimelidine dihydrochloride monohydrate (Astra Läkemedel AB) were dissolved in 0.9% NaCl in distilled water. Metergoline (Farm Italia) was dissolved in distilled water containing 0.1% ascorbic acid.

Statistics

The results were either subjected to one-way analysis of variance (ANOVA) or treated with a Kruskall-Wallis analysis of variance by ranks based on completely randomized design [30]. Post hoc pairwise comparisons were performed with the Mann-Whitney U-test (two-tailed). The 5% level of significance was maintained throughout unless where otherwise stated.

EXPERIMENT 1

The experimental evidence concerning the precise relationship between brain serotonin availability and avoidance learning is not clear at present [34]. PCA acutely produces a dose-related decrease in brain 5-HT [56] and possibly a similar dose-related increase in central 5-HT availability. If central 5-HT is involved in the PCA-induced effect, a similar dose-related effect of PCA on avoidance is predicted. Experiment 1 was designed to study if PCA administration causes similar effects on one-way avoidance acquisition and retention and if this effect of PCA is proportional to the dose given.

Procedure

Acquisition and retention testing were conducted in two separate experiments with separate groups of rats. The acquisition trials were conducted on 7 different groups of rats (n=6–10). Control groups (n=18) received saline injections 30 min prior to the first acquisition trial. Dose-response experiments were run following PCA-treatment. Rats were injected IP with either 0.08, 0.16, 0.32, 0.635, 1.25 or 2.5 mg/kg PCA. Acquisition trial started 30 min post-injection. In the retention experiments groups of rats (n=6) were injected with either saline or PCA 60 min after they had been trained to the acquisition criterion (9 consecutive avoidance responses). Retention testing started 60 min post-injection. The following PCA-doses were tested: 1.25, 2.5 and 5.0 mg/kg.

RESULTS

Avoidance Acquisition

PCA-administration produced a dose-related impairment of avoidance acquisition as computed on trials to criterion and as shown in the number of avoidances across 10 trials (Fig. 1 and Table 1). The statistical analysis (Kruskal Wallis Anova) showed significant treatment effects, $H(6)=40.5$, $p<0.001$. Subsequent pairwise testing (Mann-Whitney U-test) showed that the rats treated with PCA had significantly more trials to criterion from a dose of 0.16 mg/kg IP. PCA-rats at doses of 1.25 and 2.5 mg/kg were markedly impaired and failed to reach criterion within 30 trials and differed significantly from all the doses tested except 0.16 mg/kg. Rats in the lowest PCA dose (0.08 mg/kg) were not impaired. Other dependent variables were also affected by treatment (see Table 1). Both 0.63 and 1.25 mg/kg of PCA tended to decrease and 2.5 mg/kg clearly increased the number of trials to first avoidance. The number of shocks to criterion corresponded well to the data on learning trials to criterion. The number of response failures were generally very few and were only significantly increased by PCA 2.5 mg/kg. PCA (0.08 mg/kg) caused a significant lowering of response failures.

PCA at the doses of 0.63 and 1.25 caused a significant shortening of escape latencies on the first trial (Table 2). Analysis of escape latencies across the first 10 trials revealed that PCA-treated rats at the doses 0.63 and 2.5 mg/kg had slightly but significantly longer response latencies than the control group. Response latencies were further analyzed in terms of avoidance latencies to criterion (Table 2). PCA

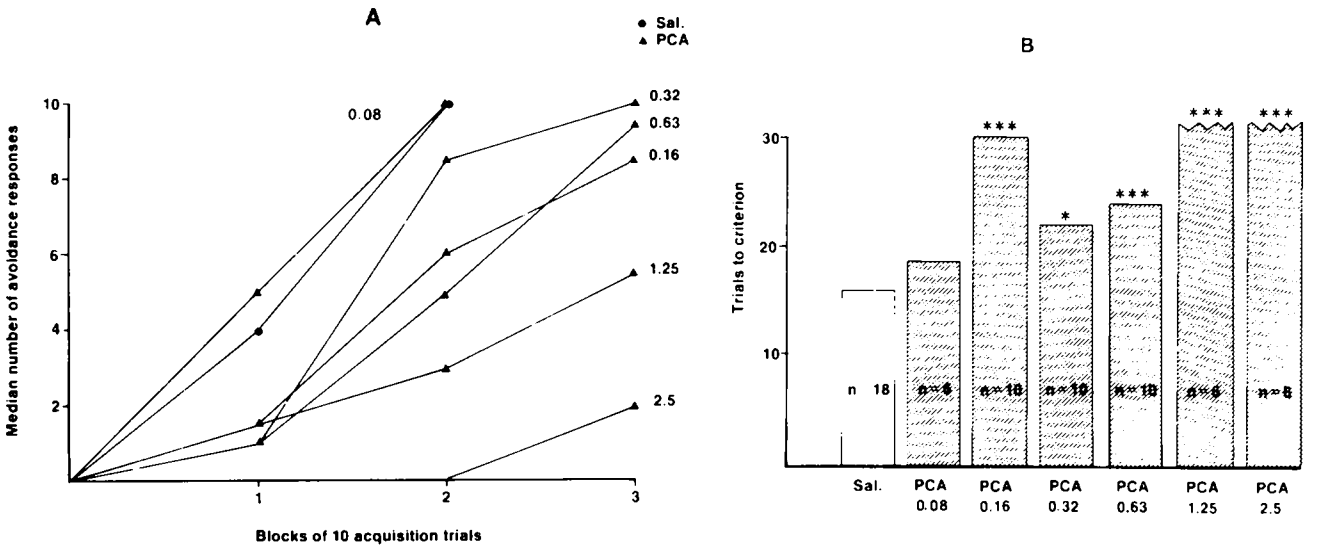

FIG. 1. Median number of avoidance responses across blocks of 10 trials during acquisition of an one-way active avoidance response for groups of rats treated with either p-chloroamphetamine (PCA) or saline (A). Dose levels of PCA (for each treatment group) are shown. The right hand portion (B) shows the median number of trials to criterion of 9 out of 10 avoidance responses for the same group of rats. The number of rats per group are depicted on each bar. * $p \leq 0.05$, *** $p \leq 0.01$, vs the saline control group (Mann-Whitney U-test).

TABLE 1

GROUP MEDIAN (Mdn) AVOIDANCE ACQUISITION PARAMETERS AND INTERQUARTILE RANGES (IQR) FOLLOWING TREATMENT WITH VARIOUS DOSES OF p-CHLOROAMPHETAMINE (PCA)

Treatment	Group Mdn (IQR) trials to 1st avoidance	acquisition/ criterion	shocks/ criterion	failures/ criterion
Saline	5.0	16.0	6.5	1.5
(n=18)	(1.0– 8.0)	(15.0–20.0)	(5.0– 9.5)	(0.0 – 2.5)
PCA 0.08 mg/kg	4.0	18.5	5.5	0.001†
(n= 6)	(1.0–10.0)	(15.5–20.5)	(4.5–10.5)	
PCA 0.16 mg/kg	5.5	30.0‡	14.5‡	1.0
(n=10)	(1.0–10.5)	(22.0–30.0)	(10.0–24.0)	(0.001– 9.5)
PCA 0.32 mg/kg	7.5	22.0*	12.0†	1.5
(n=10)	(1.0–13.0)	(17.0–29.5)	(7.5–17.5)	(0.001– 6.5)
PCA 0.63 mg/kg	1.0	24.0‡	13.0‡	3.0
(n=10)	(1.0– 9.5)	(20.5–30.0)	(9.0–19.5)	(0.001– 4.0)
PCA 1.25 mg/kg	1.0*	≥30.0‡	19.5‡	2.0
(n= 6)	(1.0– 1.5)	(30.0–32.5)	(14.5–21.0)	(1.0 – 5.5)
PCA 2.5 mg/kg	23.5‡	≥30.0‡	26.5‡	7.5†
(n= 6)	(15.0–28.0)		(25.5–29.0)	(1.5 –19.0)

Avoidance parameters are calculated as described in Experiment 1. *$p \leq 0.05$, †$p \leq 0.01$ and ‡$p \leq 0.001$ vs control (saline treatment), Mann-Whitney U-test.

produced significant increases in avoidance latencies at the doses 0.16 and 0.63–2.5 mg/kg. There was no evidence for a loss of pain sensitivity following PCA-treatment, since behavioural observation indicated a clear reactivity to shock and no freezing behaviour such as bargripping or shock-induced immobility. This is further underlined by the low number of response failures which give no evidence for response failures in PCA-treated rats due to motor impairment. Surprisingly, most PCA-rat at the doses 1.25–2.5 mg/kg returned occasionally to the "shock"-compartment following escape to the safe-compartment. This behavior was never observed in saline rats.

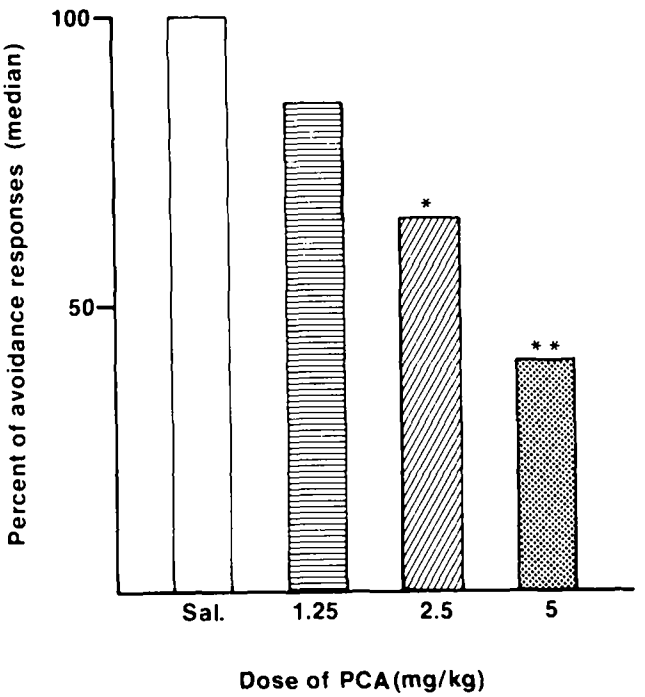

FIG. 2. The effect of PCA administration on the retention of an unsignalled active one-way avoidance response. Groups of rats (n=6) were trained to a criterion of 9 out of 10 consecutive avoidance responses and injected with saline or PCA (1.25–5 mg/kg IP). The bars represent the median in percent of 10 avoidance responses. Retention was tested 60 min after injection of saline of PCA. *$p \leq 0.05$, ** $p \leq 0.01$, vs the saline group (Mann-Whitney U-test).

