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Modulatory effect of *p*-chlorophenylalanine microinjected into the dorsal and median raphe nuclei on cocaine-induced behaviour in the rat

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Abstract

The present study examined whether a potentiation of cocaine-induced behaviour in rats following peripheral pretreatment with the 5-hydroxytryptamine (5-HT) biosynthesis inhibitor *p*-chlorophenylalanine may be due to depletion of 5-HT in the dorsal raphe nucleus and/or median raphe nucleus. Following peripheral pretreatment with *p*-chlorophenylalanine (100 mg/kg, i.p.) for 3 consecutive days, a potentiation of cocaine-induced locomotor activity and rears was observed. To investigate a possible involvement of serotonergic neurones arising in the midbrain raphe nuclei in the observed potentiation, *p*-chlorophenylalanine (0.5 μg) was microinjected in either the dorsal raphe nucleus or median raphe nucleus followed by behavioural testing 48 h later. Application of *p*-chlorophenylalanine in the dorsal raphe nucleus resulted in an enhancement of cocaine-induced locomotor activity and head bobs. In contrast, the stimulant effect of cocaine on behaviour was not altered by microinjection of *p*-chlorophenylalanine in the median raphe nucleus. Peripheral and central administration of *p*-chlorophenylalanine did not consistently alter the baseline behaviour of saline-treated animals. Biochemical results indicated only a moderate depletion of 5-HT in the midbrain raphe nuclei following peripheral *p*-chlorophenylalanine administration. Surprisingly, the central application of *p*-chlorophenylalanine in the dorsal raphe nucleus and median raphe nucleus did not alter the 5-HT levels in the midbrain raphe nucleus investigated. In addition, peripheral and central administration of *p*-chlorophenylalanine did not alter the 5-HT levels in the nucleus accumbens. In conclusion, the behavioural results suggest that the potentiation of cocaine-induced behaviour following peripheral *p*-chlorophenylalanine administration may be attributed to the dorsal raphe nucleus but not the median raphe nucleus suggesting that, serotonergic dorsal raphe nucleus neurones may normally mediate a tonic inhibitory effect on cocaine-induced behaviour. Furthermore, the biochemical data may indicate the existence of neurochemical resistance of the midbrain raphe nuclei to the 5-HT depleting effects of *p*-chlorophenylalanine.

Keywords: Cocaine; *p*-Chlorophenylalanine; Behavior; Dorsal raphe nucleus; Median raphe nucleus; Nucleus accumbens

1. Introduction

An increasing number of studies provide evidence for a modulatory influence of 5-hydroxytryptamine (5-HT) on cocaine's stimulant effect on locomotor activity in rodents. In behavioural studies following serotonergic neurotoxins it has been reported that cocaine's stimulant effect on locomotor activity is enhanced, whereas facilitating serotonergic neurotransmission reduced cocaine's stimulant effect. For example, depletion of 5-HT following peripheral administration of the 5-HT biosynthesis inhibitor *p*-chlorophenylalanine (Koe and Weissman, 1966) potentiated cocaine-induced locomotor activity in rats (Scheel-Krüger et al., 1977), whilst increasing the availability of endogenous 5-HT by pretreatment with the 5-HT precursor 5-hydroxytryptophan (5-HTP) reduced the stimulant effect of cocaine on locomotor activity (Pradhan et al., 1978). Hence, one might suggest that 5-HT plays an inhibitory role on locomotor activity elicited by cocaine (for review see Soubrié et al., 1984). However, increasing the activity of the serotonergic neurotransmitter system by administration of the selective serotonin reuptake inhibitor fluoxetine enhanced cocaine-induced locomotor activity and behaviour, suggesting a facilitatory role of 5-HT rather than an inhibitory action (Herges and Taylor, 1998a). Although it is evident from these reports that 5-HT plays a modulatory role on cocaine-induced behaviour, the apparent contradic-

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tory effects of 5-HT require further investigations to possibly identify the sites mediating the modulatory role of 5-HT.

Neuroanatomical data provide evidence for an involvement of serotonergic neurones arising in the midbrain raphe nuclei in the modulatory influence of 5-HT on cocaine-induced locomotor activity. The serotonergic projections from the dorsal raphe nucleus and the median raphe nucleus innervate the dopaminergic mesolimbic (Bobillier et al., 1976; Taber Pierce et al., 1976), which is associated with an induction of locomotor activity produced by psychostimulants (for review see Wise and Bozarth, 1987). Previous behavioural studies investigating the effect of a decreased serotonergic neurotransmission on the locomotor activity induced by the psychostimulant amphetamine have shown that, lesions of the midbrain raphe nuclei resulted in a potentiation of amphetamine-induced locomotor activity in rats (Neill et al., 1972; Costall et al., 1979; Lucki and Harvey, 1979). It has been demonstrated that selective lesions of the median raphe nucleus produced an enhancement of amphetamine-induced locomotor activity, whereas selective lesions of the dorsal raphe nucleus did not alter the behavioural response to amphetamine (Jacobs et al., 1975).

Electrochemical data may provide evidence for a possible involvement of dorsal raphe nucleus neurones in the behavioural effects of cocaine. Cocaine has been reported to suppress the spontaneous firing of dorsal raphe nucleus neurones in vivo (Pitts and Marwah, 1986, 1987; Cunningham and Lakoski, 1988, 1990). Interestingly, this inhibitory effect of cocaine on the spontaneous firing of the dorsal raphe nucleus neurones was diminished following peripheral pretreatment with *p*-chlorophenylalanine indicating that, the action of cocaine on dorsal raphe nucleus neurones may be dependent on the availability of endogenous 5-HT (Cunningham and Lakoski, 1990). Given that, selective lesions of the median raphe nucleus reportedly enhanced amphetamine-induced locomotor activity (Jacobs et al., 1975), an involvement of median raphe nucleus neurones in the behavioural effects of cocaine cannot be excluded.

The present study examined whether the previously reported potentiation of cocaine-induced behaviour (Scheel-Krüger et al., 1977) following peripheral pretreatment with *p*-chlorophenylalanine may be due to depletion of 5-HT in the dorsal raphe nucleus and/or median raphe nucleus. The peripheral and central (dorsal raphe nucleus and median raphe nucleus) *p*-chlorophenylalanine pretreatment was followed by the behavioural testing of cocaine-induced locomotor activity, rears and head bobs in rats. To demonstrate the possible existence of regional selectivity, the extent of 5-HT depletion in the dorsal raphe nucleus and median raphe nucleus in each treatment group was determined. In addition, possible changes of 5-HT levels in the nucleus accumbens following peripheral and central *p*-chlorophenylalanine administration were determined. Preliminary results of the work described were presented at the *Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists* in Canberra/Australia on the 2nd December 1997 (Herges and Taylor, 1997).

