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SOME PHARMACOLOGICAL EFFECTS OF *p*-CHLOROPHENYLALANINE UNRELATED TO TRYPTOPHAN HYDROXYLASE INHIBITION

E. MARLEY & JENNIFER E. WHELAN[1]
Department of Pharmacology, Institute of Psychiatry, De Crespigny Park, London SE5 8AF

1 Experiments were performed on a variety of tissues from different species to establish whether or not the properties of *p*-chlorophenylalanine methyl ester (PCPA) included a 5-hydroxytryptamine (5-HT)-like action which might explain the soporific action of PCPA in chicks.

2 PCPA, like 5-HT, contracted the rat fundal preparation (as did PCPA base), and in cats enhanced twitch tension of a lower limb flexor reflex, evoked adrenal medullary secretion and attenuated histamine-induced gastric secretion; the effects on the rat fundal strip and the adrenal medulla were prevented by methysergide.

3 Like 5-HT, PCPA elicited bronchoconstriction of guinea-pig lungs, isolated or *in vivo*; this was not prevented by methysergide but reduced by polyphloretin and by indomethacin. Perfusate collected from the lungs during PCPA-induced bronchoconstriction and applied to superfused isolated tissues contained a substance with prostaglandin-like activity.

4 In contrast, the effect of PCPA on the guinea-pig isolated ileum differed from that of 5-HT, since it relaxed the ileum when contracted by transmural excitation, by acetylcholine, histamine or 5-HT and contracted the ileum on wash-out.

Introduction

Most studies of *p*-chlorophenylalanine (PCPA) are concerned with its effects on the sleep-waking cycle, effects attributed to depletion of brain 5-hydroxytryptamine (5-HT) due to tryptophan hydroxylase inhibition (Koe & Weissman, 1966). For example, in various species acute administration of PCPA produced marked insomnia as construed primarily from electrophysiological data (rats—Mouret, Bobillier & Jouvet, 1967; Torda, 1967; cats— Delorme, Froment & Jouvet, 1966; Koella, Feldstein & Czicman, 1968; Johnson, Funderburk, Ruckart & Ward, 1972, and monkeys—Weitzman, Rapport, McGregor & Jacoby, 1968). Nevertheless, Torda (1967) noted that rats were less behaviourally alert after PCPA and Mouret *et al.* (1967) alluded to a dissociation between behavioural and electrocortical changes with PCPA. The rats often appeared to be behaviourally asleep despite alert electrocortical activity. However, Rechtschaffen, Lovell, Freedman, Whitehead & Aldrich (1973) were unable to induce substantial sleep decrements in rats with PCPA, while in cats (Dement, Henriksen & Ferguson, 1973) chronically treated with PCPA (6 to 37 days) wakefulness was initially increased, but substantial amounts of sleep ultimately returned. Depletion of brain 5-HT was verified in both groups.

In contrast, the immediate effects of PCPA in chicks were induction of sleep lasting up to 2 h and a lowering of body temperature (Marley & Whelan, 1974). Both these effects resembled those of 5-HT infused into the chick hypothalamus (Marley & Whelan, 1975). The intention of the present experiments was to compare the properties of PCPA with those of 5-HT on a variety of different species such as the rat (fundal strip, uterus), guinea-pig (lungs and ileum) and cat (gastric secretion, spinal cord reflex, adrenal medullae). A brief account of this work has been presented to the British Pharmacological Society (Marley & Whelan, 1974).

Methods

In vitro *experiments*

Rat isolated fundal strip In 53 experiments the fundal strip was mounted in an organ bath and in 2, superfused, the bathing fluid being Krebs solution at 37°C gassed with 5% CO_2 in O_2 (Vane, 1957, 1964); drug contact was for 90 s on a 5 min cycle. Responses

[1] Present address: Department of Pharmacology, South Parks Road, Oxford.

of this tissue, the rat uterus and colon, and the guinea-pig ileum were registered by an isotonic transducer and a pen-recorder.

Guinea-pig isolated ileum The ileum (9 animals) was suspended in Krebs solution at 37°C gassed with 5% CO_2 in O_2. For transmural stimulation (Paton, 1955), supramaximal (approx 30 V) rectangular pulses were used, of 1 ms duration at 0.1 Hz; for tetanization, excitation was for 5 to 15 s at 5 hertz. With a drug cycle of 4 min, contact and wash-out were each for 2 min; when PCPA was added to an ileum already contracted for 2 min, this too remained in contact for 2 min (i.e. a 6 min cycle).

Rat isolated uterus A uterine horn (20 rats), from a rat given stilboestrol (100 μg. i.m.) 24 h earlier, was mounted in an organ bath containing rat uterus Ringer solution at 35°C bubbled with O_2 (Ersparmer, 1954; Harvey & Pennefather, 1962). Drug exposure was for 30 s on a 2 or 3 min cycle.

Guinea-pig isolated lungs and superfused assay tissues The lungs (17 guinea-pigs) were removed, suspended in a chamber and perfused at 2.5 ml/min via the pulmonary artery with Krebs solution at 37°C, gassed with 5% CO_2 in O_2. The cannulated trachea was connected to a modified Konzett & Rossler (1940) apparatus and ventilated with oxygen by a pump (60/min, stroke volume 6–10 ml). In some experiments, a rat fundal strip and colon were mounted in series and superfused at 5 ml/min with Krebs solution containing antagonists (μmol/1: methysergide 0.05; propranolol 0.82; hyoscine 0.34; phenoxybenzamine 0.044; mepyramine 0.035) in concentrations found (Piper & Vane, 1969; 1971) to prevent response to 5-HT, catecholamines, acetylcholine and histamine. When the solution containing antagonists had superfused the tissues for at least 60 min, the lung perfusate was passed over the tissues together with the superfusing fluid (Piper & Vane, 1969). Drugs were injected into the perfusing fluid as it entered the pulmonary artery.

