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The Relative Contribution of Ascending and Descending Serotonergic Pathways in p-Chloroamphetamine-Induced Antinociception

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BJØRKUM, A. A. AND O.-G. BERGE. *The relative contribution of ascending and descending serotonergic pathways in p-chloroamphetamine-induced antinociception.* PHARMACOL BIOCHEM BEHAV 31(1) 135–140, 1988.—Systemic administration in rats of p-chloroamphetamine (PCA; 2×10 mg/kg) reduced the in vitro uptake of ^{14}C-5-hydroxytryptamine (^{14}C-5-HT) in cortical synaptosomes by 76% and in spinal cord synaptosomes by 35%. Intrathecal injection of 5,6-dihydroxytryptamine (20 μg/rat) selectively lesioned the descending serotonergic pathways (83% reduction in uptake of ^{14}C-5-HT in spinal synaptosomes, no significant change in uptake in cortical synaptosomes). Administration of PCA or 5,6-DHT did not significantly alter the uptake of ^{3}H-noradrenalin into cortical or spinal synaptosomes. The response thresholds of the rats in the increasing temperature hot plate test (1 to 7 days after administration) were unaffected by either type of lesion. Interference with the antinociceptive effect of PCA (2.5 mg/kg) was evaluated 7 days after administration of the neurotoxins. PCA pretreatment strongly reduced the peak of the PCA-induced antinociception while 5,6-DHT reduced its duration. Thus, both ascending and descending serotonergic pathways contribute to PCA-induced antinociception.

p-Chloroamphetamine 5,6-Dihydroxytryptamine Nociception Serotonin Spinal cord

DEPENDING on dosage, p-chloroamphetamine (PCA) may be used as a neurotoxin [22,35] and as a serotonin releasing agent [39]. Administration of PCA to rats in doses which have a releasing effect causes antinociception in the paw-compression, hot plate and flinch jump tests [15, 25, 28]. Results obtained with the tail flick test are less consistent [25, 28, 29]. In mice, PCA-induced antinociception has been demonstrated in the hot plate and formalin test while no effect was found in the tail flick test [18].

As a neurotoxin, PCA mainly affects serotonergic structures in the brain, sparing other monoaminergic structures and having slight or no effects in the spinal cord [18,22]. Pretreatment with neurotoxic doses of PCA attenuates the antinociceptive effects of subsequent moderate doses of PCA in rats and mice [18,25]. These findings suggest that the integrity of supraspinal serotonergic structures is essential for the expression of PCA-induced antinociception.

Some reduction in PCA-induced antinociception has also been found after selective lesions of the descending serotonergic pathways induced by intrathecal injection of 5,6-dihydroxytryptamine (5,6-DHT) [8,27]. In these studies, 5,6-DHT treatment alone enhanced the responsiveness of the tested animals, which made a definite interpretation of the results difficult. Furthermore, the antinociceptive effect of PCA was only assessed at one point after injection. Recent data suggest that PCA may affect the brain and the spinal cord with a different time course [24].

In preliminary studies, we have observed that 5,6-DHT-induced lesions of the descending serotonergic pathways fail to affect response thresholds in the increasing temperature hot plate test. In this modification of the conventional hot plate test the temperature is gradually increased from a non-noxious level until a criterion response is observed [19,25].

The increasing temperature hot plate test was therefore employed to investigate the effects of lesions of spinal and supraspinal serotonergic structures on PCA-induced antinociception. The effect of PCA was monitored for 2 hours to allow observation of any alterations in time course after the lesions.

METHOD

Animals

Male Sprague-Dawley rats (Møllegård, Denmark) were

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housed individually in conventional macrolone cages. Food was restricted to 15 g pellets per animal per day to maintain a body weight of about 250 g. Water was freely available. Retrospective analysis of data from several pharmacological experiments in which the tail flick and conventional hot plate tests have been employed indicate that this procedure does not affect the results except that confounding effects of group differences in weight gain are prevented.

The animals were housed in controlled light (lights on between 8:00 and 20:00 hours) and temperature (21–23°C) conditions and adapted to the laboratory where the testing took place for at least 18 hours before each test session. All testing took place during the light phase.

Drugs

p-Chloroamphetamine hydrochloride (PCA, Sigma Chemical Company) was dissolved in 0.9% NaCl and injected intraperitoneally (IP) in a volume of 5 ml/kg. Antinociceptive effect was induced by administration of 2.5 mg/kg. Lesions were produced by administration of 10 mg/kg on two consecutive days, 7 and 8 days before testing with the 2.5 mg/kg dose of PCA.