Avoidance Retention

The results of testing of retention performance are shown in Fig. 2 and Table 3. Retention was examined 60 min post-injection by measuring the number of avoidance responses in a total number of 10 trials. Using this measure, saline controls were at a performance level of close to 100%. The latency of the avoidance response during retention testing was approximately 1.2 sec, i.e., well below US onset. PCA produced a dose-related impairment of retention. There was a highly significant overall treatment effect, $H(3)=11.4$, $p<0.01$, Kruskall Wallis ANOVA. However, higher doses of PCA had to be given to affect retention than acquisition performance. Mann-Whitney testing revealed a significant avoidance decrement from a dose of 2.5 mg/kg. PCA in the highest dose tested (5 mg/kg) produced a 60% decrement in avoidance retention.

The mean latencies for response latencies are shown in Table 3. Again, there was a highly significant overall treatment effect, $H(3)=11.7$, $p<0.01$. PCA-treatment produced a small dose related increase in response latencies which was significant from a dose of 2.5 mg/kg (borderline significance at a dose of 1.25 mg/kg). It is notable that all PCA groups had avoidance latencies shorter than 5 sec, i.e., shorter than shock onset. There was no evidence in the retention test for impaired reactivity to shock due to freezing. There were no significant effects on response failures in any of the groups tested. PCA given at a dose of 5 mg/kg caused signs of the 5-HT syndrome (slight hindlimb abduction and occasional tremor). At a PCA dose of 2.5 mg/kg only slight hindlimb abduction was observed. The onset of the first shock in the shuttlebox generally abolished all signs of the 5-HT syndrome.

TABLE 2

GROUP MEDIAN (Mdn) ESCAPE AND AVOIDANCE LATENCIES AND INTERQUARTILE RANGES (IQR) FOLLOWING TREATMENT WITH VARIOUS DOSES OF p-CHLOROAMPHETAMINE (PCA)

	Group Mdn (IQR)		
	Escape latencies		Avoidance latencies
Treatment	1st trial	1–10 trials	Avoidance trials
Saline (n=18)	6.5 (2.4– 8.9)	5.6 (4.7–6.0)	1.4 (1.1 –1.8)
PCA 0.08 mg/kg (n= 6)	5.9 (2.2– 7.4)	4.5 (4.0–5.9)	1.6 (1.1 –2.1)
PCA 0.16 mg/kg (n=10)	8.5 (4.3–10.0)	6.4 (5.2–8.0)	1.9* (1.5 –2.4)
PCA 0.32 mg/kg (n=10)	6.2 (3.5– 7.6)	6.4 (5.1–7.9)	1.1 (0.75–1.9)
PCA 0.63 mg/kg (n=10)	2.1* (1.2– 5.6)	7.1* (5.5–7.9)	1.9* (1.3 –2.2)
PCA 1.25 mg/kg (n= 6)	2.7* (0.8– 4.9)	6.7 (5.1–7.4)	2.5‡ (2.2 –2.8)
PCA 2.5 mg/kg (n= 6)	7.0 (6.0– 8.5)	7.7‡ (7.2–8.5)	2.8‡ (2.5 –3.1)

Avoidance latencies were calculated as described in Experiment 1. *$p \leq 0.05$, †$p \leq 0.01$ and ‡$p \leq 0.001$ vs control (saline treatment), Mann-Whitney U-test.

TABLE 3

EFFECTS OF p-CHLOROAMPHETAMINE (PCA) ON RESPONSE LATENCIES OF THE TEN RETENTION TRIALS (RETENTION SESSION)

Treatment	Dose mg/kg	Mean response latency ± S.E.M. (sec) of ten trials
Saline		1.2 ± 0.2
PCA	1.25	2.1 ± 0.5
PCA	2.5	3.3 ± 0.7*
PCA	5.0	4.5 ± 0.7†

*$p<0.05$, †$p<0.01$ (Mann-Whitney U-test).

DISCUSSION

The one-way learning task employed here was easily acquired by the normal rat. PCA produced a clear dose-related impairment of both the acquistion and retention of the avoidance response. There was a more pronounced effect on avoidance acquisition than on the already established response. The very low threshold effect of PCA (0.16 mg/kg) for a significant avoidance acquisition impairment is to my knowledge the lowest dose of PCA ever shown to have significant behavioural effects. Thus the PCA-induced avoidance impairment is observed in a dose-range which is

distinctly lower than those producing behavioural signs of 5-HT release (see [47]).

In the present experiment there was no observational evidence that PCA decreased shock sensitivity or reactivity. However, both avoidance latencies (in both the acquisition and retention testing) and to some extent escape latencies were increased in the higher dose range (0.63–2.5 mg/kg) which could indicate subtle changes in shock sensitivity or reactivity. Increased response latencies could either reflect toxic, peripheral effects or an actual effect on the learning process. Several pieces of evidence suggest that altered shock sensitivity or reactivity might not account for the impaired avoidance learning produced by PCA. First, there is no response suppression on the first trial in any of the PCA doses. In fact certain doses of PCA (0.63 and 1.25 mg/kg) shortened response latency probably due to the increase in motor activity caused by PCA in these doses (see [41,64]. Second, avoidance impairment is found at doses below those which produce an increase in escape latencies on the first and on trials 1–10. Third, it is clear that all PCA groups respond to the shock in so far as they respond well within the 5 sec limit for US duration. This is also evidenced by the low number of response failures. In general, the changes in response latencies were small following PCA administration. Fourth, the fact that there was also a slight but clear increase in avoidance latencies when the animals actually did not receive shock argues against an interpretation in terms of reduced shock sensitivity, reactivity or response suppression. In addition, it is notable that there seems to exist a dissociation between the motor activity increasing effect of PCA (threshold dose 1.25 mg/kg) and the effect on response latencies. Despite the fact that PCA like amphetamine has an activity-increasing effect [12, 61, 64] it produces an increase in response latencies.

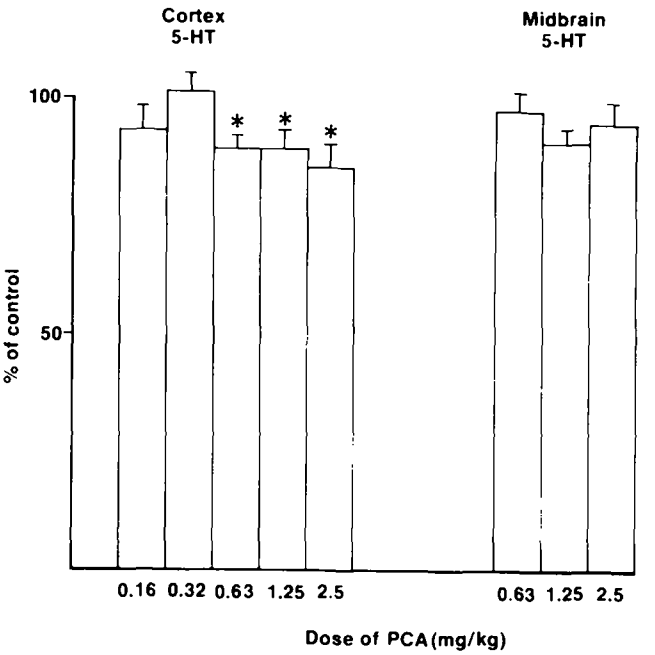

FIG. 3. The effect of p-chloroamphetamine (PCA) on 5-HT concentrations in the cerebral cortex and midbrain. Groups of six rats were treated with various doses of PCA and sacrificed 30 min after injection. For details on assay-method, see Method section. The bars represent the mean+S.E.M. in percent of respective control. Control values in ng/g wet tissue: Cerebral cortex: 5-HT=134±10; midbrain: 5-HT=345±11. * $p \leq 0.05$ vs the saline control (ANOVA).

EXPERIMENT 2

Experiment 1 showed that the avoidance impairment produced by PCA is directly related to the dose given and so possibly directly related to the amount of brain 5-HT released. However, it is not known whether the action of PCA is mediated primarily via the major ascending serotonergic pathways to the forebrain or also involves the descending 5-HT projections to the spinal cord [5, 20, 22]. Experiment 2 was designed to examine this question. The action of PCA on catecholamine (CA) neurons were also examined since it can not be excluded that changes in CA transmission may be involved in the effects of PCA (see [56].

Procedure

Similar experimental designs were used as in the behavioural studies to determine the effects of PCA treatment on monamine concentrations. Separate groups of male rats (200–250 g) were injected with either saline or PCA and decapitated at various times after administration.