In vivo *experiments*

Guinea-pigs Ten guinea-pigs (0.6–0.8 kg) were anaesthetized with urethane (10 ml/kg i.p. of a 12.5% w/v solution). The trachea was cannulated and the animals artificially ventilated, spontaneous respiration being suppressed by injection of urethane (3% w/v) intravenously. Resistance of the lungs to inflation was recorded by a modification of the Konzett & Rossler (1940) method, in which the movements of the piston-recorder were relayed to an isotonic transducer. Injections were given via a jugular cannula.

Chickens A jugular cannula, thermistor, electrocortical and electromyographic electrodes, were implanted in chicks under halothane anaesthesia (Allen, Garg & Marley, 1970). At least 24 h after recovery, behaviour, electrocortical and electromyographic activities were recorded in a temperature-controlled, soundproof box (Marley & Stephenson, 1970). Chicks tested with *p*-chlorophenethylamine were implanted with a jugular cannula only.

Cats Anaesthesia was induced with ethyl chloride and ether, and the trachea cannulated. Except for cats in which the lower limb flexor reflex was being recorded, when anaesthesia was continued with halothane (see below), anaesthesia was maintained by chloralose (80 mg/kg i.v.). Blood pressure was recorded from a carotid or a femoral artery by a blood pressure transducer and pen recorder.

Lower limb flexor reflex Cats were made spinal, by destroying the brain through the approach for the encéphale isolé described by Bradley & Key (1958); the spinal cord was then transected at the junction of the thoracic and lumbar regions and halothane anaesthesia terminated. After fixing the leg by a drill through the lower end of the femur, the reflex was elicited by stimulating the central end of the divided posterior tibial nerve with supramaximal rectangular pulses of 0.5 ms duration at 0.08–0.12 hertz. Responses of the tibialis anterior were transmitted to a Brown-Schuster myograph plate linked to an isotonic transducer and a pen-recorder.

Assay for suprarenal catecholamine secretion Cats (2.5–4.0 kg) which had undergone bilateral lumbar sympathectomy 5–14 days previously, were anaesthetized as above and artificially respired. After abdominal evisceration, the renal vessels were ligated as was the aorta immediately posterior to them. A Gordh needle was tied into the superior mesenteric artery, after retrograde insertion, and heparin (10 mg/kg) injected intra-arterially.

Blood was withdrawn from a carotid artery at 10–15 ml/min through silicone rubber-tubing by a roller-pump (Saxby, Siddiqi & Walker, 1960) driven by a Servomex motor-controller. The blood, warmed in a water-jacket at 40°C, then superfused a rat fundal strip and a chick rectum in series (Vane, 1964); these tissues had been superfused with Krebs solution containing methysergide, 0.1 μM. The blood was then collected in a reservoir and returned to the cat, part by gravity feed to one jugular vein, and part via the roller-pump to the other. The blood volume in the reservoir was kept constant by a photo-sensitive device controlling blood return via the roller-pump (Allen, 1974). 5-HT, PCPA and methysergide were injected directly to the suprarenal glands via the Gordh needle. Control responses of the tissues were obtained by injection of adrenaline via a cannula in the inferior vena cava with its tip opposite the suprarenal glands.

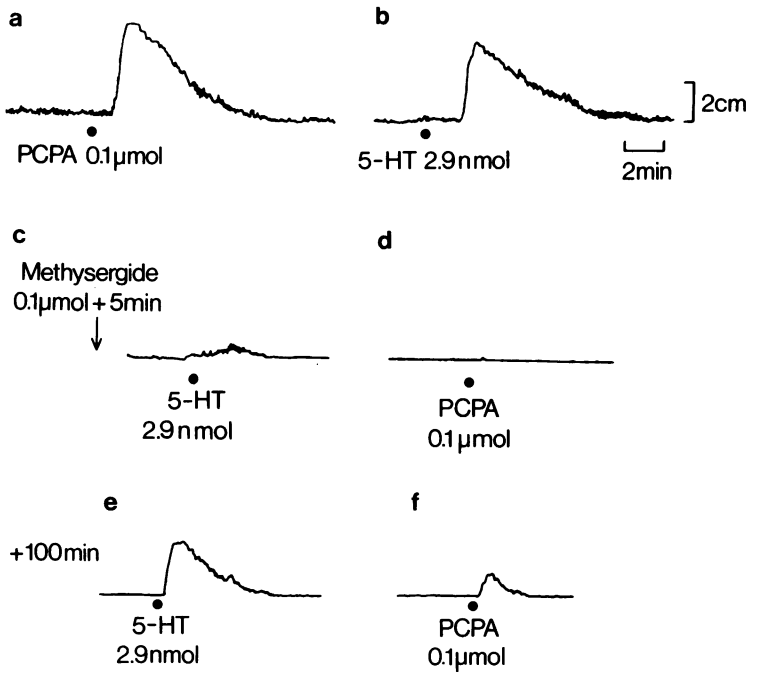

Figure 1 Contractions of the rat fundal strip, superfused with Krebs solution, by *p*-chlorophenylalanine (PCPA) and 5-hydroxytryptamine (5-HT) and their antagonism by methysergide. (a) and (b) Contractions of the rat fundal strip by PCPA, 1 μmol, and to 5-HT, 2.9 nmol. (c) and (d) Effect of PCPA was abolished, and that of 5-HT substantially reduced, by methysergide, 0.1 μmol. (e) and (f) Partial recovery of responses to 5-HT and PCPA, 100 min after methysergide.