2. Materials and methods

2.1. Animals

Female Glaxo Wistar rats weighing 200–250 g were obtained from the animal house of the Victorian College of Pharmacy, Monash University. The animals were kept in a 12 h light/dark cycle in a temperature-regulated (22–23°C) room with free access to food and water. Animals were housed in group cages of eight rats. Animals exposed to the surgery, central cannula implantation, were kept in individual cages following the surgical procedure. The experiments were conducted under the guidelines of the National Health and Medical Research Council of Australia and were approved by the, Victorian College of Pharmacy, Monash University, Animal Ethics Committee.

2.2. Peripheral p-chlorophenylalanine pretreatment

To establish a dose–response curve for cocaine under control conditions, animals were pretreated with *p*-chlorophenylalanine (100 mg/kg, 0.2 ml/100 g i.p.) or saline (0.2 ml/100 g i.p.) for 3 consecutive days followed by behavioural testing 24 h later. On the day of the behavioural testing the animals were injected with cocaine (5–30 mg/kg, 0.1 ml/100 g i.p.). To allow direct comparison with other behavioural studies conducted in our laboratory (Herges and Taylor, 1998a), which minimises total animal usage, rats were routinely injected with saline (0.1 ml/100 g i.p.) 60 and 30 min prior to cocaine or saline administration. Since saline injections prior to cocaine do not alter the rats behaviour, further reference to these injections is omitted for clarity. To enable a potentiation or attenuation of cocaine-induced behaviour to be observed cocaine was administered at the submaximal dose of 15 mg/kg. The control groups' behaviour was determined in animals treated with saline (0.1 ml/100 g i.p.) on the day of the behavioural testing preceded by saline or *p*-chlorophenylalanine pretreatment as described above.

2.3. Surgery

To enable central drug administration some groups of animals were anaesthetised with sodium methohexitone (16.7 mg/kg, 0.1 ml/100 g i.p.)/sodium amylobarbitone (27–33.3 mg/kg, 0.1 ml/100 g i.p.) and placed in a stereotaxic frame. The skull was exposed and the stereotaxic instrument was aligned above the dorsal raphe nu-

cleus or the median raphe nucleus (AP +1.2 mm, L 0 mm from the interaural zero) according to the stereotaxic atlas of Paxinos and Watson (1986). A hole (2 mm i.d.) was drilled, followed by the implantation of a 23 gauge guide cannula (V −4.2 mm) which was held in place using dental acrylic cement and 2 stainless steel screws. The guide cannula was sealed by a stainless steel stylet. The rats were injected with penicillin ticarcillin (40 mg/kg, 0.1 ml/100 g i.p.) for 3 consecutive days to prevent postoperative infections. Following the surgical procedure the animals were kept in individual cages. They were allowed 5 days of recovery prior to the central drug administration.

2.4. Central p-chlorophenylalanine pretreatment

The animals were held gently during the microinfusion. The stainless steel stylets were removed followed by the microinjection of *p*-chlorophenylalanine (0.5 μg/0.5 μl/10 s) or saline (0.5 μl/10 s) in the dorsal raphe nucleus or median raphe nucleus using a CMA/100 microinjection pump (Carnegie Medicin, Stockholm, Sweden) connected by polyethylene tubing to a stainless steel cannula inserted via the guide tube cannula (dorsal raphe nucleus: V −6.2 mm; median raphe nucleus: V −8.5 mm). The inner cannula remained in place for 3 min after the injection. After sealing the guide cannula the animals were returned to their home cage. The behavioural testing was performed 48 h after the *p*-chlorophenylalanine or saline injections. On the day of the behavioural experiment the animals were treated with cocaine or saline as described under Section 2.2.

2.5. Behavioural testing

The procedural details of the behavioural testing are described elsewhere (Herges and Taylor, 1998a). All behavioural experiments were conducted between 0830 h and 1630 h in an isolated room under light conditions. A circular photobeam activity meter (diameter 67 cm, height 79 cm) equipped with 6 horizontal infrared photocell beams located 3 cm above the wire grid floor and spaced approximately 13 cm apart was used to determine the locomotor activity. The photobeam interruptions were recorded for each behavioural session of 5 min duration. To obtain consistent basal activity, all animals were exposed to the apparatus on the days before the behavioural testing and 30 min (data not presented) and 10 min before cocaine injection. The behavioural sessions 10, 30 and 60 min after cocaine administration were videotaped using a Panasonic WV-BL 200 video camera connected to a Panasonic Super-VHS FS 90 video cassette recorder. The video was stored for the later scoring of the behaviours rears (standing up on hind legs) and head bobs (lateral and upward head movements).

2.6. Histology

On completion of the behavioural experiments the animals were anaesthetised and dye (0.5 μl/10 s) was microinjected as described under Section 2.4. The brains were quickly removed and frozen at −15°C. The location of dye within the dorsal raphe nucleus and median raphe nucleus was defined histologically. The data included in this study were obtained from animals with the confirmed correct placement of the cannula in the particular midbrain raphe nucleus investigated. From the 112 animals that underwent the surgery, 24 animals had to be excluded because of incorrect placement of the cannula in the particular midbrain raphe nucleus (dorsal raphe nucleus: 7 rats; median raphe nucleus: 17 rats).

2.7. Biochemical analysis

To determine the extent of 5-HT depletion, one group of animals was peripherally pretreated with *p*-chlorophenylalanine or saline as described under Section 2.2. Another group of animals was exposed to the surgery followed by microinjection of *p*-chlorophenylalanine or saline in the dorsal raphe nucleus or median raphe nucleus as described under Section 2.4. The animals were killed by decapitation with a guillotine 24 and 48 h following the peripheral and central *p*-chlorophenylalanine or saline pretreatment, respectively. Brains were quickly removed and chilled at −15°C for 20 min prior to dissection according to the stereotaxic atlas by Paxinos and Watson (1986). After removing the cerebellum, two sagittal sections lateral to the midline (L 3.2 mm), one vertical section 4.2 mm anterior to the optic chiasma and one horizontal section at the level of the corpus callosum were removed. The brains were then cut in several coronal 400 μm slices anterior to the cerebellum using a McIlwain tissue chopper (The Mickle Laboratory Engineering). The slices containing the midbrain raphe nuclei (AP +0.7 to +1.5 mm) were quickly dissected into dorsal raphe nucleus and median raphe nucleus on an ice cold petri dish. The remaining brain tissue was returned to the freezer prior to sectioning the forebrains into several coronal 400 μm slices. The nucleus accumbens was dissected from the appropriate slices (R +1.8 to +2.6 mm). Following dissection the tissue samples were immediately placed in 200 μl 0.4 M $HClO_4$ containing 0.1 μg/ml *n*-acetylserotonin as internal standard. The tissue samples were disrupted using a Vibracell sonicator (Sonics and Materials, CT, USA) with an ultrasonic power of 40 W delivered to the probe for 10 s followed by centrifugation (Beckman, Microfuge™ 12, USA, 12,000 rpm for 5 min at room temperature). The aliquots were analysed on the same day for 5-HT in the dorsal raphe nucleus, median raphe nucleus and nucleus accumbens by high performance liquid chromatography

(HPLC). The pellets were resuspended by sonicating with an ultrasonic power of 40 W delivered to the probe for 10 s. Fifteen microliters of these samples were collected and 0.1 ml 1 M NaOH was added to dissolve the precipitated protein (Lowry et al., 1951). After 30 min, the protein levels were determined with the folin phenol reagent according to Lowry et al. (1951).