5,6-Dihydroxytryptamine creatinine sulfate (5,6-DHT, Sigma Chemical Company) was dissolved in 0.9% NaCl containing 0.2 mg/ml ascorbic acid. A dose of 20 μg (calculated as the base) was injected intrathecally in a volume of 10 μl. In addition, 4.5 μl vehicle was used to flush the catheter.

The radioisotopes ^{14}C-5-hydroxytryptamine creatinine sulfate (^{14}C-5-HT) and l-^{3}H-noradrenalin hydrochloride (^{3}H-NA) were obtained from The Radiochemical Centre (Amersham, U.K.).

Surgery and 5,6-DHT Administration

Intrathecal catheterization was performed as described in detail elsewhere [5]. The animals were anesthetized with a combination of pentobarbital (40 mg/kg) and chloral hydrate (180 mg/kg) given IP. A polyethylene catheter (PE10, Clay Adams) previously stretched to reduce the dead space to less than 4.5 μl was inserted through a slit in the atlanto-occipital membrane. The tip of the catheter was located in the subarachnoid space at the rostral end of the lumbar enlargement. A minimum of 7 days was allowed for recovery before administration of 5,6-DHT. 5,6-DHT was injected at a rate of 7.5 μl per min by means of a perfusion pump. Control animals were injected with vehicle only. During the infusion, the animals were free to move in the home cage and showed no or only minor signs of discomfort.

Biochemical Evaluation of the Lesions

The animals were sacrificed by decapitation and the brains and spinal cords rapidly removed and dissected on ice [5]. The simultaneous uptake of radiolabeled NA and 5-HT into crude synaptosomes prepared from the lumbar spinal cord and the frontal cortex was determined as described in detail previously [13]. Briefly, after preincubation of the crude synaptosomal fraction in a modified Krebs-Ringer bicarbonate buffer for 3 min at 37°C, ^{14}C-5-HT and ^{3}H-NA were added to bring the final concentrations of isotopes in the incubation medium to 100 nM and 10 nM respectively. After 10 min the incubation was terminated by filtration. The samples were analyzed in a Packard Tri-Carb 460 CD liquid scintillation spectrometer. Uptake in the presence of cocaine (1 mM) was subtracted from the total uptake.

Antinociceptive Testing

The increasing temperature hot plate test was conducted with an IITC Export Inc. Model 35-D Analgesiameter. The day before each test session, the animals were adapted to the cold apparatus for 1 min. During testing, the temperature of the plate was gradually increased from 43°C at a rate of 2.5°C per min. The temperature when the animal started licking a hind paw was recorded as the response temperature. A detailed evaluation of the testing procedure has been published [19].

The effects of 5,6-DHT and PCA-induced lesions were evaluated in separate experiments using the same overall design. In each experiment half of the animals received neurotoxic treatment. The other half was identically treated except that vehicle only was injected. The groups were further subdivided to receive either PCA or vehicle on the final day of testing.

The animals were tested once immediately prior to administration of neurotoxin. Testing was then carried out once a day on the first, third and fifth day after administration of 5,6-DHT or PCA. The antinociceptive effect of PCA was evaluated on the seventh day when each animal was tested 15, 30, 60 and 120 min after administration of 2.5 mg/kg PCA or vehicle.

Statistics

The data were examined by analysis of variance (ANOVA) as detailed in the results or by Student's t-test when the analysis was restricted to two means. Unless otherwise stated, significance was accepted at the 5% level. All results are given as mean±S.E.M.

RESULTS

Biochemical Verification of the Lesions

The biochemical analysis was carried out 2 to 3 weeks after administration of neurotoxin. Only animals that received vehicle as test treatment were used, to avoid confounding neurotoxic effects of the 2.5 mg/kg test dose. PCA significantly reduced the uptake of 5-HT into cortical synaptosomes and to a lesser extent the upake into spinal synaptosomes (Table 1). 5,6-DHT significantly reduced the uptake in the spinal cord but did not affect the uptake into cortical synaptosomes. Neither treatment altered the uptake of noradrenalin in spinal or cortical synaptosomes.

Effects of PCA-Induced Lesions

Prior to administration of the neurotoxic doses of PCA, and on the first, third and fifth day afterwards, no difference in response temperature was observed between the PCA and vehicle pretreated rats (data not shown) and statistical analysis (ANOVA, 2 treatments × 4 trials) revealed no tendency towards treatment effect, $F(1,18)<1$, or treatment × trial interaction, $F(3,54)<1$.