Gastric secretion Three cats (2.5–4.0 kg) were allowed water but deprived of food overnight. The cats were then anaesthetized, the abdomen opened, the pyloroduodenal junction ligated and a cannula (2.5 cm o.d.) tied into the gastric greater curvature. An arterial cannula was passed in the aorta, so that its tip lay immediately anterior to the origin of the coeliac artery. The distal end of the oesophagus was then ligated and the vagi divided in the neck. After washing out the stomach with warm saline, the abdomen was closed round the cannula and the animal laid on its side. Histamine (3.0 nmol $kg^{-1}min^{-1}$) was then continuously infused via a femoral vein and gastric juice collected in graduated tubes. When the volume of histamine-induced gastric secretion was reasonably constant (4–6 ml/15 min), 5-HT or PCPA was infused via the aortic cannula for 30 minutes. For estimation of free and total HCl, the specimens were titrated with 0.1N NaOH after the addition of Topfers reagent (Bayliss, 1954).

Drugs

The drugs used included the hydrochlorides of β-phenethylamine, phenoxybenzamine, (±)-phenylisopropylhydrazine, (±)-propranolol, Ro4-4602 (N^1-(DL-seryl)-N^2(2,3,4 trihydroxybenzylhydrazine)), and tryptamine. Also used were acetylcholine perchlorate, (−)-hyoscine hydrobromide, 5-hydroxytryptamine creatinine sulphate, indomethacin, (±)-mebanazine oxalate, mepyramine maleate, methysergide bimaleate, *p*-chlorophenethylamine, *p*-chloro-(±)DL-phenylalanine and its methyl ester, L-phenylalanine and its methyl ester, phenelzine hydrogen sulphate and tetrodotoxin. Prostaglandin E_1 and prostaglandin $F_{2\alpha}$ tromethamine salt (0.2 mg/ml) were dissolved in ethanol and 0.9% saline containing sodium carbonate, (Bennett & Posner, 1971). Polyphloretin phosphate (100 mg/ml) was dissolved in distilled water and the pH adjusted to 7.0 with sodium carbonate.

Results

Section A. 5-hydroxytryptamine-like effects of p-*chlorophenylalanine*

Rat stomach strip The superfused rat fundal strip which is extremely sensitive to 5-HT (Vane, 1957) was contracted by PCPA in the presence of hyoscine and almost identical contractions could be elicited by

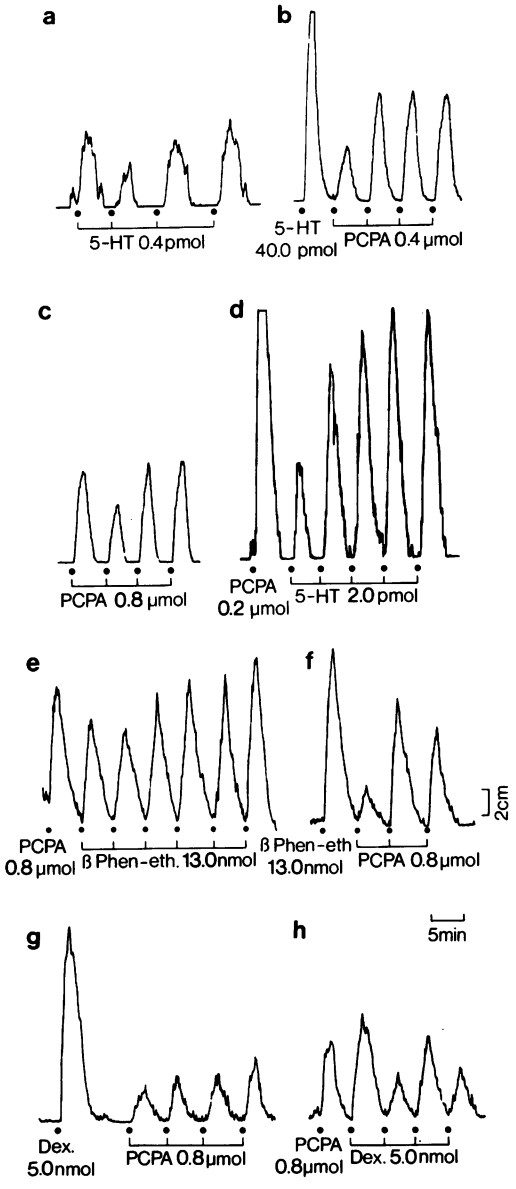

Figure 2 Responses of 8 rat fundal strips (a–h) to 5-hydroxytryptamine (5-HT), *p*-chlorophenylalanine (PCPA), β-phenethylamine (βPhen-eth) and dexamphetamine (Dex). The effect of an initial (priming) dose of one drug was observed on subsequent responses to the same or other drugs. (a) Initial diminution, with subsequent recovery of response to 5-HT, 0.4 µmol, following a priming dose of 5-HT. (The first dose interval was 5 min, but duration of subsequent dose-intervals was increased.) (b) Initial diminution, with subsequent increase of response to PCPA, 0.4 µmol, following a priming dose of 5-HT, 40 pmol. (c), (d) and (e). Similar recovery patterns as in (a) or (b), but priming doses of PCPA, 100 nmol and 5-HT, 2.9 nmol (Figures 1a and b). The responses to PCPA and 5-HT were virtually abolished by a single injection of methysergide, 0.1 µmol (Figures 1c and d), and partial recovery occurred 100 min later (Figures 1e and f).