2.8. Chromatography for 5-HT determination

The samples were analysed by reverse-phase ion-pair HPLC consisting of a Waters 510 pump (Waters, Division of Millipore, MA, USA) operating at a pressure of about 2500 PSI, a Reodyne BH 7125 injector, a guard column (Newguard RP-18, Applied Biosystems, CA, USA) and a reverse phase Spherisorb 5 ODS column (250 mm × 4.6 mm i.d. Phase Sep., Deeside, UK), which was encapsulated in a temperature-controlled oven set at 28°C (Waters, Division of Millipore). The HPLC was coupled to a LC-4B dual electrode electrochemical detector equipped with a TL-5a glassy carbon working electrode (Bioanalytical Systems, IND, USA). The analysis of 50 μl samples was performed at a flow rate of 1.3 ml/min and a detector potential of 750 mV (vs. Ag/AgCl/3 M NaCl) with a sensitivity of 20.0 nA FS. The peaks were recorded using a BBC Goerz Metrawatt SE 120 dual pen recorder and the peak height was determined manually.

The mobile phase consisted of 12% v/v methanol in 0.15 M sodium phosphate buffer containing 0.1 mM ethylendiaminetetraacetic acid (EDTA), 0.5 mM sodium octane sulphonic acid. After adjusting the pH of the mobile phase to 3.6 with 10 M NaOH, the mobile phase was filtered through a 0.45 μm Durapore membrane filter and degassed under vacuum (Waters, Division of Millipore).

For every chromatographic run a standard curve of 5-HT (0.25–20 ng) with *n*-acetylserotonin (5 ng) as the internal standard was established. The 5-HT standards were freshly prepared from a 5-HT stock solution prior to the chromatographic analysis. The internal standard *n*-acetylserotonin (0.1 mg/ml) was added to each 5-HT standard solution. The 5-HT content of the samples was calculated from the ratios of the peak heights of 5-HT to the internal standard *n*-acetylserotonin.

2.9. Statistical analysis

Statistical analysis of cumulative scores of cocaine (5–30 mg/kg)-induced behaviour after saline or *p*-chlorophenylalanine pretreatment were analysed by non-parametric Kruskal–Wallis one way analysis of variance (ANOVA on ranks) followed by post-hoc pairwise multiple comparison by using the Student–Newman–Keuls test. The effects of *p*-chlorophenylalanine on baseline behaviour and cocaine-induced behaviour and the biochemical data were

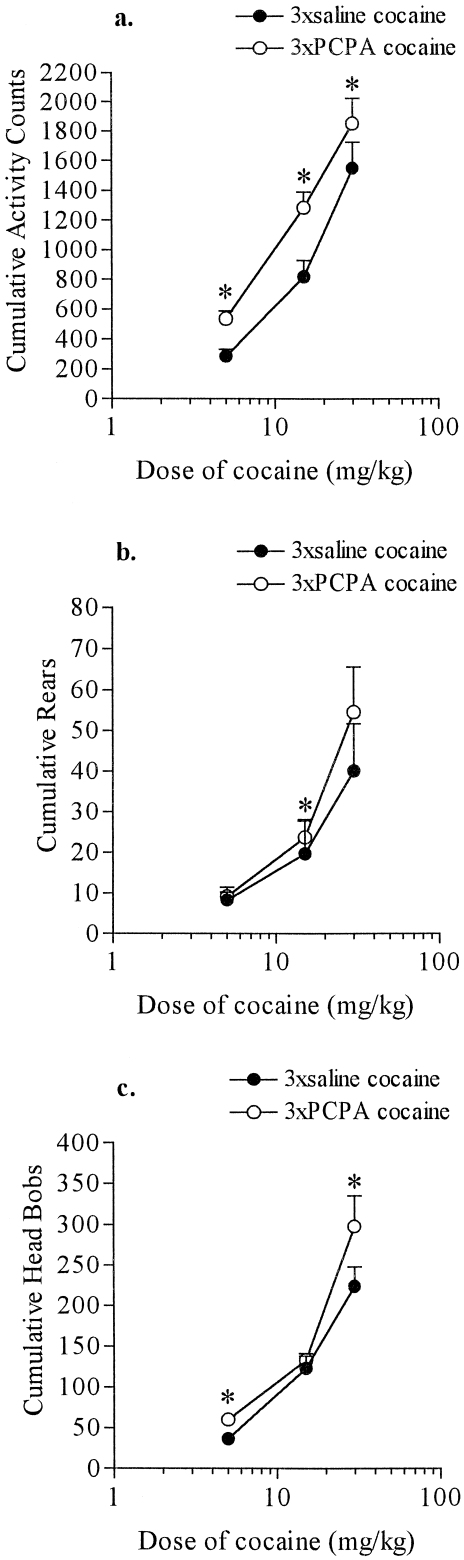


Fig. 1. (a) The locomotor activity, (b) rears and (c) head bobs induced by cocaine at doses of 5, 15 and 30 mg/kg in rats pretreated with 100 mg/kg *p*-chlorophenylalanine (3×PCPA) or saline (3×saline) for 3 consecutive days. The graphs represent the cumulative counts at 10, 30 and 60 min per 5 min after cocaine injections. The values represent mean scores ± S.E.M. of 12 rats. $^{*}P < 0.05$ (analysis of median values by non-parametric Kruskal–Wallis test), 3×PCPA cocaine compared to 3×saline cocaine (dose comparison).

analysed by the parametric *t*-test or the non-parametric Mann–Whitney rank sum test. The probability level of the statistical analyses was 5%. The behavioural counts presented in the graphs represent the mean ± S.E.M.

2.10. Drugs

The following drugs were used: cocaine HCl (Glaxo Wellcome, Australia), DL-*p*-chlorophenylalanine methyl ester (Sigma, St. Louis, MO, USA), methohexitone sodium (Eli Lilly, West Ryde, Australia), amylobarbitone sodium (Eli Lilly) and ticarcillin sodium (SmithKline Beecham, Melbourne, Australia). Cocaine HCl and DL-*p*-chlorophenylalanine methyl ester were dissolved in saline 0.9% W/V. Methohexitone, amylobarbitone and ticarcillin were prepared in water for injection.