RESULTS

Monoamine Concentration

PCA treatment (0.16–2.5 mg/kg) reduced 5-HT concentrations in the cerebral cortex in a dose-related manner when the animals were sacrificed 30 min postinjection (Fig. 3). A significant decrease was found in the dose range 0.63–2.5 mg/kg (11–15% decrease). No significant reduction in 5-HT was observed in the midbrain in the same dose range. PCA treatment (2.5 mg/kg IP, 60 min before sacrifice) significantly reduced 5-HT concentrations in the cerebral cortex by 37%, in the hippocampus by 21% and in the striatum by 29% of the value for the saline control group (Fig. 4). No significant 5-HT reduction was observed in the hypothalamus, midbrain or spinal cord at this dose of PCA. PCA treatment elevated NA concentration in the cerebral cortex (37% increase), caused a slight significant reduction (10%) in the spinal cord but did not significantly affect NA concentrations in other areas. PCA failed to affect DA concentrations in the striatum and hypothalamus but caused a nonsignificant increase in the midbrain and the spinal cord.

DISCUSSION

The time- and dose-range at which PCA affects avoidance learning corresponds to a decrease of 5-HT in the forebrain. DA levels were generally unaffected or tended to be slightly elevated and NA levels were elevated in the cerebral cortex. The elevation of NA levels and the trend for a similar elevation of DA in some regions may be due to the potent inhibitory effects on brain MAO produced by PCA in vivo [52]. Taken together, these findings are consistent with previous reports showing no or insignificant reductions in CA concentrations following PCA injection [56]. Thus, the acute effects of PCA on avoidance appears to be related both in dose and time to the action on 5-HT but not to an action on catecholamine intraneuronal stores. The effect of PCA on avoidance learning and 5-HT concentration is directly proportional to the dose given and may also be directly related to the amount of 5-HT released from intraneuronal stores. Only small

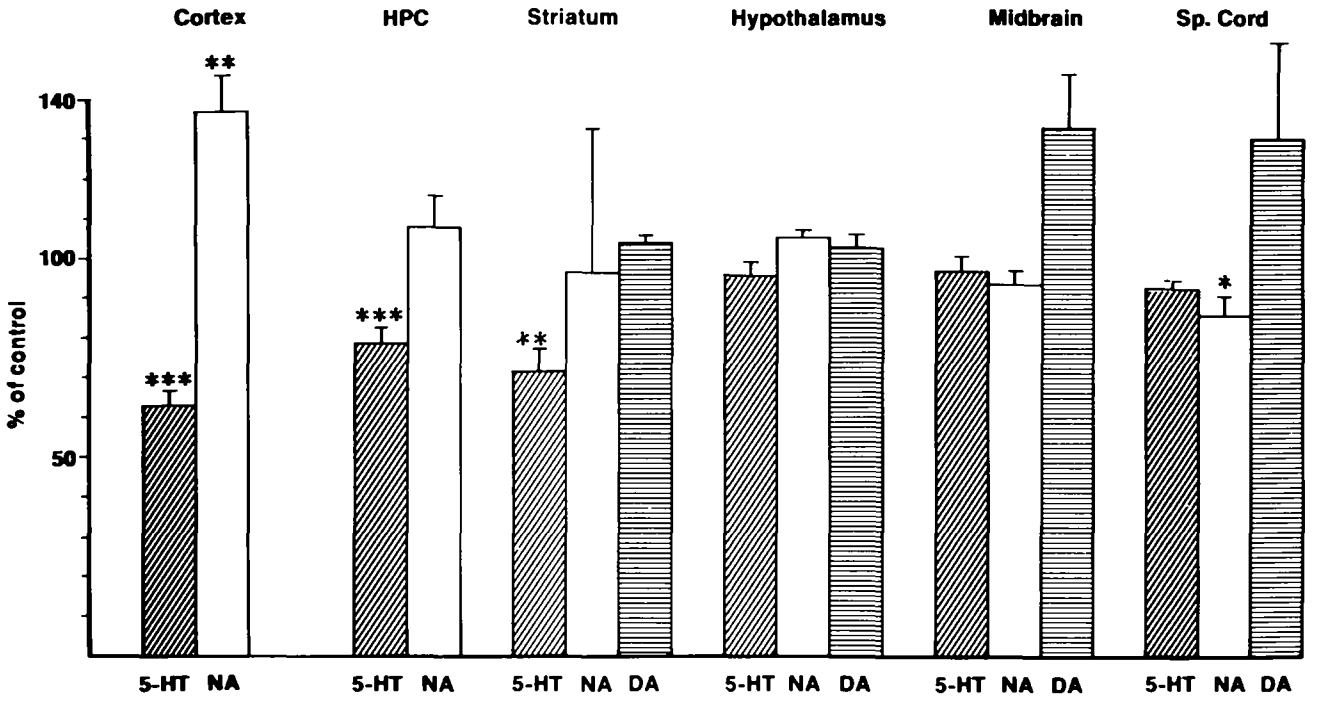

FIG. 4. The effects of p-chloroamphetamine (PCA) on monoamine concentrations in several rat brain regions. PCA (2.5 mg/kg) or saline was injected to groups of six rats which were sacrificed 60 min after injection. NA, DA and 5-HT were assayed as described by Atack and Magnusson [4]. The bars represent the means+S.E.M. in percent of each control group. Control values in ng/g wet tissue: Cortex: NA=177±8, 5-HT=159±6; Hippocampus (HPC): NA=329±19, 5-HT=237±5; Corpus Striatum: NA=30±10, DA=5452±327, 5-HT=271±12; Hypothalamus: NA=1817±62, DA=443±17, 5-HT=559±24; Midbrain: NA=496±24, DA=33±8, 5-HT=311±13; Spinal Cord: NA=279±11, DA=18±3, 5-HT=297±11. * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$ vs control (ANOVA).

changes of 5-HT concentration at the receptors seem to be required in order to produce an effect on avoidance learning.

Experiment 2 also showed that acute PCA treatment produced differential effects on the ascending and descending 5-HT pathways. PCA treatment reduced 5-HT concentrations in brain regions (cortex, hippocampus, striatum) innervated by the two major ascending 5-HT pathways. PCA, thus, affects both the mesostriatal serotonergic projection arising from the dorsal (B7) as well as the mesolimbic serotonergic axons derived from the median (B8) raphe nuclei [5, 20, 22]. PCA had less pronounced effects on the hypothalamic region which is innervated by the median raphe nuclei and possibly also from caudally located raphe nuclei [22,34]. PCA also had also marginal effects on the midbrain innervation and on the spinal cord which receives a 5-HT innervation from the caudally located raphe nuclei (B1–B3 cell groups) [22,34].

Thus, acute PCA treatment in the doses used here affects all 5-HT systems known to innervate the forebrain while having less effects on the descending 5-HT pathways. The preferential effects of acute PCA on forebrain 5-HT have been observed earlier by Costa and coworkers [12]. Thus, (−)-PCA was found to reduce both 5-HT and 5-HT turnover rate most potently in the tel-diencephalic area while corresponding values in the brain stem were only slightly affected. It is notable that also the long-term neurotoxic action of PCA on central 5-HT neurons shows a similar regional specificity [32].

EXPERIMENT 3

The purpose of experiment 3 was to further determine whether the avoidance impairment caused by PCA was primarily related to presynaptic 5-HT release. In addition to its effects on 5-HT release PCA is also a potent 5-HT uptake blocker [37, 52, 53] and as a consequence an enhanced postsynaptic effect is known to occur. The effects of PCA was therefore compared with the specific 5-HT uptake inhibitors zimelidine [51] and fluoxetine [18] and the NA uptake inhibitor desipramine. These drugs were also injected prior to PCA-administration. If PCA acts via presynaptic 5-HT release, zimelidine and fluoxetine pretreatment would attenuate the PCA-induced effect since they have been shown to block the 5-HT decrease produced by PCA [19,50].

Procedure

Avoidance acquisition was performed using identical experimental conditions as described for Experiment 1. Groups of rats (each n=8 were treated with either PCA (2.5 mg/kg, 30 min prior to testing), zimelidine (10 mg/kg, 90 min prior to testing) desipramine (10 mg/kg, 60 min prior to testing), zimelidine (10 mg/kg) + PCA, (2.5 mg/kg), desipramine (10 mg/kg) + PCA (2.5 mg/kg) and saline. Zimelidine (10 mg/kg) was injected 90 min before acquisition or 60 min prior to the saline or PCA (2.5 mg/kg) injection while corresponding values for desipramine was 60 and 30 min, respectively. In a separate experiment four groups of rats were treated with