In experiments with the rat fundal strip bathed in a conventional organ bath, PCPA base (0.2, 0.4 and 2.5 µmol) was also found to contract the tissue, and as with the methyl ester, its effects were prevented by methysergide although antagonism was surmounted by increasing the dose of PCPA (base). The possibility that PCPA and 5-HT contracted the fundal strip by reacting with the same receptors was explored further by testing the tissue's response to serial doses of PCPA and of 5-HT, and by examining the interaction of PCPA with β-phenethylamine and dexamphetamine, substances thought to contract the fundal strip by activation of 5-HT receptors (Vane, 1960). The results are illustrated in Figure 2. Indeed, PCPA exhibited cross-tachyphylaxis with 5-HT (Figures 2b, d), with β-phenethylamine (Figures 2e, f) and possibly with dexamphetamine (Figures 2g, h). Whereas serial doses of PCPA led initially merely to brief reduction followed by recovery in amplitude of responses (Figure 2c), continued serial dosage adversely affected the tissue, the magnitude of responses waning until they eventually disappeared, when contractions evoked by 5-HT were difficult to elicit. This decline in effect of PCPA was not due to inability of the muscle to respond, since the response to acetylcholine, 8 pmol, was unaffected. Following even a single 'priming' dose of PCPA, the magnitude of the responses to dexamphetamine became erratic (Figure 2h). The addition of hyoscine (0.1 µM), mepyramine (10 µM) and occasionally propranolol (0.6 µM) to the bathing fluid, did not alter the contractions to 5-HT, PCPA, β-phenethylamine or dexamphetamine.

Since desensitization by PCPA not only affected responses to 5-HT but also to dexamphetamine and β-phenethylamine, dose-response data were obtained to these four substances. The results are given in Table 1. Dose-response curves for 5-HT, β-phenethylamine and PCPA were reasonably parallel, further evidence

PCPA (0.8 µmol, 0.2 µmol and 0.8 µmol respectively) with subsequent doses of PCPA, 0.8 µmol; 5-HT, 2 pmol, or β-phenethylamine, 13 nmol, respectively). (f) After a priming dose of β-phenethylamine, responses to PCPA, 0.8 µmol, became erratic; erratic responses also to dexamphetamine in (h) following a priming dose of PCPA. (g) Gradual increase in response to PCPA, 0.8 µmol, following a priming dose of dexamphetamine, 5 nmol; interval between dose of dexamphetamine and first dose of PCPA was 10 minutes. Doses in this Figure and Figures 4 and 5 are expressed as the final drug concentration per ml of bath fluid.

of an action mediated via 5-HT receptors, whereas that for dexamphetamine was unexpectedly flatter.

There remains the difficulty of explaining how PCPA, a zwitterion in which the algebraic sum of the electric charges on the basic and acidic substituents on the α-carbon atom of the side-chain should approximate zero at neutral or near neutral pH, could exert spasmogenic effects. Four possibilities were examined.

Effects of pH on the response to p-chlorophenylalanine Experiments were performed with two rat fundal strips bathed in Krebs solution over the pH range, 2.0 to 8.5. With solutions of pH 2.0 to 7.0, responses to PCPA, 10 µmol, varied between +8% and −6% from the mean, with peak effects at pH 6.0. Evidence favouring the importance of ionization of the amine group came from results obtained at higher pH values, since response to PCPA declined at pH 8.0 to 66% of the mean and was zero at pH 8.5, a pH at which the carboxyl but not the amino group was likely to be ionized.

Decarboxylation of p-chlorophenylalanine A net cationic charge at the amino group would also be achieved if PCPA was rapidly decarboxylated to one of its metabolites, *p*-chloro-phenethylamine, a substance which contracts the fundal strip. To exclude this possibility, 6 rats were pretreated with a decarboxylase inhibitor, Ro4-4602 (335 µmol/kg i.p. 4 h previously), to prevent such decarboxylation. However, effects of PCPA (10 µmol) were unimpaired on fundal strips taken from these animals.

Possible release of intraneuronal 5-hydroxytryptamine by p-chlorophenylalanine There remained a possibility that PCPA evoked contractions of the rat fundus by release of intraneuronal 5-HT. This possibility was excluded by 2 types of test: (1) Pretreatment with PCPA. Rats were pretreated for 3 days with PCPA, 120 µmol/kg intraperitoneally, a dose that reduces gut 5-HT concentration to less than 30% (Koe & Weissman, 1966). The response of the fundal strip to 5-HT or PCPA was scarcely altered. (2) Monoamine oxidase inhibition. If contraction of the rat fundal strip by PCPA was mediated by the release of intraneuronal 5-HT, the response to PCPA but not that to exogenous 5-HT would be potentiated by a monoamine oxidase inhibitor. However, exposure of the rat stomach strip to a monoamine oxidase inhibitor, mebanazine (5 nmol/ml) added to the bathing solution, only slightly enhanced responses to 5-HT and PCPA. In contrast, the response to tryptamine was greatly enhanced proving that sufficient mebanazine was present to bring about inhibition of the monoamine oxidase. Similar results were obtained with two other monoamine oxidase inhibitors, phenylisopropylhydrazine (3 nmol/ml) and phenelzine (3 nmol/ml). Consequently, the likelihood is that the contractor effects of PCPA were due to its 5-HT-like properties.

Rat uterus Whereas the rat uterus was extremely sensitive to 5-HT and contracted to doses of 1 nmol or less added to a 10 ml bath, addition of PCPA up to a maximum of 2 µmol only rarely contracted a spontaneously active uterus, but neither relaxed nor contracted uterine horns which showed no spontaneous activity.