3. Results

3.1. Dose-dependent effect of cocaine on locomotor activity, rears and head bobs

Cocaine (5–30 mg/kg i.p.) increased the locomotor activity, the number of rears and the number of head bobs in a dose-dependent manner following *p*-chlorophenylalanine (3 × PCPA cocaine) and saline (3 × saline cocaine) pretreatment, respectively (Fig. 1a,b,c). The *p*-chlorophenylalanine pretreatment produced a shift to the left of the behavioural cocaine dose–response curves [locomotor activity: $H_{(5)} = 51.7$, $P < 0.001$; rears: $H_{(5)} = 21.3$, $P < 0.001$; head bobs: $H_{(5)} = 56.6$, $P < 0.001$] (Fig. 1a,b,c). Following *p*-chlorophenylalanine pretreatment the cumulative counts for cocaine-induced locomotor activity at 5–30 mg/kg were significantly higher compared to the respective saline pretreated cocaine groups (Fig. 1a). A significantly higher number of rears following *p*-chlorophenylalanine pretreatment was observed in the cocaine 15 mg/kg group in comparison to the number of rears induced by cocaine 15 mg/kg in saline pretreated animals (Fig. 1b). Following *p*-chlorophenylalanine pretreatment the number of cumulative counts for cocaine-induced head bobs was significantly greater for cocaine 5 and 30 mg/kg compared to the respective saline pretreated cocaine groups (Fig. 1c). The results of the following experiments were obtained from animals treated with cocaine at the dose of 15 mg/kg, which enables a potentiation or attenuation of cocaine-induced behaviour to be observed.

3.2. Effect of peripheral p-chlorophenylalanine pretreatment on cocaine-induced locomotor activity, rears and head bobs

A statistically significant difference between the behaviour of saline and *p*-chlorophenylalanine pretreated

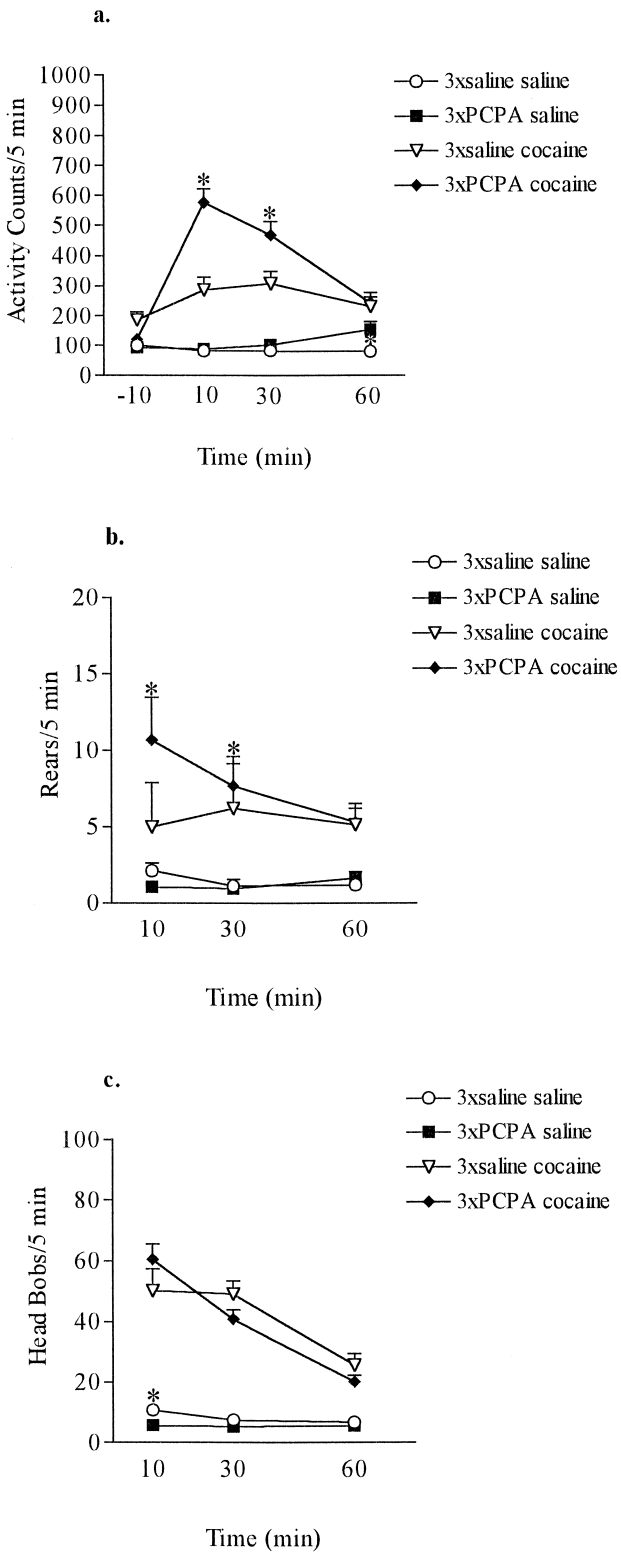


Fig. 2. (a) The locomotor activity, (b) rears and (c) head bobs induced by cocaine (15 mg/kg) or saline in rats pretreated with 100 mg/kg *p*-chlorophenylalanine (3×PCPA) or saline (3×saline) for 3 consecutive days. The values represent mean scores ± S.E.M. of 14–16 rats. $^{*}P < 0.05$ (analysis of mean values by parametric *t*-test or median values by non-parametric Mann–Whitney test), 3×PCPA saline compared to 3× saline saline, 3×PCPA cocaine compared to 3×saline cocaine.

animals was observed for the locomotor activity and head bobs at 60 and 10 min, respectively (Fig. 2a,c). Peripheral *p*-chlorophenylalanine pretreatment did not alter the number of rears of saline-treated animals (Fig. 2b). The stimulant effect of cocaine on locomotor activity and rears was significantly enhanced in the *p*-chlorophenylalanine pretreated group (3 × PCPA cocaine) in comparison to the cocaine control group (3 × saline cocaine) at 10 and 30 min (Fig. 2a,b). The *p*-chlorophenylalanine pretreatment did not significantly increase the cocaine-induced head bobs (Fig. 2c).

3.3. Effect of central p-chlorophenylalanine pretreatment on cocaine-induced behaviours

3.3.1. Effect of p-chlorophenylalanine pretreatment in the dorsal and median raphe nuclei on cocaine-induced locomotor activity

Pretreatment with *p*-chlorophenylalanine in the dorsal raphe nucleus and median raphe nucleus (PCPA saline) did not affect the locomotor activity of saline-treated animals (saline saline) (Fig. 3a,b). The stimulant effect of cocaine was greater in rats microinjected with *p*-chlorophenylalanine in the dorsal raphe nucleus (PCPA cocaine) compared to animals microinjected with saline in the dorsal raphe nucleus (saline cocaine) (Fig. 3a). This potentiation reached statistical significance at 10 and 30 min. Microinjection of *p*-chlorophenylalanine in the median raphe nucleus (PCPA cocaine) did not alter the cocaine-induced locomotor activity of the saline pretreated group (saline cocaine) (Fig. 3b).