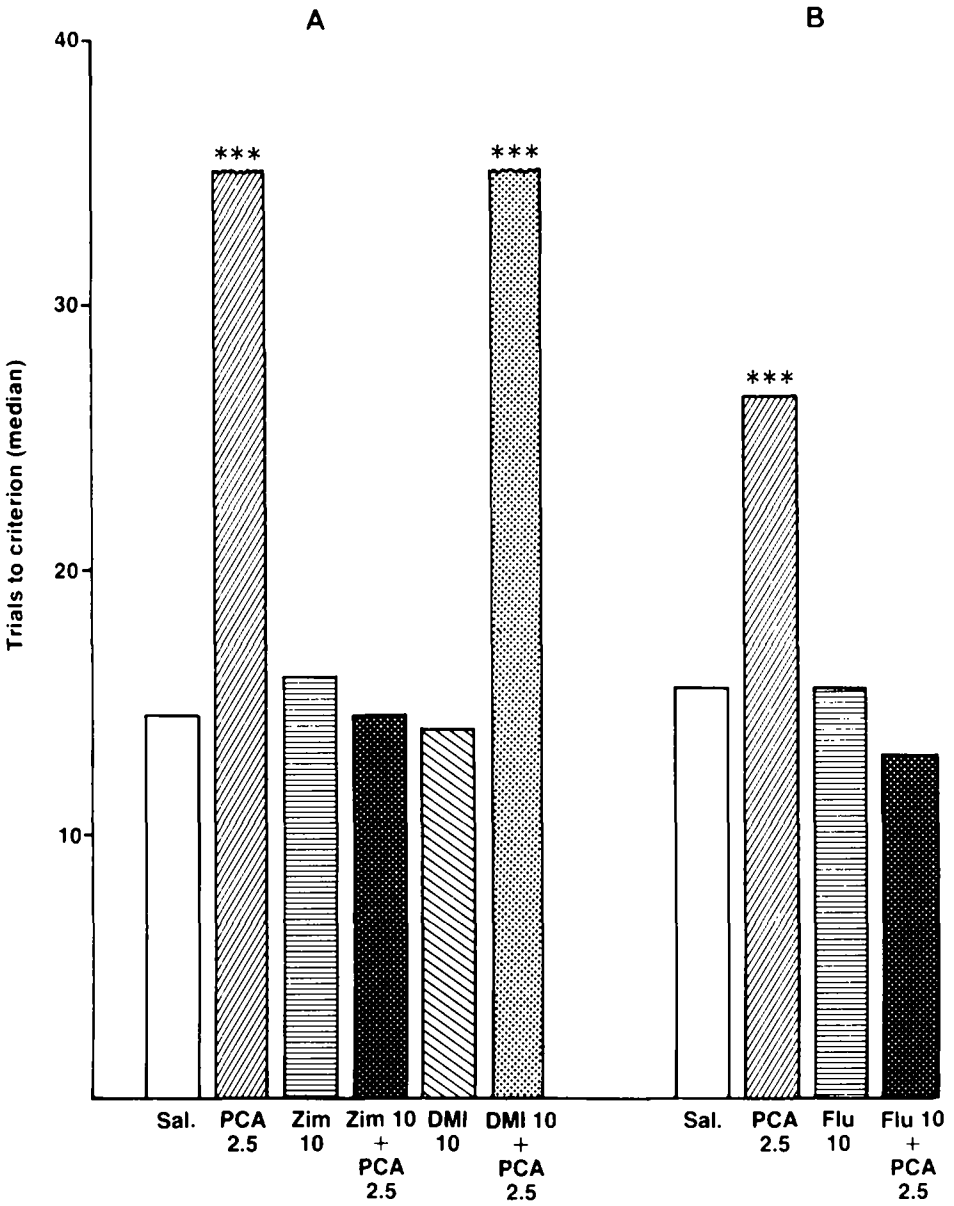

FIG. 5. Blockade of the p-chloramphetamine (PCA)-induced avoidance acquisition deficit by pretreatment with zimelidine (Zim) and fluoxetine (Flu). In experiment A Zim (10 mg/kg) was injected 90 min before acquisition or 60 min before PCA (2.5 mg/kg). Desipramine (DMI, 10 mg/kg) was injected 60 min before acquisition or 30 min before PCA. In experiment B Flu (10 mg/kg) was injected 60 min before acquisition or 30 min prior to PCA (2.5 mg/kg). Acquisition started 30 min after the saline or PCA injection. The results are the group median values of 8 rats. *** $p \leq 0.001$ vs saline (Mann-Whitney U-test).

either PCA (2.5 mg/kg, IP), fluoxetine (10 mg/kg, 60 min prior to testing), fluoxetine (10 mg/kg, 30 min before PCA) + PCA (2.5 mg/kg) or saline. Saline and PCA (2.5 mg/kg) were injected 30 min prior to the first acquisition trial. Similar experimental design as in Experiment 1 was used in retention testing. Groups of rats (each n=6–8) were treated with either PCA (5 mg/kg), fluoxetine (10 mg/kg), fluoxetine (10 mg/kg) + PCA (5 mg/kg) and saline. Fluoxetine was injected 30 min prior to PCA. Retention testing occurred 60 min after PCA or saline injection.

RESULTS

Avoidance Acquisition

The results are shown in Fig. 5. A two-tailed Mann-Whitney U-test based on the mean number of trials to criterion revealed that desipramine and zimelidine treatment at a dose of 10 mg/kg did not produce any significant effect. Zimelidine, but not desipramine, pretreatment, however, significantly blocked the avoidance impairment caused by PCA (2.5 mg/kg). The PCA-group differed from all the other

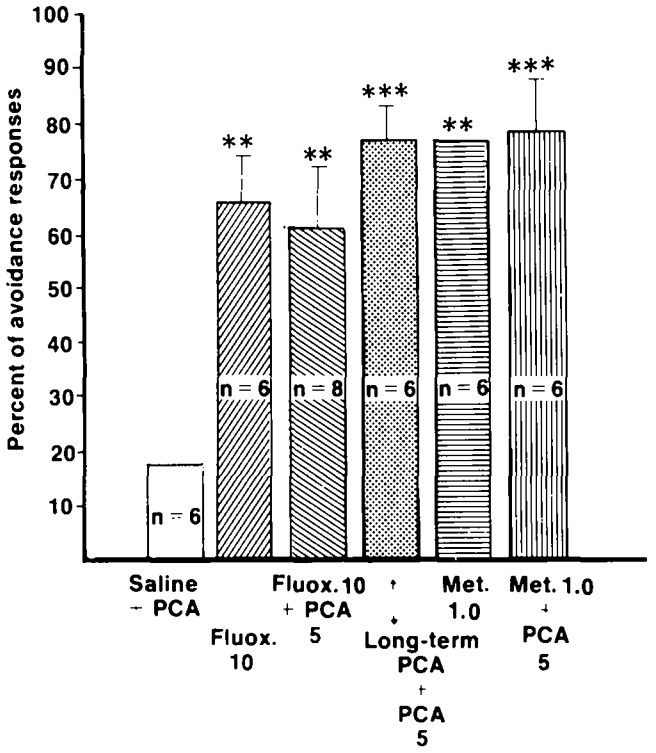

FIG. 6. Blockade of p-chloroamphetamine (PCA)-induced avoidance retention deficit by pretreatment with fluoxetine, metergoline and by degeneration of 5-HT neurons produced by the long-term action of PCA. Fluoxetine (10 mg/kg IP) and metergoline (1 mg/kg) were injected 60 and 1 min respectively prior to PCA (5 mg/kg). In a separate group of rats PCA was given on two consecutive days at a dose of 10 mg/kg IP to produce a long-term degeneration of 5-HT neurons. Acute PCA treatment (5 mg/kg) was given 7 days after the last PCA-injection. A concurrent saline control was also run. Retention was tested 60 min after the injection of saline of PCA (5 mg/kg). Number of rats are depicted on each bar which represents the mean+S.E.M. in percent of 10 avoidance responses. ** $p \leq 0.01$, *** $p \leq 0.001$ vs PCA control (Mann-Whitney U-test).

groups tested ($p \leq 0.001$). It is notable that the avoidance performance of the zimelidine (10 mg/kg) + PCA (2.5) group is at control level while the performance of the desipramine (10 mg) + PCA (2.5) group does not differ from the PCA group.

In a separate study fluoxetine was given to rats treated with PCA 2.5 mg/kg IP (Fig. 5). Fluoxetine (10 mg/kg) did not change acquisition. Fluoxetine pretreatment, however, completely blocked the PCA induced deficit and the fluoxetine + PCA group had a nonsignificant lower number of trials to criterion than the fluoxetine and saline groups. The Mann-Whitney u-test showed significant differences between the PCA group and each of the other three groups.

Avoidance Retention

Fluoxetine (10 mg/kg) failed to significantly affect avoidance retention as previously was shown for zimelidine. Similar to zimelidine [44] fluoxetine pretreatment clearly blocked the retention deficit induced by the PCA (5 mg/kg) injection (Fig. 6).

DISCUSSION

Pretreatment with compounds (zimelidine and fluoxetine) given at doses which produce a specific and potent inhibition of 5-HT uptake was shown to block the avoidance learning deficit caused by PCA. This finding suggests a specific role for 5-HT in the PCA-induced deficit. It is also notable that unlike PCA neither zimelidine nor fluoxetine caused any avoidance learning deficit. In this context it should be pointed out that PCA is considerably more potent as a 5-HT releaser than as a 5-HT uptake blocker [54] while both fluoxetine and zimelidine are "pure" 5-HT uptake inhibitors which do not increase 5-HT release [18, 51, 53, 54]. Thus, the increase in postsynaptic activity by inhibition of the removal of the transmitter from the synaptic cleft is generally not sufficient to cause a retention impairment or affect acquisitional processes (see general discussion). Desipramine pretreatment, on the other hand, failed to block the behavioural effect as well as the acute 5-HT decrease induced by PCA [44]. These findings seem to exclude a direct role of released NA in the PCA-deficit.

EXPERIMENT 4

Experiment 3 indicated that the deficiency in active avoidance acquisition after acute PCA administration was related to an action on 5-HT neurons and due to release of endogenous 5-HT. Lowering of 5-HT concentration prior to the acute PCA administration via degeneration of central 5-HT neurons, particularly the ascending 5-HT pathways would, therefore, block the avoidance deficit caused by PCA. Thus, central serotonin neurons were degenerated by pretreatment with high doses of PCA (2×10 mg/kg IP) as earlier described [32,42] 7 days prior to the acute PCA injection.

Procedure

Avoidance acquisition was performed as described for Experiment 1. Four groups of rats (each n=8) were treated with either saline or PCA (10 mg/kg). Each saline or PCA injection was given on two consecutive days, the last injection 7 days prior to acquisition. Each of these groups was injected with saline or PCA (2.5 mg/kg). In a separate experiment groups of rats (each n=6) were pretreated with either PCA (10 mg/kg given on two consecutive days last injection 7 days prior to testing) and a similar injection of saline. Following acquisition (see Experiment 1) these groups received an acute injection of PCA (5 mg/kg) or saline. Retention was tested 60 min after the injection of saline or PCA.