Seven days after administration of neurotoxic doses, PCA (2.5 mg/kg) had a pronounced antinociceptive effect in vehicle pretreated animals, particularly 15 and 30 min after injection (Fig. 1). The peak effect was considerably weaker in the PCA pretreated rats, although at all time points, either of the groups that received PCA as test treatment responded at higher temperatures than the groups that received vehicle.

ANOVA (2 test treatments × 2 pretreatments × 4 trials) demonstrated a significant interaction between trials and pretreatments, $F(3,105)=5.78$, $p<0.002$, trials and test

TABLE 1

EFFECTS OF PCA AND 5,6-DHT ON ACCUMULATION OF ^{14}C-5-HT AND ^{3}H-NA IN SYNAPTOSOMES FROM FRONTAL CORTEX AND LUMBAR SPINAL CORD

	^{14}C-5-HT		^{3}H-NA	
Group	Cortex	Spinal Cord	Cortex	Spinal Cord
Vehicle	100.0 ± 16.3	100.0 ± 16.2	100.0 ± 8.9	100.0 ± 8.4
PCA	23.8 ± 3.2*	65.1 ± 3.4†	103.9 ± 8.5	98.1 ± 6.2
Vehicle	100.0 ± 2.3	100.0 ± 5.3	100.0 ± 6.5	100.0 ± 8.4
5,6-DHT	94.2 ± 4.4	16.9 ± 4.1*	103.3 ± 6.7	96.1 ± 6.2

All data given in percent of control values. The actual control values were 487–588 d.p.m. for the 5-HT isotope and 5205–14935 d.p.m. for the NA isotope. The data represents V_{max} for the uptake as determined in separate studies.

*$p<0.001$, compared to vehicle group, Student's t-test.

†NS, $0.05<p<0.10$, compared to vehicle group, Student's t-test.

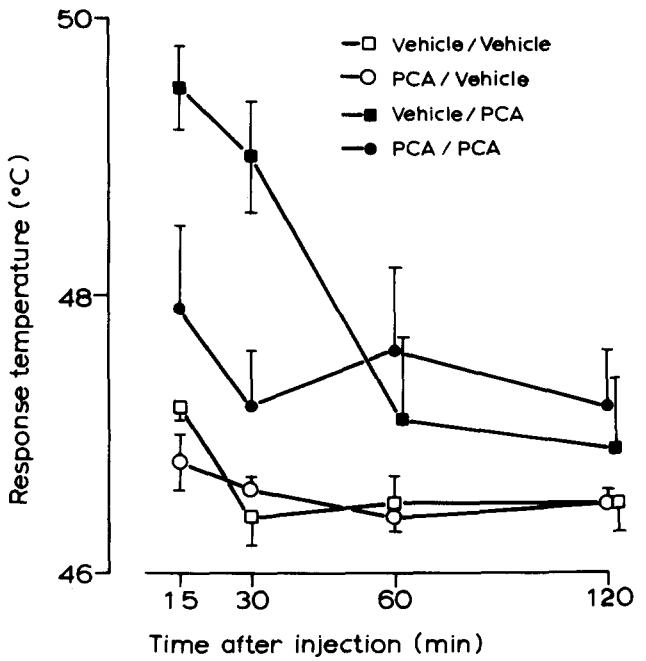

FIG. 1. The effects of PCA pretreatment (2×10 mg/kg) on PCA-induced antinociception (mean±S.E.M, n=8–12 in each group). Testing was performed 7 days after the last pretreatment dose of PCA or vehicle. The test injection consisted of 2.5 mg/kg PCA or vehicle.