3.3.2. Effect of p-chlorophenylalanine pretreatment in the dorsal and median raphe nuclei on cocaine-induced rears

p-Chlorophenylalanine pretreatment in the dorsal raphe nucleus and median raphe nucleus (PCPA saline) had no effect on the number of rears of saline-treated animals (saline saline) (Fig. 3c,d). The number of rears in the *p*-chlorophenylalanine cocaine group (PCPA cocaine) tended to be greater compared to saline cocaine treated animals, although this difference did not reach statistical significance (Fig. 3c). *p*-Chlorophenylalanine pretreatment in the median raphe nucleus (PCPA cocaine) did not significantly alter the number of rears induced by cocaine in animals microinjected with saline in the median raphe nucleus (saline cocaine) (Fig. 3d).

3.3.3. Effect of p-chlorophenylalanine pretreatment in the dorsal and median raphe nuclei on cocaine-induced head bobs

Pretreatment with *p*-chlorophenylalanine in the dorsal raphe nucleus and median raphe nucleus (PCPA saline) had no effect on the number of head bobs of saline-treated animals (saline saline) (Fig. 3e,f). A potentiation of cocaine-induced head bobs (saline cocaine) was observed following microinjection of *p*-chlorophenylalanine in the dorsal raphe nucleus (PCPA cocaine) (Fig. 3e). The enhancing effect of *p*-chlorophenylalanine pretreatment in the dorsal raphe nucleus reached statistical significance 10 and 30 min after the cocaine injection. The stimulant effect of cocaine on the number of head bobs (saline cocaine) was not altered by microinjection of *p*-chlorophenylalanine in the median raphe nucleus (PCPA cocaine) (Fig. 3f).

3.4. 5-HT levels

3.4.1. Effect of peripheral p-chlorophenylalanine pretreatment on 5-HT levels in the dorsal and median raphe nuclei and the nucleus accumbens

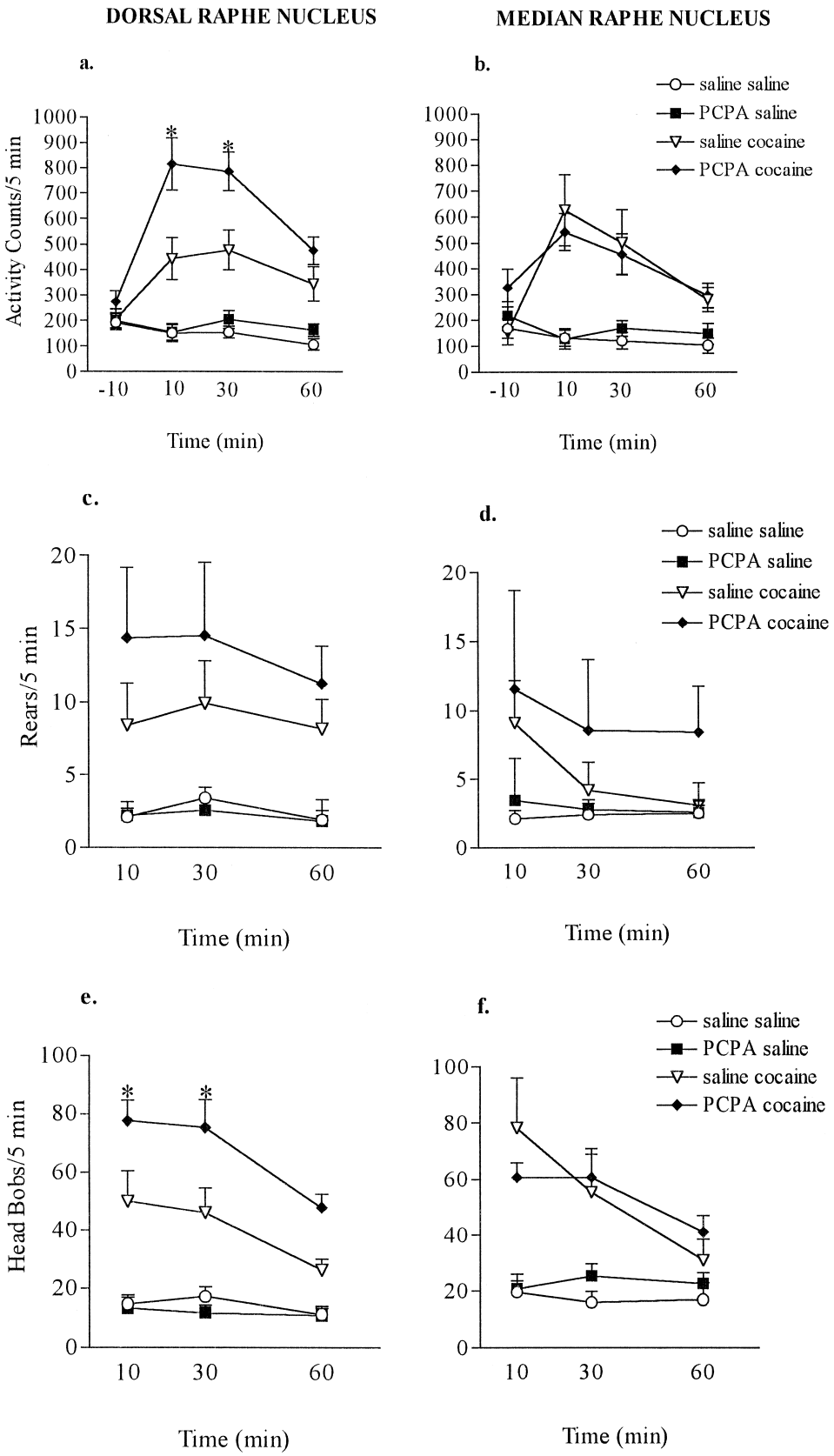
Peripheral pretreatment with *p*-chlorophenylalanine (100 mg/kg/day for 3 consecutive days) produced a reduction of 5-HT in the dorsal raphe nucleus to 74.9% of control (33.5 ± 5.35 vs. 44.7 ± 7.93 ng/mg), although this did not reach statistical significance (Fig. 4a). The 5-HT content of the median raphe nucleus was significantly reduced by *p*-chlorophenylalanine pretreatment to 55.9% of control (18.4 ± 3.05 vs. 32.9 ± 4.15 ng/mg) (Fig. 4a). *p*-Chlorophenylalanine pretreatment had no depleting effect on the 5-HT content of the nucleus accumbens (20.5 ± 7.74 vs. 16.6 ± 1.60 ng/mg) (Fig. 4a).