RESULTS

Avoidance Acquisition

Figure 7 shows that the long-term treatment with PCA (2×10 mg/kg) completely blocked the impaired acquisition caused by the acute PCA (2.5) administration. The PCA (2×10) groups treated with saline or PCA (2.5 mg/kg) were not impaired compared to the saline group but, in fact, tended to show lower number of trials to criterion than the acutely injected saline group.

Avoidance Retention

In a separate experiment PCA was given in a high dose (2×10 mg/kg IP), the last dose 7 days before PCA injection (see Fig. 6). The longterm treatment of PCA failed to affect avoidance retention. This type of treatment, however, significantly blocked the retention impairment caused by acute PCA (5 mg/kg) treatment.

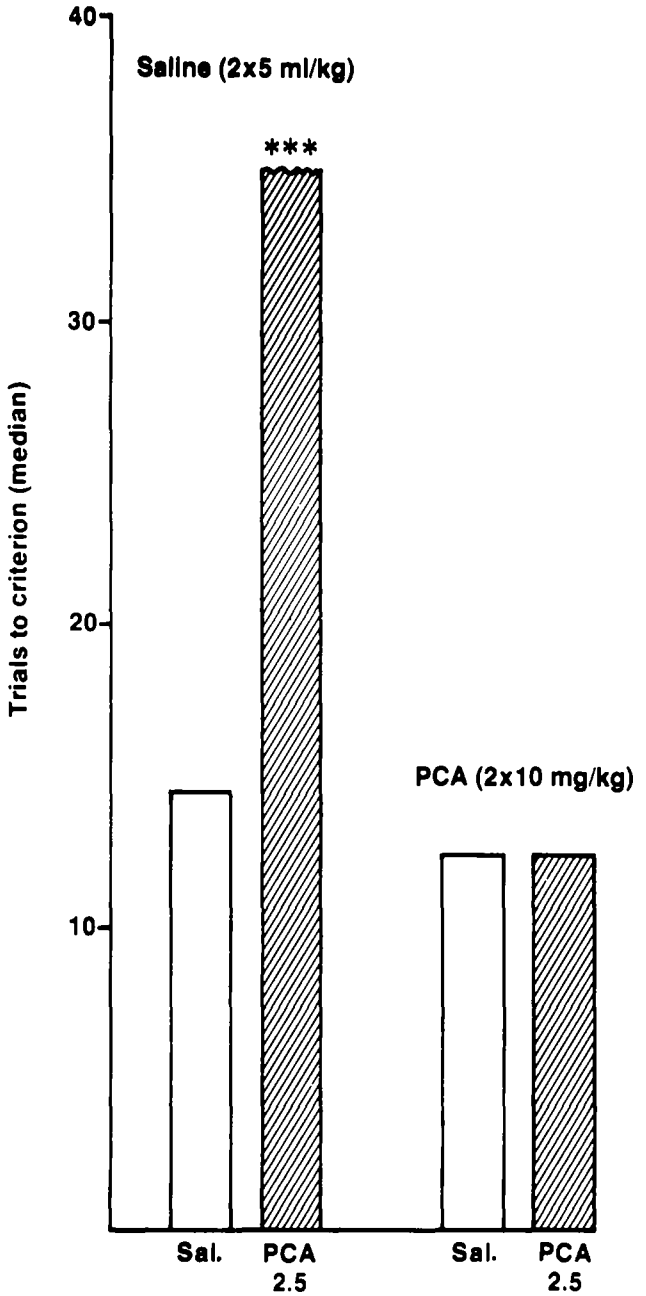

FIG. 7. Blockade of the p-chloroamphetamine (PCA) induced one-way acquisition deficit by prior degeneration of 5-HT neurons achieved by the long-term action of PCA on 5-HT neurons. Groups of rats (each n=8) were injected with saline or PCA (10 mg/kg) on two consecutive days, the last injection 7 days prior to acquisition. Acquisition started 30 min after the injection of PCA (2.5) or saline. *** $p \leq 0.001$ vs saline. (Mann-Whitney U-test).

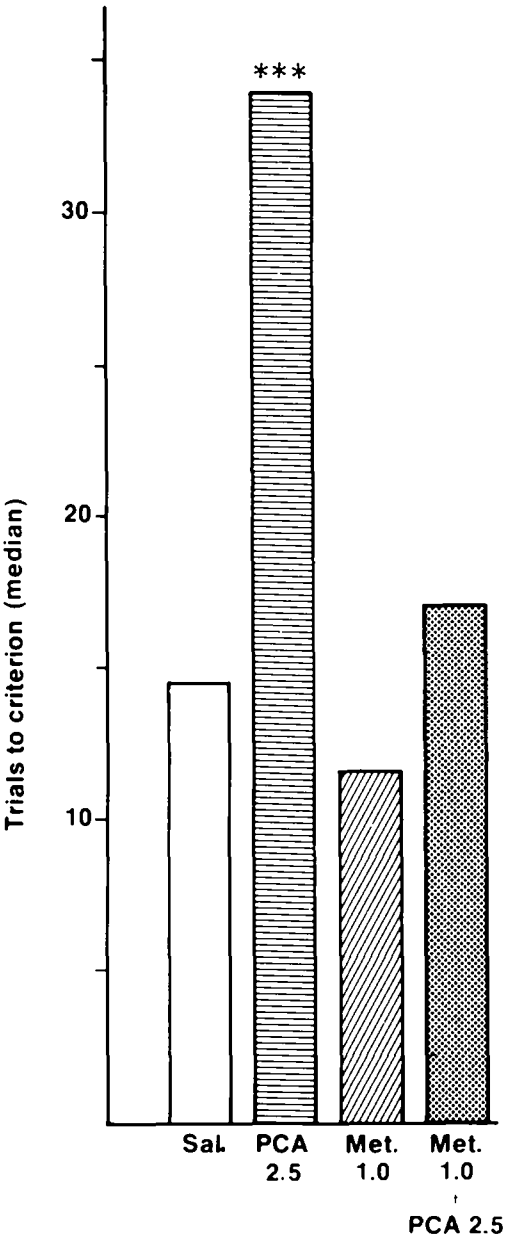

FIG. 8. Metergoline pretreatment blocks the p-chloramphetamine (PCA)-induced acquisition deficit. Metergoline (1 mg/kg), saline or PCA (2.5 mg/kg) were injected 30 min before testing. The results are the group median values of 8 rats. Pairwise comparisons revealed that the PCA group was significantly impaired compared with the saline and the other two groups (*** $p \leq 0.001$).

TABLE 4

GROUP MEDIAN (Mdn) AVOIDANCE ACQUISITION PARAMETERS AND INTERQUARTILE RANGES (IQR) FOLLOWING TREATMENT WITH p-CHLOROAMPHETAMINE (PCA) AND/OR METERGOLINE

Treatment	Group Mdn (IQR) trials to 1st avoidance	acquisition/ criterion	shocks/ criterion	failures/ criterion
Saline	4.5† (3.5– 9.0)	14.5‡ (13.0–17.5)	5.0‡ (4.0– 8.5)	0.5* (0 – 1.0)
PCA 2.5 mg/kg	14.0 (9.5–16.5)	34.0 (30 –35.5)	18.5 (12.5–21.5)	4.0 (1.0–17.5)
Metergoline 1 mg/kg	2.0‡ (2.0– 3.5)	11.5‡ (11.0–17.0)	2.5† (2.0– 7.0)	0.0† (0 – 0)
Metergoline 1 mg/kg+ PCA 2.5 mg/kg	5.5† (1.0– 7.0)	17.0‡ (15.5–18.5)	7.5† (5.5– 9.0)	1.0 (0 – 2.0)

Avoidance parameters were calculated as described in Experiment 1. *$p \leq 0.05$, †$p \leq 0.01$ and ‡$p \leq 0.001$ vs PCA (2.5), Mann-Whitney U-test.

DISCUSSION

The demonstration that lowering of brain 5-HT concentration by prior degeneration of 5-HT neurons completely blocked the avoidance decrement by acute PCA administration indicates that the acute PCA effect involves 5-HT neurons. This finding is important since the acute action of PCA also involves CA neurones [12, 56, 64]. Thus, the action of PCA is dependent upon intact 5-HT neurons and a certain amount of a releasable pool of 5-HT available in the presynaptic neurons.

Previous studies have demonstrated that PCA produced a preferential degeneration of the ascending 5-HT pathways involving both the dorsal and median raphe projections (cortex, hippocampus, striatum, thalamus) while having marginal effects on the descending 5-HT neurons [32,40]. Thus, the present data support the previous findings that the action of PCA is mediated via 5-HT terminal systems in the forebrain. Finally, the acute 5-HT releasing action on avoidance is completely different from the long-term depletion of brain 5-HT achieved either by synthesis inhibition or by degeneration of forebrain 5-HT neurons. Thus, neither the 5-HT synthesis inhibitor PCPA [31] nor long-term PCA treatment by themselves caused any avoidance learning deficit [40].

EXPERIMENT 5

The impairment of avoidance learning produced by PCA appears to be a result of enhanced post-synaptic activity in some serotonergic pathways. Thus, compounds which block postsynaptic 5-HT receptor sites would attenuate the action of PCA. The present study was designed to determine whether metergoline, which blocks post-synaptic 5-HT receptor sites in the CNS [23], would block the PCA-induced impairment of avoidance learning.