Secretion of adrenaline by the cat adrenal medulla In cats, the close arterial injection of 5-HT to the adrenal medulla caused secretion of assayable quantities of catecholamines (Reid, 1952). The present experiments were undertaken in 5 cats with chronically denervated adrenal glands, anaesthetized with chloralose and with blood superfused assay tissues in an extracorporeal circuit. Adrenaline, 1.5 and 3 nmol, injected into the inferior vena cava (hereafter, intravenous), relaxed the blood-superfused chick rectum but had no effect on the rat fundal strip; intravenous noradrenaline 5 and 10 nmol was ineffective. Following 5-HT, 0.06 µmol, and later, PCPA 2 µmol, injected to the adrenal glands via the superior mesenteric artery (hereafter, intra-arterial), the chick rectum relaxed as after adrenaline and the rat fundal strip contracted to 5-HT but did not respond to PCPA. The intra-arterial injections of 5-HT and PCPA elevated the cats' blood pressure. Following intra-arterial methysergide (0.213 µmol) the chick rectum no longer relaxed to 5-HT, 0.06 µmol, or PCPA, 2 µmol, given by the same route even though

Table 1 Analysis of dose-response data

Drug	n	*Dose-ranges*	*Gradient*	*s.d.*
5-hydroxytryptamine	5	*0.29 – 5.8 nmol*	*2.9*	±0.06
p-Chlorophenylalanine	4	*1.0 –10.0* µmol	*2.18*	±0.09
Dexamphetamine	6	0.005– 0.542 µmol	*1.03*	±0.05
β-Phenethylamine	5	0.005– 0.588 µmol	*2.36*	±0.09

* Using a 4 × 4 latin square, giving submaximal doses in random order at 20 min intervals. Each experiment was with a fresh rat fundal strip; *n* = number of experiments.

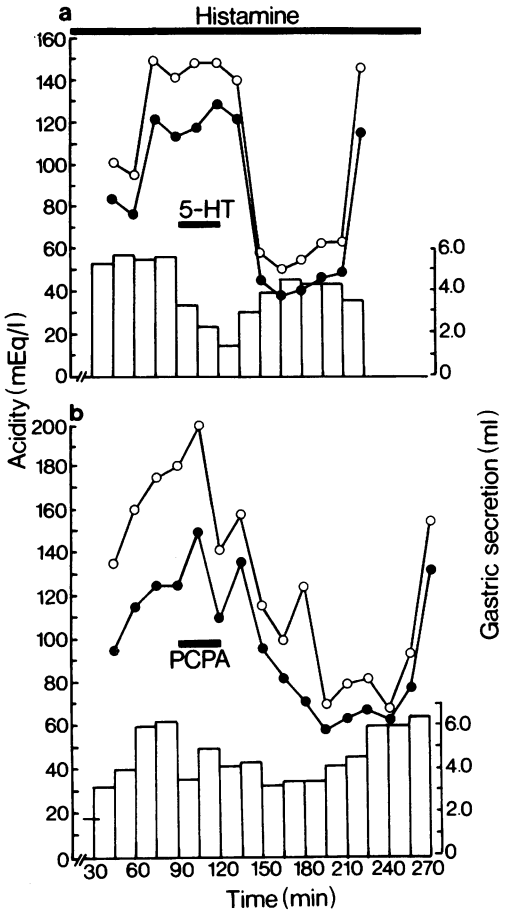

Figure 3 Histograms of gastric secretion and graphs of free and total acidity induced by histamine infused intravenously (3 nmol kg^{-1} min^{-1}) in two cats (a, b) anaesthetized with chloralose. Duration of infusion indicated by black bar. Gastric secretion is expressed as ml on the right of each graph; free acidity (●) and total acidity (○) are expressed as mEq/l HCl on the left. Reduction of free and total acidity, with subsequent recovery, following infusion for 30 min into the aorta (see methods section) of 5-hydroxytryptamine (5-HT), 2.6 nmol kg^{-1} min^{-1} in one cat and of *p*-chlorophenylalanine (PCPA), 0.6 μmol kg^{-1} min^{-1} in another.

relaxation of the chick rectum by intravenous adrenaline was unaltered.

Gastric secretion In 3 cats anaesthetized with chloralose the increase in total and free acidity of gastric secretion evoked by histamine was greatly attenuated following infusion of either 5-HT (2.6 nmol $kg^{-1}min^{-1}$) or PCPA (0.6 μmol $kg^{-1}min^{-1}$) (Figures 3a, b), and the volume of gastric juice also diminished.

Spinal cord flexor reflex. In the 4 spinal cats tested, 5-HT and PCPA increased twitch tension of the reflexly excited anterior tibialis muscle. The effects of both substances developed immediately but 5-HT was considerably more potent than PCPA. 5-HT, 2.75 μmol/kg intravenously elevated basal and peak tensions for 10 min whereas a similar elevation of peak tension for 6 min required 240 μmol/kg of PCPA; PCPA did not elevate basal tension. In 2 further cats given strychnine 0.125 μmol/kg, considerable elevation of the peak tension was obtained with as little as 18 μmol/kg of PCPA. 5-HT and PCPA were ineffective after division of the anterior tibial nerve. In contrast, intravenous 5-HT and PCPA in the above doses depressed reflex peak tension in two spinal cats also given chloralose (60 mg/kg i.v.).

In the spinal cats, 5-HT and PCPA elevated blood pressure; in the chloralosed cats they lowered blood pressure before increasing it. These phenomena were probably mediated by different mechanisms, since in the chloralosed cats following division of the vagi in the neck, 5-HT became pressor whereas the effect of PCPA was unaltered.

Behavioural, electrocortical and electromyographic effects in chicks Thirty-one 10–16-day chicks at an ambient temperature of 24°C were given PCPA (8, 10 or 12 μmol/100 g i.v. or i.p.). Behavioural and electrocortical sleep developed within 1 to 2 min, and lasted 1 to 2 h, with disappearance of electromyographic activity from the dorsal neck muscles. Brief arousal was obtainable with sensory stimuli. The development of sleep could not be attributed to formation *in vivo* of *p*-chlorophenethylamine, a centrally active metabolite of PCPA, since *p*-chlorophenethylamine (10 μmol/100 g i.v.) evoked excitation. This developed within 1 min, lasted 5–10 min and resembled that induced by dexamphetamine; *viz* there was behavioural arousal, vocalization, wing abduction, the normal slope of the spine was altered so that the trunk became horizontal, and the respiratory rate increased up to 140/minute.