3.4.2. Effect of central (dorsal raphe nucleus or median raphe nucleus) p-chlorophenylalanine pretreatment on 5-HT levels in the dorsal and median raphe nuclei and the nucleus accumbens

The 5-HT content of the dorsal raphe nucleus was not altered 48 h after the microinjection of *p*-chlorophenylalanine in the dorsal raphe nucleus compared to saline administration in the dorsal raphe nucleus (65.4 ± 10.7 vs. 71.3 ± 15.6 ng/mg) (Fig. 4b). Microinjection of *p*-chlorophenylalanine in the dorsal raphe nucleus significantly

Fig. 3. (a–b) Locomotor activity, (c–d) rears and (e–f) head bobs induced by cocaine (15 mg/kg) or saline in rats. (a–b) Effect of *p*-chlorophenylalanine (PCPA) microinjected in the dorsal raphe nucleus (a) and median raphe nucleus (b) on cocaine-induced locomotor activity. (c–d) Effect of *p*-chlorophenylalanine (PCPA) microinjected in the dorsal raphe nucleus (c) and median raphe nucleus (d) on cocaine-induced rears. (e–f) Effect of *p*-chlorophenylalanine (PCPA) microinjected in the dorsal raphe nucleus (e) and median raphe nucleus (f) on cocaine-induced head bobs. The values represent mean scores ± S.E.M. of 9–13 rats. $^{*}P < 0.05$ (analysis of mean values by parametric *t*-test or median values by non-parametric Mann–Whitney test), PCPA cocaine compared to saline cocaine.

reduced the 5-HT level in the median raphe nucleus to 53.6% of control (39.2 ± 5.43 vs. 73.2 ± 9.37 ng/mg) (Fig. 4b). The 5-HT level of the nucleus accumbens was not affected by microinjection of *p*-chlorophenylalanine in the dorsal raphe nucleus (28.1 ± 4.30 vs. 33.0 ± 14.1 ng/mg) (Fig. 4b).



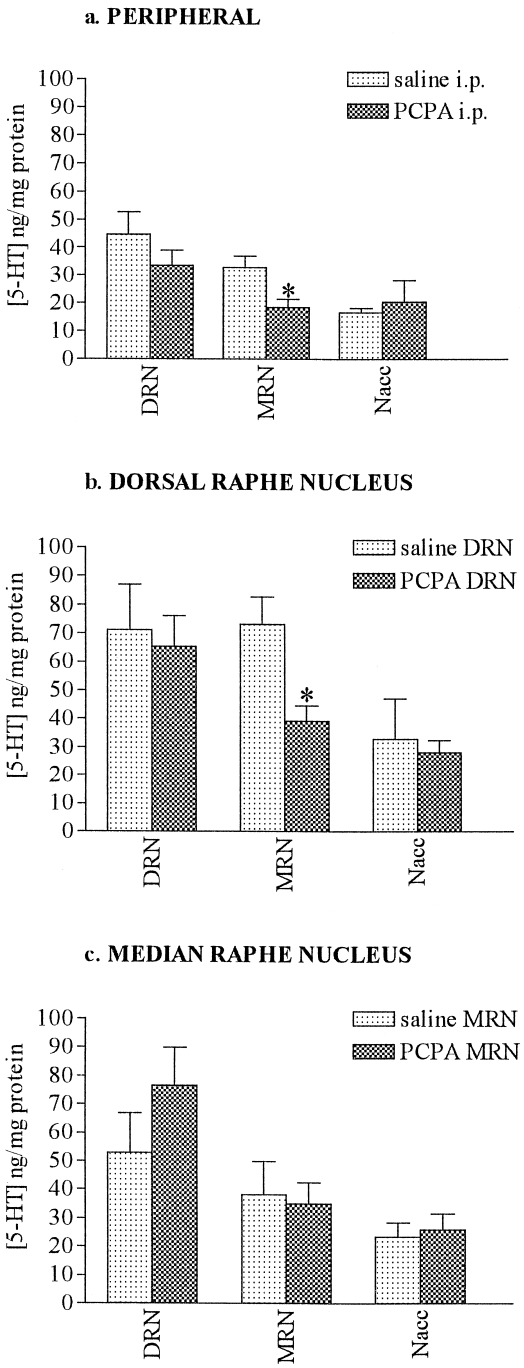


Fig. 4. (a) Effect of *p*-chlorophenylalanine and saline administered i.p. (PCPA i.p., saline i.p.), (b) microinjected in the dorsal raphe nucleus (PCPA DRN, saline DRN) and (c) microinjected in the median raphe nucleus (PCPA MRN, saline MRN) on 5-HT levels (ng/mg protein) in the dorsal raphe nucleus (DRN), median raphe nucleus (MRN) and nucleus accumbens (Nacc). The values represent mean levels ± S.E.M. of 6–12 rats. $^{*}P < 0.05$ (analysis of mean values by parametric *t*-test or median values by non-parametric Mann–Whitney test), PCPA i.p. compared to saline i.p. and PCPA DRN compared to saline DRN.

The 5-HT levels in the dorsal raphe nucleus and median raphe nucleus were not altered 48 h after microinjection of *p*-chlorophenylalanine in the median raphe nucleus in comparison to saline administration in the median raphe nucleus (dorsal raphe nucleus: 76.7 ± 13.1 vs. 53.0 ± 13.9 ng/mg; median raphe nucleus: 35.1 ± 7.28 vs. 38.1 ± 11.8 ng/mg) (Fig. 4c). Microinjection of *p*-chlorophenylalanine in the median raphe nucleus did not alter the 5-HT content of the nucleus accumbens compared to control (25.9 ± 5.71 vs. 23.6 ± 4.85 ng/mg) (Fig. 4c).

4. Discussion

The results of the present study provide further evidence for a potentiation of cocaine-induced behaviours in rats following 5-HT depletion. In agreement with published studies, a potentiation of cocaine-induced locomotor activity in rats was observed following peripheral pretreatment with the 5-HT biosynthesis inhibitor *p*-chlorophenylalanine (Scheel-Krüger et al., 1977). The reported potentiation, apparently following whole brain 5-HT depletion, has been interpreted as indicative of the serotonergic neurotransmitter system having an inhibitory role on cocaine's stimulant effect on behaviour (for review see Soubrié et al., 1984). In support of an inhibitory role is the observation that, the 5-HT precursor 5-HTP attenuated locomotor activity induced by cocaine in rats (Pradhan et al., 1978). Similar results have been reported for the other psychostimulant amphetamine. Following *p*-chlorophenylalanine pretreatment, the amphetamine-induced locomotor activity was potentiated (Mabry and Campbell, 1973; Breese et al., 1974; Segal, 1976), which was prevented by administration of 5-HTP (Mabry and Campbell, 1973). In contrast to an inhibitory role of 5-HT on cocaine-induced behaviour is our previous observation that, the selective serotonin reuptake inhibitor fluoxetine potentiated the cocaine-induced behaviour in rats suggesting a facilitatory role of the serotonergic neurotransmitter system (Herges and Taylor, 1998a). These apparent contradictory behavioural data may imply that the serotonergic neurotransmitter system exerts both an excitatory and inhibitory effect on cocaine-induced behaviour.