Procedure

Avoidance acquisition was carried out as described for Experiment 1. Four groups of rats (n=8) were treated with either saline, PCA (2.5 mg/kg), metergoline (1 mg/kg, 30 min before acquisition) or metergoline (1 mg/kg) + PCA (2.5 mg/kg). Metergoline was injected 1 min before PCA. Saline or PCA injection were given 30 min prior to testing. Retention was tested in a separate group of animals as described for Experiment 1. One group of rats (n=10) received metergoline (1 mg/kg) 1 min prior to PCA (5 mg/kg). The other three groups (n=6) received PCA (5 mg/kg), metergoline (1 mg/kg) or saline 60 min before retention testing.

RESULTS

Acquisition

The acquisition data are shown in Fig. 8. A Kruskall-Wallis ANOVA based on the number of trials to criterion showed a highly significant treatment effects, $H(3)=18.6$, $p \leq 0.001$. The PCA (2.5 mg/kg) treated rats was highly impaired as shown previously. Subsequent pairwise testing revealed that the PCA group differed significantly from saline, metergoline and the metergoline + PCA group ($p \leq 0.001$). Metergoline failed to significantly affect acquisition. The metergoline treatment, however, completely blocked the PCA induced avoidance impairment and this group did not differ from the saline treated rats. The other response parameters are shown in Table 4. These data correspond well to the findings in Experiment 1. PCA (2.5 mg/kg) increased both trials to criterion, shocks to criterion and produced a slight but significant increase in response failures. All these increases were significantly blocked by metergoline.

Retention

Metergoline (1 mg/kg) by itself did not significantly affect avoidance retention (Fig. 6). Metergoline pretreatment, however, blocked the avoidance deficit caused by PCA (5 mg/kg).

DISCUSSION

Metergoline was used to test the possibility of an enhanced postsynaptic 5-HT activity as a casual factor in the effect of PCA on avoidance. Behavioural [23], biochemical

[9,23] and electrophysiological [57,67] studies indicate that metergoline is a relatively specific 5-HT antagonist in the brain. Recent binding studies indicate that metergoline in the hippocampus may selectively label a class of 5-HT receptors which are very similar to the high-affinity binding site for ^{3}H-5-HT [25]. Metergoline also inhibits the high-affinity binding of ^{3}H-5-HT to brain membranes in the nanomolar range in the hippocampus [25] and dorsal neocortex [23,39]. In view of the recent evidence for multiple 5-HT receptors in the CNS [46] it should be noted that metergoline also inhibits the binding of the mixed 5-HT agonist-antagonist ^{3}H-d-LSD [43]. With the proposed terminology of Peroutka and Snyder [46] metergoline, thus, seems to act on both 5-HT$_1$ and 5-HT$_2$ receptors.

The present experiment demonstrates that pretreatment with metergoline blocked the effect of PCA on avoidance learning without effecting avoidance acquisition or retention when given alone. This result and previous findings shows that the PCA-induced deficit most likely is due to an enhancement of postsynaptic 5-HT acitivty secondary to 5-HT release. At present, the relationhip between the 5-HT receptor sites and serotonergic synaptic transmission is not known. However, in view of the recent binding-data with metergoline it is possible that metergoline blocks the functional effect of increased 5-HT concentration at the 5-HT receptors via inhibition of the high-affinity binding sites of 5-HT which may not correspond to the 5-HT receptor coupled to 5-HT stimulated adenylate cyclase [25]. The results with metergoline alone indicate that blockade of 5-HT receptors and possibly reduction of 5-HT neurotransmission does not facilitate or impair avoidance learning of this type. These results are, thus, consistent with previous findings using PCPA or high doses of PCA to produce a destruction of forebrain terminals. Both these procedures, which markedly decrease 5-HT concentration in the CNS and possibly decrease 5-HT synaptic activity, do not in general facilitate or impair one-way avoidance [40].

GENERAL DISCUSSION

The results of the present experiments have shown that parenteral PCA administration to the rat produces an active avoidance learning impairment. The major findings of the present paper indicate that the avoidance impairment following acute PCA-administration is specifically related to the acute release of endogenous 5-HT from presynaptic nerve endings resulting in stimulation of postsynaptic 5-HT receptors. The main evidence may be summarized as follows: (1) The threshold dose and dose range for the avoidance decrement parallels the acute 5-HT release produced by PCA. No such correlation between the action of PCA on NA and DA mechanisms and the behavioural response was found. (2) Administration of zimelidine and fluoxetine, a procedure shown to block the acute 5-HT release caused by PCA, also blocked the avoidance deficiency. (3) Depletion of central serotonin by preferential degeneration of forebrain 5-HT terminal systems by a neurotoxic dose of PCA (2×10 mg/kg) also blocked the acute effect of PCA on avoidance. These findings indicate that 5-HT but not NA and/or DA availability is required for the learning deficit caused by PCA. The effect of PCA on avoidance is thus completely opposite to that of amphetamine which produces a facilitation of avoidance learning [16]. The amphetamine facilitation has been shown to be dependent on intact CA synthesis and mediated via release of newly synthetized NA or DA [49]. (4) The central 5-HT receptor blocking agent metergoline blocked the behavioural action of PCA. (5) Recent studies show that the time-course of the PCA-induced avoidance deficiency (unpublished findings) parallels the time-course for PCA-induced 5-HT release *in vivo* as shown in the striatum and hippocampus by *in vivo* voltammetry [36]. Thus, an avoidance impairment is evident about 15 min after PCA-injection and lasts about 90–120 min at a dose of 2.5 mg/kg. Maximal effect is observed in the time range of 30–60 min. This time-course parallels the time-course for 5-HT dependent behaviours induced by PCA [36, 43, 66], which are maximal about 20–30 min after injection of PCA (5–10 mg/kg). In general, the doses of PCA causing avoidance impairments are clearly lower than those producing the PCA-induced serotonergic syndrome in Sprague-Dawley rats (5–10 mg/kg) which is an indicator of serotonin receptor stimulation [36,66]. Taken together, these data suggest that release of 5-HT leading to increased 5-HT availability and to increased stimulation of serotonin receptors interferes with the acquisition and retention of the avoidance response in rats.

Compounds which inhibit uptake or produce a release of 5-HT increase the concentration or availability of the neurotransmitter in the synaptic cleft. As a consequence an enhancement of 5-HT activity at postsynaptic 5-HT receptor sites seems to occur. In view of the importance of the neuronal reuptake mechanism for the biological inactivation of 5-HT, the differences between zimelidine and fluoxetine on one hand and PCA on the other are important. The failure of 5-HT uptake inhibitors to induce an acquisition and a retention deficit and to induce a marked 5-HT syndrome in mice and rats [43,44] and their relative weak electrophysiologic "5-HT receptor" response *in vivo* [36] is consistent with the view that enhancement of central 5-HT activity via interference with uptake produces a less marked activation of postsynaptic activity than 5-HT release. On the other hand, both zimelidine and fluoxetine have been shown to block the effects on avoidance and the acute 5-HT depletion caused by PCA possibly by interfering with the 5-HT release process *in vivo* [53,54] or by blocking the entry of PCA into presynaptic 5-HT neurons [18,37]. Since there is no direct evidence that PCA is actively transported into presynaptic neurons [53,54], the 5-HT uptake inhibitors may interfere with the 5-HT release mechanism and block the hypothetized outward active transport of 5-HT [53]. These two compounds also inhibit the PCA-induced 5-HT dependent behaviours such as head-twitches in both mice and rats [43] but not the behavioural effects and avoidance deficits (unpublished results) induced by the 5-HT agonists d-LSD and 5-Methoxy-N,N-dimethyltryptamine (see [63]).

The long-term effect of PCA was found to completely block the acute action of PCA. Thus, lowering of 5-HT by PCA eliminates that 5-HT pool which is available for rapid release by PCA and which is required for the postsynaptic 5-HT activation resulting in the acute avoidance deficit. It is not known, however, whether the action of PCA is mediated via a 5-HT pool which corresponds to synthetized 5-HT available for neuronal release. It should be noted that PCA most likely releases 5-HT from both vesicular and non-vesicular storage sites [53,54] and the PCA-induced 5-HT release, therefore, may not occur via exocytosis in contrast to the 5-HT release which is due to neuronal activity. PCA appears to produce at least some behavioural effects via release of 5-HT from extravesicular sites since PCA can induce the serotonergic syndrome in reserpinized mice [43]. It is

therefore possible particularly in view of the very small changes in 5-HT concentration required to produce the avoidance deficit that the amount of 5-HT released by acute PCA administration and which is of behavioural significance corresponds partly to the 5-HT pool available for neuronal release. This hypothesis is currently under investigation in animals with partially depleted brain 5-HT stores.

There are several alternative interpretations of the PCA-induced avoidance deficit. First, it has to be considered whether alterations in nonassociative processes (locomotion, pain perception) are responsible for the observed effects. The avoidance deficit is not readily explained on the basis of either reduced motor activity, nonspecific toxic behavioural effects or motor suppression (freezing). Effects on acquisition were found at doses lower than those affecting locomotor activity. In fact, the increased motor activity in PCA-treated rats [56,61] should favour one-way avoidance acquisition. Escape and avoidance latencies as well as the subjective observations failed to provide any evidence for PCA-induced interference with the actual reactivity to the shock. Taken together, the present data do not support an interpretation in terms of drug-induced changes of nonassociative processes. The present findings raise the possibility that the PCA-disruption may result from modifications of processes involved in the maintenance of recently acquired information (short-term memory or STM-system), or memory-storage or consolidation processes. It is conceivable that PCA-administration or the increase in 5-HT activity also may interfere with processes involving retrieval of stored information. Note, however, that higher doses of PCA are required to impair avoidance retention than acquisition. Thus, PCA administration which causes an increase in 5-HT activity appears to be more detrimental during the early phases of information processing before the actual consolidation has occurred. In favor of this interpretation is also the difference between the 5-HT uptake inhibitors zimelidine and fluoxetine vs PCA. None of the uptake inhibitors, which cause a less marked 5-HT activation than PCA, produced any retention deficit.