Section B. Other actions of p-*chlorophenylalanine*

Guinea-pig ileum As noted by Gaddum & Picarelli (1957), 5-HT contracts the longitudinal muscle of the guinea-pig isolated ileum (Figure 4a). In contrast, PCPA 0.5 mmol caused a decrease in basal tone followed by an after-contraction on wash-out (Figure 4b); basal tone was subsequently increased, and even 20 min after the wash-out, tone was still slightly raised. The height and duration of the wash-out contractions were dose-dependent and were unaffected by the addition to the bath of hyoscine, (10 μM), mepyramine, (0.1 μM) or propranolol (2 μM). The

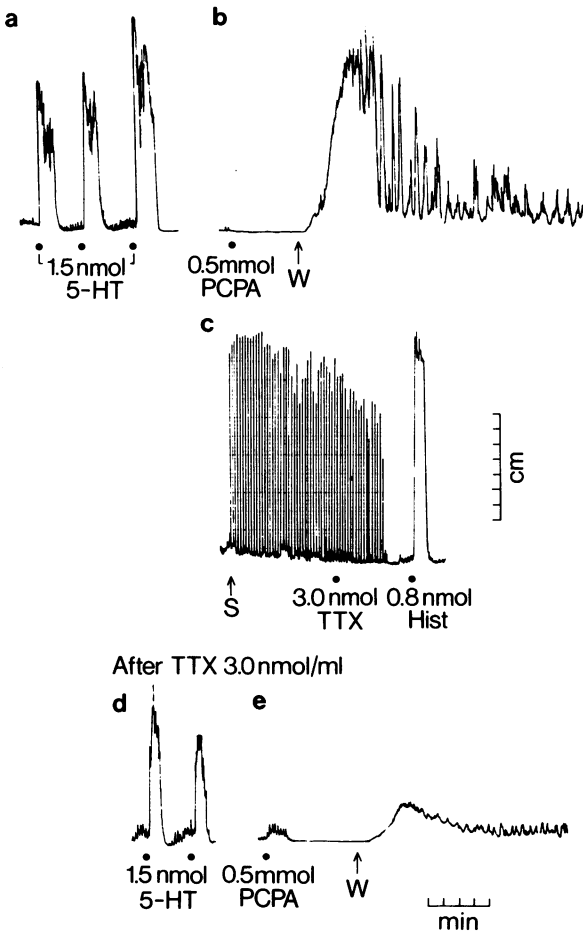

Figure 4 Effects of 5-hydroxytryptamine (5-HT) and *p*-chlorophenylalanine (PCPA) on the longitudinal muscle of a guinea-pig isolated ileum. (a) Contractions to three successive doses of 5-HT, 1.5 nmol. (b) In contrast, PCPA 0.5 mmol slightly reduced resting tone, followed on wash-out of the drug by contraction and increase in basal tone and spontaneous activity. (c) Twitch responses of the electrically excited (S, 0.1 Hz) guinea-pig ileum were abolished by tetrodotoxin (TTX), 3.0 nmol/ml, although the tissue contracted still to histamine (Hist) 0.8 nmol. Following tetrodotoxin, there was (d) diminished response to 5-HT and (e) reduction in amplitude and duration of the wash-out contraction to PCPA.

after-contraction to PCPA, 0.5 μmol, (compare Figures 4b, e) and contraction to 5-HT, 1.5 nmol (compare Figures 4a, d) were considerably impaired by the addition of tetrodotoxin (3 nmol/ml) to the bath in sufficient concentration to abolish the response of the tissue to electrical stimulation with coaxial electrodes, yet leave the response to histamine intact.

In addition to decreasing basal tone of the ileum, PCPA, 1 mmol, caused immediate relaxation of the ileum contracted by acetylcholine (6 pmol, Figure 5a; 0.2 nmol, Figure 5f), histamine (0.8 nmol, Figure 5b), 5-HT (3 nmol, Figure 5e), or by coaxial stimulation at 0.1 Hz or 5 Hz (Figures 5c, d).

Bronchoconstriction in guinea-pigs in vivo 5-HT causes bronchoconstriction in intact guinea-pigs (Konzett & Rossler, 1940; Herxheimer, 1953; Konzett, 1956). Sustained bronchoconstriction developed immediately after an intravenous injection of 5-HT, 0.06 μmol; however, PCPA, 6 mmol intravenously caused an immediate bronchoconstriction which first waned over the next 1 to 2 min and then redeveloped even more intensely. Following methysergide (6 μmol i.v.) the bronchoconstrictor effect of 5-HT (0.06 μmol i.v.) was prevented while that of PCPA (6 mmol i.v.) was almost unaffected.

Bronchoconstrictor effects of PCPA (6 mmol i.v.)