It could be suggested that different serotonergic neurones are involved in these contradictory actions of 5-HT. In view of the existence of serotonergic projections from the midbrain raphe nuclei to the dopaminergic mesolimbic system (Bobillier et al., 1976; Taber Pierce et al., 1976), the proposed site of action for cocaine to induce locomotor activity (Wise and Bozarth, 1987), it could be suggested that these serotonergic neurones are involved in modulating cocaine-induced behaviour. Furthermore, electrophysiological studies have shown that the spontaneous firing of dorsal raphe nucleus neurones in rats is inhibited by cocaine (Pitts and Marwah, 1986, 1987; Cunningham and Lakoski, 1988, 1990), which may contribute to its behavioural effects. The inhibitory action of cocaine on the firing of dorsal raphe nucleus neurones was reported to be

abolished by peripheral pretreatment with *p*-chlorophenylalanine suggesting that endogenous 5-HT mediates cocaine's inhibitory action (Cunningham and Lakoski, 1990). Since 5-HT depletion diminished cocaine's inhibitory action on dorsal raphe nucleus neurones, whilst cocaine-induced behaviour was potentiated by 5-HT depletion, it may be suggested that the dorsal raphe nucleus neurones may normally inhibit the stimulant effect of cocaine on behaviour. However, an involvement of median raphe nucleus neurones in the present study cannot be excluded given that, the reduction of the 5-HT level in the median raphe nucleus was greater than in the dorsal raphe nucleus following peripheral and local (dorsal raphe nucleus) administration of *p*-chlorophenylalanine. It is of interest to note that although peripheral administration of *p*-chlorophenylalanine at a dose of 100 mg/kg for three consecutive days reportedly reduced the whole brain 5-HT level by 89% (Koe and Weissman, 1966), the same dosing regimen of *p*-chlorophenylalanine in the present study only resulted in a 5-HT depletion of 25% and 44% in the dorsal raphe nucleus and median raphe nucleus, respectively. It appears that the midbrain raphe nuclei, especially the dorsal raphe nucleus, are more resistant to 5-HT depletion following *p*-chlorophenylalanine. This apparent resistance of the midbrain raphe nuclei to the depletion of 5-HT by *p*-chlorophenylalanine has also been observed in reserpinized rats (Kuhn et al., 1985). However, despite the only moderate reduction of 5-HT in the midbrain raphe nuclei the stimulant effect of cocaine on behaviour was potentiated by peripheral pretreatment with *p*-chlorophenylalanine.

Although the behavioural evidence suggests an inhibitory role of serotonergic dorsal raphe nucleus neurones on cocaine-induced behaviour, this is not accompanied by a significant alteration of the 5-HT levels in the dorsal raphe nucleus 48 h after the local administration of *p*-chlorophenylalanine in the dorsal raphe nucleus. Following a single dose of peripheral *p*-chlorophenylalanine the activity of the rate-limiting enzyme tryptophan hydroxylase appears to be lowest after 2 days (Meek and Neff, 1972), which was the same time allowed after central *p*-chlorophenylalanine administration before the behavioural testing in the present study. Due to the close proximity of the midbrain raphe nuclei it may be argued that *p*-chlorophenylalanine injected into the dorsal raphe nucleus diffused to the median raphe nucleus, suggesting that the observed potentiation of cocaine-induced behaviour may be attributed to inhibition of the firing of serotonergic median raphe nucleus neurones. Also, since the experimental procedure of the biochemical assay did not allow verification of the placement of the microinjection needle, the possibility that it was located in the median raphe nucleus instead of the dorsal raphe nucleus cannot be excluded. However, it is unlikely that incorrect positioning of the cannula can explain these results, since in the behavioural experiments incorrect placement of the cannula in the dorsal raphe nucleus was determined to only occur in 14% of the rats. In addition, the involvement of the median raphe nucleus in modulating behaviour cannot be ruled out, since following selective lesions of the median raphe nucleus a potentiation of amphetamine-induced locomotor activity has been reported (Jacobs et al., 1975). In contrast, selective lesions of the dorsal raphe nucleus did not alter the behavioural response to amphetamine (Jacobs et al., 1975). Although these reports may suggest a modulatory role of median raphe nucleus serotonergic neurones on amphetamine-induced locomotor activity, it has been argued that in view of an increased baseline behavioural activity following median raphe nucleus lesions (Jacobs et al., 1974; Geyer et al., 1976), this may have an additive effect with the amphetamine-induced hyperactivity (Jacobs et al., 1975). A similar additive effect can be excluded in the present study, since peripheral and central administration of *p*-chlorophenylalanine in the midbrain raphe nuclei did not produce a consistent increase in baseline activity of saline-treated animals.

Another possible explanation for the discrepancy between the behavioural and biochemical results is that the 5-HT level in the dorsal raphe nucleus had returned to near normal 48 h after the microinjection of *p*-chlorophenylalanine. It has previously been suggested that 80–90% of brain 5-HT is presynaptically stored in a reserve pool and the remaining 10–20% of 5-HT is stored in a cytoplasmic functional pool containing the newly synthesised 5-HT (see Kuhn et al., 1985), which would be expected to be depleted by *p*-chlorophenylalanine. It has been postulated that the level of 5-HT in the small functional pool regulates the 5-HT biosynthesis (Shields and Eccleston, 1972). Following pretreatment with *p*-chlorophenylalanine, a reduction in the level of 5-HT in the functional pool may result in stimulation of a feedback mechanism activating the still functional tryptophan hydroxylase (Shields and Eccleston, 1972). An increased activity of tryptophan hydroxylase may account for the assumed restored 5-HT levels in the dorsal raphe nucleus following *p*-chlorophenylalanine pretreatment proposed in the present study. Alternatively, the apparent resistance of the midbrain raphe nuclei to the 5-HT depleting effect of *p*-chlorophenylalanine may be due to the synthesis of new tryptophan hydroxylase in the neuronal cell bodies. This has been previously suggested to explain the observation that 5-HT levels in the midbrain raphe nuclei were increased after tryptophan and pargyline administration despite *p*-chlorophenylalanine pretreatment (Aghajanian et al., 1973). Although the midbrain raphe nuclei appear to be resistant to 5-HT depletion following *p*-chlorophenylalanine, the inhibitory responses of serotonergic dorsal raphe nucleus and median raphe nucleus neurones have been reduced or completely prevented 24–72 h after peripheral *p*-chlorophenylalanine pretreatment (Segal, 1975; Wang and Aghajanian, 1978).