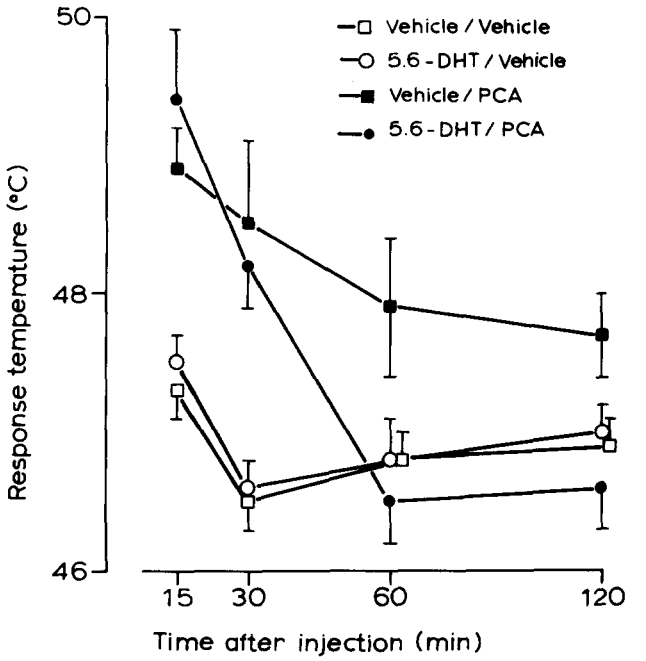

FIG. 2. The effects of 5,6-DHT pretreatment (20 μg) on PCA-induced antinociception (mean±S.E.M, n=8–9 in each group). Testing was performed 7 days after intrathecal administration of 5,6-DHT or vehicle. The test injection consisted of 2.5 mg/kg PCA or vehicle.

treatments, $F(3,105)=5.76$, $p<0.002$, as well as between all three factors, $F(3,105)=8.27$, $p<0.0001$.

When the data from the two groups that received vehicle as test treatment were analyzed separately, no significant group effect, $F(1,18)<1$, or group × trial interaction, $F(3,54)=1.49$, $p>0.20$, was found, indicating a lack of effect of PCA-induced lesions on response temperatures, in line with the findings reported above. ANOVA applied to the data of the two groups that received PCA as test treatment demonstrated a significant group effect, $F(1,17)=5.94$, $p<0.025$, and group × trial interaction, $F(3,51)=9.56$, $p<0.0001$, showing that the pretreatment significantly affected the PCA-induced antinociception.

Effects of 5,6-DHT-Induced Lesions

No difference in response temperature was observed between the 5,6-DHT and the vehicle pretreated rats during the week before testing with PCA. ANOVA (2 treatments × 4 trials) revealed no tendency towards treatment effect, $F(1,15)<1$, or treatment × trial interaction, $F(3,45)<1$.

Seven days after administration of 5,6-DHT, PCA (2.5 mg/kg) had a pronounced antinociceptive effect in both vehicle pretreated and 5,6-DHT pretreated rats (Fig. 2). The peak effect was similar in both groups but the duration appeared to be shorter in the lesioned animals.

ANOVA (2 test treatments × 2 pretreatments × 4 trials)

demonstrated a significant interaction between trials and pretreatments, $F(3,90)=3.28$, $p<0.025$, trials and test treatments, $F(3,90)=11.36$, $p<0.0001$, and a tendency towards overall interaction between the three factors, $F(3,90)=2.40$, $0.05<p<0.10$.

Separate analysis of the two groups that received vehicle as test treatment showed no significant group effect, $F(1,15)<1$, or group × trial interaction, $F(3,45)<1$, confirming that 5,6-DHT treatment lacked any direct effect on response temperatures. ANOVA applied to the data of the two groups that received PCA as test treatment demonstrated no significant group effect, $F(1,15)=2.15$, $p>0.15$, but a significant group × trial interaction, $F(3,45)=3.59$, $p<0.025$, showing that the 5,6-DHT pretreatment significantly affected the PCA-induced antinociception.

DISCUSSION

Animals pretreated with PCA showed approximately 75% reduction of synaptosomal accumulation of serotonin in the frontal cortex. The rats showed no direct change in response temperature in the increasing temperature hot plate test, but were less sensitive to the antinociceptive action of PCA. Animals pretreated with 5,6-DHT showed more than 80% reduction in synaptosomal accumulation of serotonin in the lumbar spinal cord, reduced duration of PCA-induced antinociception but no direct alteration in response temperature.

Former studies have shown the utility of PCA for lesioning of supraspinal serotonergic structures [33,34]. Catecholaminergic neurones and spinal serotonergic terminals appear to be quite resistant to the neurotoxic effects of the drug [22,35]. The findings reported here are in line with the earlier studies, although a tendency to lesioning of the descending serotonergic pathways was noted.

The effect of intrathecal administration of 5,6-DHT is also in good agreement with earlier studies with regard to selectivity and reduction in synaptosomal uptake [5]. Thus, the lesioning methods used appear to be selective for serotonergic neurones. However, coexistence of 5-HT and other neurotransmitters (e.g., Substance P and TRH) in the same neurones has been demonstrated [12,16]. It is likely that these transmitters are also affected by the lesions, but the functional implication is uncertain.