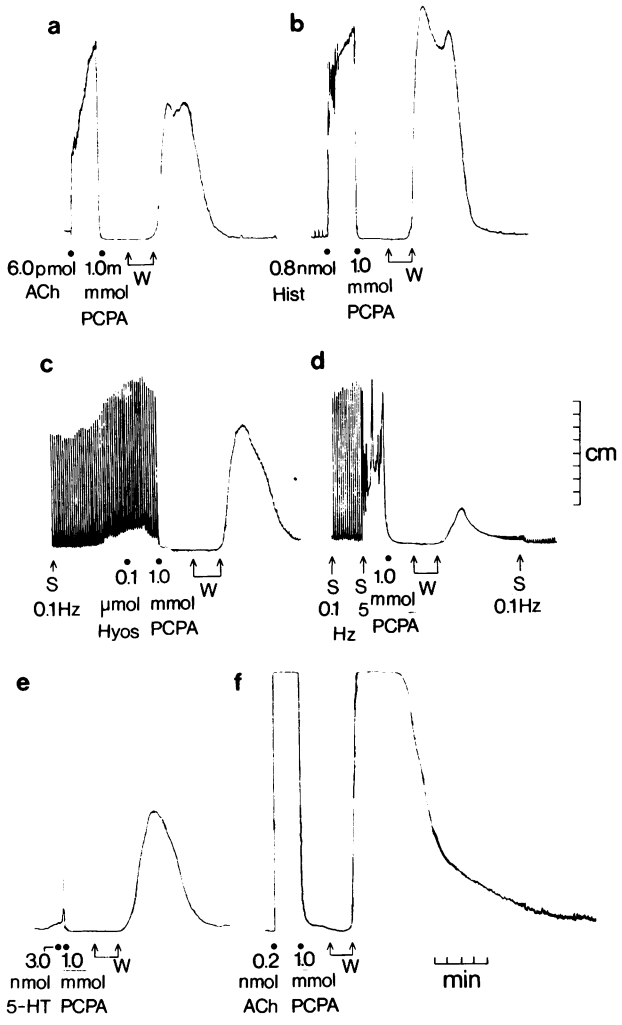

Figure 5 Abolition by *p*-chlorophenylalanine (PCPA), 1 mmol of contractions of different guinea-pig ilea to acetylcholine (ACh), 6 pmol (a) and 0.2 nmol (f); to histamine (Hist), 0.8 nmol (b); to coaxial electrical excitation (S) at 0.1 Hz (c) and 5 Hz (d); and to 5-hydroxytryptamine (5-HT) 3 nmol (e). In (c) Hyos = hyoscine. Wash-out contractions developed in each case.

could not be attributed to histamine release, for whereas mepyramine (25 nmol i.v.) abolished bronchoconstriction due to intravenous histamine (33 nmol), a ten-fold increase in the dose of mepyramine failed to alter the effect of intravenous PCPA (6 mmol). Prostaglandin $F_{2\alpha}$ (13.4 nmol i.v.) mimicked the bronchoconstrictor effects of PCPA. Moreover, the effects of prostaglandin $F_{2\alpha}$ and PCPA were abolished by a prostaglandin antagonist, polyphloretin phosphate (40–45 µg i.v.). In control experiments, intravenous injection of 0.5 ml 70% ethanol, the solvent for prostaglandin $F_{2\alpha}$, lacked effect on bronchial tone.

Bronchoconstriction in guinea-pig isolated lungs 5-HT and PCPA injected into the perfused pulmonary artery induced effects resembling those obtained with intravenous injection *in vivo*. Thus 5-HT, 0.06 µmol and PCPA, 6 mmol induced bronchoconstriction (Figures 6a, b). Addition of methysergide, 6 to 14 µmol/ml, to the perfusate abolished the bronchoconstrictor action of 5-HT (Figure 6c), and slowed the onset of bronchoconstriction due to PCPA, 6 µmol (Figure 6d). Perfusion of the lungs for 30 min with Krebs solution containing indomethacin, 3 nmol/ml, substantially diminished the response to PCPA (6 µmol); bronchoconstriction lasted merely