The lack of correlation between the 5-HT levels in the midbrain raphe nuclei and the observed potentiation of

cocaine-induced behaviour following *p*-chlorophenylalanine pretreatment may also indicate possible changes of postsynaptic 5-HT receptor sensitivity which may be responsible for the increased behavioural response. Results from binding studies have shown an increase of the apparent affinity of [^{3}H]5-HT binding to rat forebrain membrane preparations following short-term *p*-chlorophenylalanine administration (Bennett and Snyder, 1976; Steigrad et al., 1978; Fleisher et al., 1979) and lesions of the midbrain raphe nuclei (Bennett and Snyder, 1976). The number of 5-HT binding sites were not significantly altered (Steigrad et al., 1978). Complicating interpretation of the apparent increased affinity is the report that *p*-chlorophenylalanine and its metabolite *p*-chlorophenylacetic acid increased the [^{3}H]5-HT binding in vitro (Fleisher et al., 1979). Although the enhanced activity response to cocaine in the present study may result from development of postsynaptic supersensitivity, a number of behavioural studies do not report development of supersensitivity of postsynaptic 5-HT receptors (Trulson et al., 1976; Dourish et al., 1986). For example, short- and long-term administration of *p*-chlorophenylalanine did not affect the 5-HT behavioural syndrome elicited by the 5-HT$_{1A}$ receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) or the 5-HT precursor 5-HTP (Trulson et al., 1976; Dourish et al., 1986).

It is interesting to note that the 5-HT level in the nucleus accumbens was not altered after peripheral *p*-chlorophenylalanine pretreatment. The synthesis of 5-HT in terminal projections of the midbrain raphe nuclei has been reported to be enhanced after electrical stimulation of the serotonergic neurones arising in the midbrain raphe nuclei (Shields and Eccleston, 1972), which may be attributed to an increased activity of tryptophan hydroxylase (Boadle-Biber et al., 1983). Interestingly, after peripheral pretreatment with *p*-chlorophenylalanine the synthesis of 5-HT following electrical stimulation of the midbrain raphe nuclei was increased in the same manner as in control animals (Shields and Eccleston, 1972). This finding was suggested to be due to an increased activity of tryptophan hydroxylase (Shields and Eccleston, 1972). The reported increased enzyme activity of tryptophan hydroxylase in forebrain regions (Boadle-Biber et al., 1983) following *p*-chlorophenylalanine would be expected to result in an enhanced 5-HT biosynthesis and consequently reduced 5-HT depletion, compensating for the depleting effect of *p*-chlorophenylalanine in the terminal regions of serotonergic neurones.

The observation that local administration of *p*-chlorophenylalanine in the dorsal raphe nucleus enhanced cocaine-induced locomotor activity and head bobs, whereas microinjection of *p*-chlorophenylalanine in the median raphe nucleus did not, is in keeping with the proposed hypothesis that serotonergic dorsal raphe nucleus neurones may normally mediate a tonic inhibitory effect on cocaine's stimulant action on behaviour. Consistent with the suggested involvement, in the action of the dorsal raphe nucleus is our previous observation that blockade of somatodendritic 5-HT$_{1A}$ autoreceptors by WAY1000635 in the dorsal raphe nucleus potentiated the stimulant effect of cocaine on behaviour (Herges and Taylor, 1998b). It would be expected that a decreased activity of dorsal raphe nucleus neurones leads to a decreased release of 5-HT. Recently, it has been reported using in vivo microdialysis studies that, selective serotonin reuptake inhibitors produce a decrease of 5-HT release in forebrain regions. For example, although fluoxetine increased extracellular 5-HT levels in forebrain regions, it produced a measurable decrease in the release of 5-HT (Rutter and Auerbach, 1993). Fluoxetine has also been reported to inhibit the spontaneous firing of dorsal raphe nucleus neurones (Clemens et al., 1977; Cunningham and Lakoski, 1990; Smith and Lakoski, 1997), suggesting that the decreased release of 5-HT in forebrain regions may be associated with an activation of the serotonergic feedback mechanism (Rutter and Auerbach, 1993). Like fluoxetine cocaine has been reported to inhibit the 5-HT reuptake (Ross and Renyi, 1969; Koe, 1976) and suppress the firing of dorsal raphe nucleus neurones (Pitts and Marwah, 1986, 1987; Cunningham and Lakoski, 1988, 1990). Hence cocaine may have similar effects to those of fluoxetine on the extracellular 5-HT levels in forebrain regions. The potentiation of cocaine-induced locomotor activity following 5-HT depletion in the dorsal raphe nucleus may be attributed to an attenuation of the serotonergic feedback mechanism resulting in higher extracellular 5-HT levels in forebrain regions. Since infusion of 5-HT in the ventral tegmental area and nucleus accumbens increased extracellular dopamine levels in the nucleus accumbens (Guan and McBride, 1989; Parsons and Justice, 1993) and electrolytic lesions of the dorsal raphe nucleus neurones increased the dopamine turnover in the nucleus accumbens (Hervé et al., 1979), one might suggest that the observed potentiation of locomotor activity may be attributed to an enhanced dopaminergic neurotransmission in the mesolimbic system.

The lack of effect of *p*-chlorophenylalanine microinjected in the median raphe nucleus on cocaine-induced behaviour might be attributed to differential regional sensitivities of the midbrain raphe nuclei. Electrophysiological and neurochemical data suggest a greater sensitivity of the dorsal raphe nucleus, compared to the median raphe nucleus, to the selective 5-HT$_{1A}$ receptor agonist 8-OH-DPAT (Sinton and Fallon, 1988; Blier et al., 1990; Invernizzi et al., 1991). The greater inhibition of serotonergic dorsal raphe nucleus firing, resulting in 5-HT release in the nucleus accumbens following administration of 8-OH-DPAT may be due to increased 5-HT$_{1A}$ receptor sensitivity (Invernizzi et al., 1991). This greater sensitivity of the dorsal raphe nucleus may result from the higher density of 5-HT$_{1A}$ receptors in the dorsal raphe nucleus compared to those in the median raphe nucleus (Weissmann-Nanopoulos et al., 1985) and the greater number of dorsal raphe

nucleus neurones projecting to the various brain regions (Invernizzi et al., 1991).

In conclusion, the results of our study indicate an inhibitory role of 5-HT on the stimulant effect of cocaine on behaviour, which appears to originate from the serotonergic dorsal raphe nucleus. Whilst both peripheral and central (dorsal raphe nucleus) *p*-chlorophenylalanine administration potentiated cocaine's stimulant effect, this was not accompanied by a change in the level of 5-HT in the dorsal raphe nucleus or the nucleus accumbens. The lack of correlation between the 5-HT levels and the pharmacological response to *p*-chlorophenylalanine pretreatment may implicate that only partial inhibition of 5-HT biosynthesis is sufficient to produce significant behavioural changes. In addition, it may also indicate the existence of serotonergic neurones resistant to *p*-chlorophenylalanine induced depletion of 5-HT. On the other hand, a complex compensatory mechanism of the serotonergic neurotransmitter system may play a role in the unchanged 5-HT level in the nucleus accumbens after *p*-chlorophenylalanine pretreatment.

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