It should be noted that total lesions are not obtained by administration of neurotoxins and that functional recovery may occur, possibly by a process involving spared fibers and development of receptor supersensitivity [3,14]. In the reported study, the interval between administration of neurotoxin and investigation of PCA-induced antinociception was chosen as to allow maximum neurotoxic effect and minimum development of functional recovery.

This report is the first in which the effects of lesions of ascending and descending serotonergic pathways have been investigated by means of the increasing temperature hot plate test. Some earlier studies using less selective lesioning procedures and measuring responsiveness to electrical stimulation have suggested that ascending serotonergic projections may exert tonic inhibition on nociceptive functions (for references see [7]). The lack of effect of PCA-induced lesions in the present study is, however, in general agreement with several studies using other tests and various selective lesioning procedures for ascending serotonergic pathways [9–11, 17, 25, 28].

A substantial amount of experimental evidence shows that the descending serotonergic pathways exert tonic inhibition on nociception as measured with the tail flick, flinch jump and conventional hot plate tests [2, 8, 27, 30, 31]. However, in the formalin test, mice lesioned by intrathecal injection of 5,6-DHT showed less responsiveness during the initial phase of the test and appeared to have normal pain responses during the second phase [13]. The initial phase of the formalin test probably represents acute, chemical pain while the second phase represents inflammatory pain [20]. The formalin test and the increasing temperature hot plate test are sensitive to both weak analgesics and opiates and may therefore be better models for clinical pain than the conventinal hot plate test and the tail flick test, both of which are insensitive to nonnarcotic analgesics [19].

Several factors may contribute to the difference between tests with regard to the role of the descending serotonergic pathways. Tonic inhibitory activity is most reliably demonstrated when the stimulus is of brief duration and may thus be related to regulation of protective reflexes and responses. However, lesions of serotonergic pathways may also have indirect effects in nociceptive tests. Lesions enhance or reduce "stress-induced analgesia" depending on the parameters of the stress [21,38]. Recent studies have, furthermore, demonstrated that the tail flick test is highly sensitive to the temperature of the skin at the start of stimulation [6] and that the hyperalgesia found in the tail flick test after lesions of the descending serotonergic pathways may be accounted for by a concomitant increase in skin temperature [37], most likely caused by a reduction in vasomotor tone. Due to technical difficulties in obtaining reliable temperature recordings from the paws without interfering with the required behavioral responses, data relating paw temperature to responsiveness in the hot plate tests are not yet available. It seems likely, however, that the conventional hot plate test is similarly affected as the tail flick test. In both cases the response occurs as the exposed skin reaches a critical temperature, and the time required to obtain this temperature would probably be shorter at elevated initial skin temperature also in the hot plate test. The importance of initial skin temperature would be minimized in the rising temperature paradigm since the response latency is irrelevant for the results.

Thus, the present results are in line with the contention that the tonic inhibition on nociception exerted by the descending serotonergic pathways is secondary to alterations in skin temperature. Whether the formalin and flinch jump tests are confounded by vasomotor effects of lesions remain to be elucidated.

Acutely, PCA enhances serotonergic neurotransmission by neuronal release [35,39] and inhibition of reuptake [32]. Only weak effects have been reported with regard to other monoamine neurotransmitters [23,24] and substantial evidence suggests that release of 5-HT from supraspinal neuronal terminals is essential for the expression of PCA-induced antinociception in the conventional hot plate test [25,26]. The findings reported here suggest that this is also the case with the increasing temperature hot plate test.

PCA probably affects terminal regions of all ascending serotonergic pathways although some regions, particularly the hypothalamus, appear to be less susceptible to its neurotoxic effects [22]. Supraspinal serotonergic projections to the hippocampus [36] and the thalamus [1] have been hypothesized to play a role in the modulation of pain. Further research is clearly needed to determine if any single ascending projection is critical for the antinociceptive effect of PCA.

The present study confirms and extends previous results suggesting a role for the descending serotonergic pathways

in PCA-induced antinociception [8]. Whether release of serotonin in the spinal cord is sufficient to produce antinociception in the increasing temperature hot plate test is, however, uncertain. Experiments in mice have shown that direct injections of PCA into the spinal subarachnoid space had antinociceptive effects in the tail flick test, but not in the increasing temperature hot plate test [4].

In conclusion, neither descending nor ascending serotonergic pathways appear to exert tonic antinociceptive effects in the increasing temperature hot plate test. Both systems do, however contribute to the antinociceptive action of PCA.

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