The present data raise questions as which components of short-term memory system or the memory-storage process are affected by the increase in postsynaptic 5-HT activity. Recent studies have shown that PCA (5 mg/kg) did not acutely affect the acquisition of fear conditioning, but markedly blocked classically conditioned fear retention when tested 24 hours later [2,3]. The fear retention deficit was also blocked by pretreatment with zimelidine and fluoxetine but not with desipramine and was not only observed when PCA was injected prior to acquisition but also when the injection occurred 30 min before the retention test [3]. This fear retention impairment indicated an involvement of 5-HT in both storage and retrieval processes [2,3]. The effects of fear retention correlate well both with the time and with the dose range in which PCA causes an avoidance retention deficit (threshold dose 1.25 mg/kg). Thus, it seems that PCA administration does not affect the actual acquisition or encoding of fear but affected the consolidation of the information regarding situational cues associated with shock. Since the acquisition of fear is certainly not consolidated at the time of PCA-administration, these data indicate that the fear retention deficit is probably mediated via a process which is beyond the operation of a STM-system. It must be emphasized, however, that the active avoidance task in contrast to fear acquisition involves multitrial learning sessions where a STM-system has to operate between trials and memory storage probably to a large extent occurs during training or between trials. It is conceivable that PCA-administration or increase in 5-HT activity in some way could interfere with the memory-storage processing which occurs between trials. In this context, the behaviour of PCA-rats are of considerable interest. PCA treated rats unlike saline rats occasionally returned to the shock-compartment in the intertrial period suggesting a temporary loss of memory for the aversive properties of the shock compartment established during conditioning. Experience of shock in the next trial reestablished avoidance behaviour which, however, generally after a few number of trials was followed by another crossing to the shock compartment. It seems as if PCA-rats had forgotten the aversive properties of the shock-compartment either via a deficit in the reactivation of arousal of stimuli associated with shock or via a deficit in a fear mediational process linked to the instrumental response (avoidance). It is currently being investigated whether or not the effects of PCA-administration are mediated via one or several factors such as memorial storage and/or retriaval processes and the possible role of intertrial-memory storage processing involving eg. contextual cues (see [45]) and intertrial interference.

The accumulated evidence from the present investigations suggests that 5-HT neurons may have a role in processes involving encoding, storage and retrieval of (recently) acquired information. However, the mechanism behind this activation of postsynaptic 5-HT receptors and its relationship to learning processes still remains to be elucidated. The finding that metergoline pretreatment block the avoidance impairment is in this context particularly interesting. Metergoline is a potent inhibitor of both 5-HT_1 and 5-HT_2 receptors in forebrain regions [23,25]. It is notable, however, that neither metergoline, PCPA nor degeneration of forebrain 5-HT terminals by high doses of PCA appear to generally facilitate one-way avoidance learning [40]. Serotonin neurons may, thus, play a modulatory role in avoidance learning, i.e., changes in 5-HT receptor activity may only under certain defined conditions alter aversive learning processes. Thus, it is possible that serotonin neurons may modulate aversive learning and memory processes by causing changes in more general physiological functions such as arousal or attention which also have been linked to CA neuronal activity (see [26,35]. In this context it is notable that the role of 5-HT neurons in avoidance learning appear to be inhibitory and thus opposite to the role suggested for central NA and DA pathways (see [26]). Although the present data seems to exclude a direct role of CA mechanisms in the PCA-induced deficit, it cannot be excluded that serotonin neurons may indirectly alter avoidance learning or memory-storage mechanisms by modifying neurotransmission in catecholamine neurons. There is evidence for direct anatomical connections between the raphe system and the locus coeruleus NA pathways and the nigroneostriatal DA pathways [8]. Pharmacological studies also indicate functional interactions between serotonin and CA neurons [55].

REFERENCES

1. Aprison, M. H. and J. M. Hingtgen. Neurochemical correlates of behaviour. *Int. Rev. Neurobiol.* **13:** 325–342, 1970.
2. Archer, T. Serotonin and fear retention in the rat. Submitted.
3. Archer, T., S. O. Ögren and C. Johansson. The acute effect of p-chloroamphetamine on the retention of fear conditioning in the rat: Evidence for a role of serotonin in memory consolidation. *Neurosci. Lett.* **25:** 75–81, 1981.
4. Atack, C. and T. Magnusson. A Procedure for the isolation of noradrenaline (together with adrenaline), dopamine, 5-hydroxytryptamine and histamine from the same tissue sample using a single column of strongly acidic cation exchange resin. *Acta pharmac. tox.* **42:** 35–57, 1978.
5. Azmitia, E. C. and M. Segal. An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J. comp. Neurol.* **179:** 641–668, 1978.
6. Blakely, T. A. and L. F. Parker. The effects of parachlorphenylalanine on experimentally induced conflict behaviour. *Pharmac. Biochem. Behav.* **1:** 609–613, 1973.
7. Blanchard, R. J. and D. C. Blanchard. Crouching as an index of fear. *J. comp. physiol. Psychol.* **67:** 370–375, 1969.
8. Bobillier, P., B. D. Lewis, S. Sequin and J. F. Pujol. Evidence for direct anatomical connections between the raphe system and other aminergic groups of the central nervous system as revealed by auto-radiography. In: *Interactions Between Putative Neurotransmitters in the Brain*, edited by S. Garattini, J. F. Pujol and R. Samanin. New York: Raven Press, 1978, pp. 343–354.
9. Bourgoin, S., F. Artaud, J. Bockaert, F. Héry, J. Glowinski and M. Hamon. Paradoxical decrease of brain 5-HT turnover by metergoline, a central 5-HT receptor blocker. *Naunyn-Schmiedeberg's Arch. Pharmac.* **302:** 313–321, 1978.
10. Brody, J. F. Behavioral effects of serotonin depletion of p-chlorophenylalanine (a serotonin depletor) in rats. *Psychopharmacologia* **17:** 14–33, 1970.
11. Chase, T. N. and D. L. Murphy. Serotonin and central nervous system function. *A. Rev. Pharmac.* **13:** 181–197, 1973.
12. Costa, E., K. M. Naimzada and A. Revuelta. Effect of phenmetrazine, aminorex and (±)p-chloroamphetamine on the motor activity and turnover rate of brain catecholamines. *Br. J. Pharmac.* **43:** 570–579, 1971.
13. Costa, E. and A. Revuelta. (−)-p-Chloroamphetamine and serotonin turnover in rat brain. *Neuropharmacology* **11:** 291–295, 1972.
14. Essman, W. B. Some neurochemical correlates of altered memory consolidation. *Trans. N.Y. Acad. Sci.* **32:** 948–973, 1970.
15. Essman, W. B. Neuromolecular modulation of experimentally induced retrograde amnesia. *Confinia neurol.* **35:** 1–22, 1973.
16. Evangelista, A. M., R. C. Gattoni and I. Izquierdo. Effect of amphetamine, nicotine and hexamethonium on performance of a conditioned response during acquisition and retention trials. *Pharmacology* **3:** 91–96, 1970.
17. Fibiger, H. C., F. G. Lepiane and A. G. Phillips. Disruption of memory produced by stimulation of the dorsal raphe nucleus: mediation by serotonin. *Brain Res.* **155:** 380–386, 1978.
18. Fuller, R. W., K. W. Perry and B. B. Molloy. Effect of 3-(p-trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine on the depletion of brain serotonin by 4-chloroamphetamine. *J. Pharmac. exp. Ther.* **193:** 796–803, 1975.
19. Fuller, R. W., K. W. Perry and B. B. Molloy. Reversible and irreversible phases of serotonin depletion by 4-chloroamphetamine. *Eur. J. Pharmac.* **33:** 119–124, 1975.
20. Fuxe, K. Evidence for the existence of monoamine neurons in the central nervous system. IV. Distribution of monoamine nerve terminals in the central nervous system. *Acta physiol. scand.* **64:** Suppl. 247, 37–85, 1965.
21. Fuxe, K., L. L. Butcher and J. Engel. Dl-5-hydroxytryptophan-induced changes in central monoamine neurons after peripheral decarboxylase inhibition. *J. Pharm. Pharmac.* **23:** 420–424, 1971.
22. Fuxe, K. and G. Jonsson. Further mapping of central 5-hydroxytryptamine neurons: studies with the neurotoxic dihydroxytryptamines. *Adv. Biochem. Pharmac.* **10:** 1–12, 1974.
23. Fuxe, K., S. O. Ögren, L. F. Agnati and G. Jonsson. Further evidence that methergoline is a central 5-hydroxytryptamine receptor blocking agent. *Neurosci. Lett.* **9:** 195–200, 1978.
24. Graeff, F. G. and R. I. Schoenfeld. Tryptaminergic mechanisms in punished and nonpunished behaviour. *J. Pharmac. exp. Ther.* **173:** 277–283, 1970.
25. Hamon, M., M. Mallat, A. Herbet, D. L. Nelson, M. Audinot, L. Pichat and J. Glowinski. [^{3}H] Metergoline: A new ligand of serotonin receptors in the rat brain. *J. Neurochem.* **36:** 613–626, 1981.
26. Hunter, B., S. F. Zornetzer, M. E. Jarvik and J. L. McGaugh. Modulation of learning and memory: Effects of drugs influencing neurotransmitters. In: *Handbook of Psychopharmacology*, vol. 8, edited by L. L. Iversen, S. D. Iversen and S. H. Snyder. New York: Plenum Press, 1977, pp. 531–577.
27. Jacobs, B. L. Minireview: An animal behavior model for studying central serotonergic synapses. *Life Sci.* **19:** 777–786, 1976.
28. Joyce, D. and H. M. B. Hurwitz. Avoidance behaviour in the rat after 5-hydroxytryptophan (5-HTP) administration. *Psychopharmacologia* **5:** 424–430, 1964.
29. Kehr, W., M. Lindqvist and A. Carlsson. Distribution of dopamine in the rat cerebral cortex. *J. Neural Transm.* **38:** 173–180, 1976.
30. Kirk, R. E. *Experimental design: Procedures for the Behavioral Sciences*. Belmont, CA: Brooks/Cole., 1968.
31. Koe, K. B. and A. Weissman. p-Chlorophenylaline: A specific depletor of brain serotonin. *J. Pharmac. exp. Ther.* **154:** 499–516, 1966.
32. Köhler, C., S. B. Ross, B. Srebro and S. O. Ögren. Long-term biochemical and behavioural efects of p-chloroamphetamine in the rat. *Ann. N.Y. Acad. Sci.* **305:** 645–663, 1978.
33. Lorens, S. A. and L. M. Yunger. Morphine analgesia, two-way avoidance, and consummatory behavior following lesions in the midbrain raphe nuclei of the rat. *Pharmac. Biochem. Behav.* **2:** 215–221, 1974.
34. Lorens, S. A. Some behavioural effects of serotonin depletion depend on method: A comparison of 5,7-dihydroxytryptamine, p-chlorophenylalanine, p-chloroamphetamine and electrolytic raphe lesions. *Ann. N.Y. Acad. Sci.* **305:** 532–555, 1978.
35. Mabry, P. O. and B. A. Campbell. Serotonergic inhibition of catecholamine-induced behavioural arousal in the rat. *J. comp. physiol. Psychol.* **86:** 193–201, 1974.
36. Marsden, C. A., J. Conti, E. Strope, G. Curzon and R. N. Adams. Monitoring 5-hydroxytryptamine release in the brain of the freely moving unanaesthetized rat using in vivo voltammetry. *Brain Res.* **171:** 85–99, 1979.
37. Meek, J. L., K. Fuxe and A. Carlsson. Blockade of p-chloromethamphetamine induced 5-hydroxytryptamine depletion by chlorimipramine, chlorpheniramine and meperidine. *Biochem. Pharmac.* **20:** 707–709, 1971.
38. Modigh, K. Central and peripheral effects of 5-hydroxytryptophan on motor activity in mice. *Psychopharmacologia* **23:** 48–54, 1972.
39. Ögren, S. O., K. Fuxe, L. F. Agnati, J. Á. Gustafsson, G. Jonsson and A. C. Holm. Reevaluation of the indoleamine hypothesis of depression. Evidence for a reduction of functional activity of central 5-HT systems by antidepressant drugs. *J. Neural Transm.* **46:** 85–103, 1979.
40. Ögren, S. O., K. Fuxe, T. Archer, H. Hall, A. C. Holm and C. Köhler. Studies on the role of central 5-HT neurons in avoidance learning: A behavioural and biochemical analysis. In: *Serotonin: Current Aspects of Neurochemistry and Function*, edited by B. Haber, S. Gabay, M. R. Issidorides and S. G. A. Alivisatos. New York: Plenum Press, 1981, pp. 681–705.

41. Ögren, S. O. and C. Johansson. Serotonin and one-way active avoidance learning in the rat. Separating the associative and nonassociative effects of 5-HT release induced by p-chloroamphetamine. Submitted.
42. Ögren, S. O., C. Köhler, S. B. Ross and B. Srebro. 5-Hydroxytryptamine depletion and avoidance acquisition in the rat. Antagonism of the long-term effects of p-chloroamphetamine with a selective inhibitor of 5-hydroxytryptamine uptake. *Neurosci. Lett.* **3:** 341–347, 1976.
43. Ögren, S. O. and S. B. Ross. Substituted amphetamine derivatives. II Behavioural effects in mice related to monoaminergic neurons. *Acta pharmac. tox.* **41:** 353–368, 1977.
44. Ögren, S. O., S. B. Ross, A. C. Holm and L. Baumann. 5-Hydroxytryptamine and avoidance performance in the rat. Antagonism of the acute effect of p-chloroamphetamine by zimelidine, an inhibitor of 5-hydroxytryptamine uptake. *Neurosci. Lett.* **7:** 331–336, 1977.
45. Olton, D. S. and R. L. Isaacson. Importance of spatial location in active avoidance tasks. *J. comp. physiol. Psychol.* **65:** 535–539, 1968.
46. Peroutka, S. J. and S. H. Snyder. Multiple serotonin receptors: Differential binding of [^{3}H] 5-hydroxytryptamine, [^{3}H] lysergic acid diethylamide and [^{3}H] spiroperidole. *Molec. Pharmac.* **16:** 687–699, 1979.
47. Pfister, W. R., V. Noland, M. T. Lowry, D. E. Nichols and K. W. Yim. Comparison of the behavioural effects of para-chloroamphetamine, chlordimeform, quipazine and intraventricular serotonin in the rat. *Communs Psychopharmac.* **2:** 287–296, 1978.
48. Rake, A. V. Involvement of biogenic amines in memory formation: The central nerous system indole amine involvement. *Psychopharmacologia* **29:** 91–100, 1973.
49. Roffman, M. and H. Lal. Role of brain amines in learning associated with "Amphetamine-State." *Psychopharmacologia* **25:** 195–204, 1972.
50. Ross, S. B. Antagonism of the acute and long-term biochemical effects of 4-chloroamphetamine on the 5-HT neurons in the rat brain by inhibitors of the 5-hydroxytryptamine uptake. *Acta pharmac. tox.* **39:** 456–476, 1976.
51. Ross, S. B., S. O. Ögren and A. L. Renyi. (Z)-Dimethylamino-1-(4-bromophenyl)-1-(3-pyridyl) propene (H 102/09), a new selective inhibitor of the neuronal 5-hydroxytryptamine uptake. *Acta pharmac. tox.* **39:** 152–166, 1976.
52. Ross, S. B., S. O. Ögren and A. L. Renyi. Substituted amphetamine derivatives. I. Effect on uptake and release of biogenic monoamines and on monoamine oxidase in the mouse brain. *Acta. pharmac. tox.* **41:** 337–352, 1977.
53. Ross, S. B. and D. Kelder. efflux of 5-hydroxytryptamine from synaptosomes of rat cerebral cortex. *Acta physiol. scand.* **99:** 27–36, 1977.
54. Ross, S. B. Interactions between reserpine and various compounds on the accumulation of [^{14}C]5-hydroxytryptamine and [^{3}H]noradrenaline in homogenates from rat hypothalamus. *Biochem. Pharmac.* **28:** 1085–1088, 1979.
55. Samanin, R. and S. Garattini. The serotonergic system in the brain and its possible functional connections with other aminergic systems. *Life Sci.* **17:** 1201–1210, 1975.
56. Sanders-Bush, E. and L. R. Steranka. Immediate and long-term effects of p-chloroamphetamine on brain amines. *Ann. N. Y. Acad. Sci.* **305:** 208–221, 1978.
57. Sastry, B. S. R. and J. W. Phillis. Metergoline as a selective 5-hydroxytryptamine antagonist in the cerebral cortex. *Can. J. Physiol. Pharmac.* **55:** 130–133, 1977.
58. Schlesinger, K. and R. A. Schreiber. Effects of p-chorphenylalanine on conditioned avoidance learning. *Psychon. Sci.* **11:** 225–226, 1968.
59. Srebro, B. and S.A. Lorens. Behavioural effects of selective midbrain raphe lesion in the rat. *Brain Res.* **89:** 303–325, 1975.
60. Steranka, L. R. and R. J. Barrett. Facilitation of avoidance acquisition by lesion of the median raphe nucleus: evidence for serotonin as a mediator of shock-induced suppression. *Behav. Biol.* **11:** 205–213, 1974.
61. Steranka, L. R., R. J. Barrett and E. Sanders-Busch. Facilitation of Sidman avoidance performance by p-chloramphetamine: role of biogenic amines. *Neuropharmacology* **16:** 751–759, 1977.
62. Stevens, D. A., L. D. Fechter and O. Resnick. The effects of p-chlorophenylalanine, a depletor of brain serotonin on behaviour: II. Retardation of passive avoidance learning. *Life Sci.* **8:** 379–385, 1969.
63. Stoff, D. M., D. A. Gorelick, T. Bozewicz, W. H. Bridger, J. C. Gillin and R. J. Wyatt. The indole hallucinogens N,N-dimethyltryptamine (DMT) and 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT) have different effects from mescaline on rat shuttlebox avoidance. *Neuropharmacology* **17:** 1035–1040, 1978.
64. Strada, S., E. Sanders-Busch and F. Sulser. p-Chloroamphetamine: Temporal relationship between psychomotor stimulation and metabolism of brain norepinephrine. *Biochem. Pharmac.* **19:** 2621–2629, 1970.
65. Tenen, S. S. The effects of p-chlorophenylalanine, a serotonin depletor, on avoidance acquisition, pain sensitivity and related behaviour in the rat. *Psychopharmacologia* **10:** 204–219, 1967.
66. Trulson, M. E. and B. L. Jacobs. Behavioural evidence for the rapid release of CNS serotonin by PCA and fenfluramine. *Eur. J. Pharmac.* **36:** 149–154, 1976.
67. Wang, R. Y. and G. K. Aghajanian. Collateral inhibition of serotonergic neurones in the rat dorsal raphe nucleus: Pharmacological evidence. *Neuropharmacology* **17:** 819–825, 1978.
68. Wolley, D. W. and T. Van der Hoeven. Alteration in learning ability caused by changes in cerebral serotonin and catecholamines. *Science* **139:** 610–611, 1963.