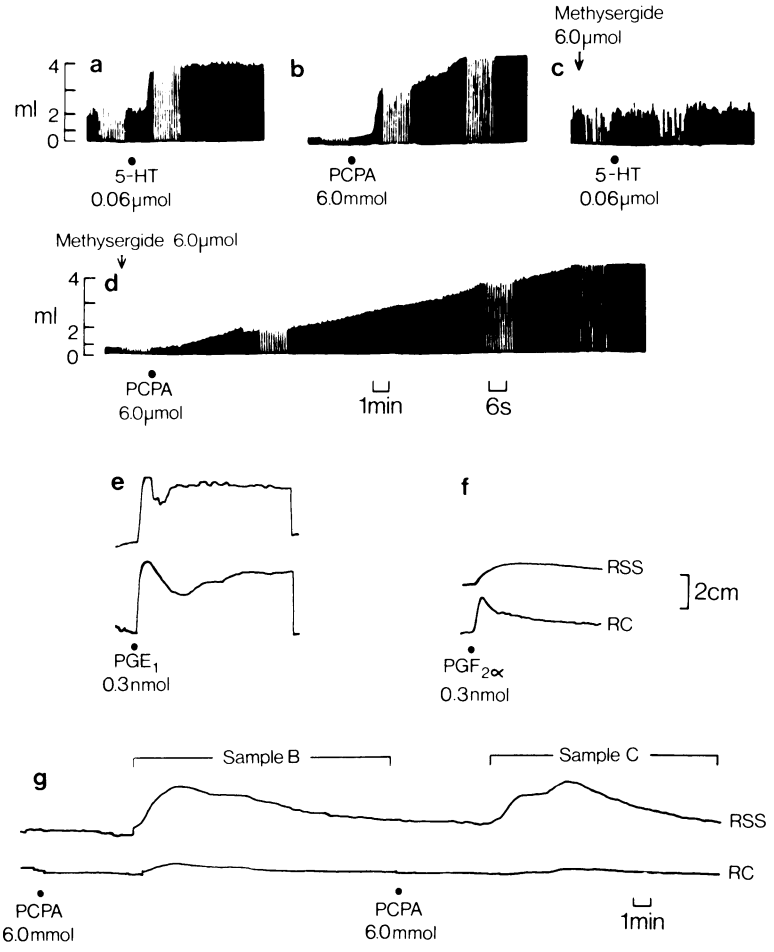

Figure 6 Responses above (a–e) of guinea-pig isolated lungs showing resistance to inflation, and below (e–g) of isolated tissues superfused by perfusate (containing a mixture of antagonists: see methods sections) from another guinea-pig's isolated lungs (RSS = rat fundal strip; RC = rat colon). (a) and (b) Increased resistance to inflation evoked by 5-hydroxytryptamine (5-HT), 0.06 μmol and to *p*-chlorophenylalanine (PCPA), 6 μmol injected into the perfusate entering the pulmonary artery. (c) Effects of 5-HT were abolished by methysergide, 6 μmol, whereas (d) the response to PCPA was slowed. (Responses registered at slow and fast paper speeds.) (e) and (f) Responses of the rat fundal strip and colon to prostaglandins E_1 (PGE_1) and $F_{2\alpha}$ ($PGF_{2\alpha}$), 0.3 nmol, injected directly into the superfusing fluid. (g) Response of the superfused tissues, which developed 5 min after doses of PCPA, 6 mmol, into the perfused pulmonary artery, showing considerable contractions of the rat fundal strip and smaller, simultaneous contractions of the rat colon.

30 s, a finding compatible with PCPA releasing a prostaglandin-like substance capable of evoking bronchoconstriction.

To ascertain whether PCPA released pulmonary prostaglandins, the perfusate from the lungs was superfused over a rat fundal strip and colon (see methods section). These tissues contracted to prostaglandin E_1, 0.07 and 0.3 nmol added to the superfusate, but whereas the rat colon responded to doses of prostaglandin $F_{2\alpha}$ as small as 0.075 nmol, up to 0.3 nmol was required to contract the rat fundal strip (Figures 6e, f).

PCPA (6 mmol) injected into the superfusate passing directly to the rat fundal strip and rat colon had no effect (since methysergide was present), but when injected into the perfusate to the lungs, it increased bronchial resistance and after a delay of 5 min, of which 4 min was required for the perfusate leaving the lungs to reach the superfused tissues, it contracted these (Figure 6g, Sample B). A second dose of PCPA (6 mmol) perfused through the lungs 20 min later, contracted the rat fundal strip but had a smaller

effect on the rat colon (Figure 6g, Sample C) and bronchoconstriction was not further intensified.

Discussion

The present research was begun in an attempt to explain the soporific action of PCPA in chicks (Marley & Whelan, 1974), particularly since previous emphasis had been on the 'wakefulness' produced in various other species by this drug. Experiments *in vitro* and *in vivo* revealed at least three hitherto undescribed actions of PCPA.

First, the actions of PCPA *in vitro* on the rat fundal strip and *in vivo* on the cat adrenal medulla, and the antagonism of both by methysergide, are presented as evidence for a direct action of PCPA on 5-HT-like receptors. The effects of PCPA in cats on gastric secretion (see also Fjalland, 1973) and a spinal cord reflex, together with those on behaviour and electrocortical activity in chickens are compatible with 5-HT-like properties (Marley & Whelan, 1974). Evidence that the cationic head of PCPA was crucial for its 5-HT-like activity, derived from experiments using the rat fundal strip. Thus, PCPA still contracted this tissue removed from rats pretreated with Ro4-4602, a substance which inhibits L-aromatic amino acid decarboxylating enzymes. Moreover, PCPA retained its potency on the fundal strip in bathing fluids at acid pH favouring ionization of the amino group, but became ineffective at an alkaline pH at which ionization of the carboxyl group occurs.

PCPA base dissolved in dilute HCl also contracted the tissue, thus excluding the methyl ester moiety as important for the drug's 5-HT-like properties. Release of 5-HT by PCPA was also excluded. The rapid onset of the effects of PCPA and its methyl ester, *in vitro* and *in vivo*, made it unlikely that a metabolite was responsible. Indeed, the only contaminant of PCPA methyl ester revealed by thin-layer chromatography was free PCPA (see Appendix).

Next, whereas 5-HT contracted the guinea-pig ileum there was merely slight relaxation of this tissue to PCPA, although large doses abolished contractions evoked by acetylcholine, histamine, 5-HT or by transmural stimulation. Relaxation of the tissue by PCPA was followed by contraction on wash-out, an effect attenuated by tetrodotoxin, so implicating neuronal as well as smooth muscle components. These phenomena could be explained by a non-specific hyperpolarizing effect of PCPA on the ileal excitable membranes, since the evidence was against interference with calcium influx or an increase in membrane permeability. The different innervation patterns of the guinea-pig ileum and rat stomach would also need consideration in any explanation of these phenomena.

Finally, PCPA elicited bronchoconstriction in guinea-pigs as did 5-HT, but methysergide prevented that to 5-HT but not that to PCPA. However, bronchoconstriction evoked by PCPA was substantially attenuated by indomethacin, and since indomethacin inhibits prostaglandin synthesis (Ferreira, Moncada & Vane, 1971), these results and those of the effect of the lung perfusate on isolated tissues, were compatible with release of a prostaglandin-like substance by PCPA. Although the lungs can fairly readily be provoked into releasing both prostaglandin E_2 and $F_{2\alpha}$ (Piper & Vane, 1971), prostaglandin $F_{2\alpha}$ elicits bronchoconstriction and prostaglandin E_2 bronchodilation (Baum, Wendt, Peters, Butz & Shropshire, 1974), so the fact that PCPA caused bronchoconstriction suggested release of prostaglandin $F_{2\alpha}$, or that release of this substance predominated.

In conclusion, PCPA and its methyl ester possess a number of hitherto undescribed pharmacological properties, in particular a 5-HT-like action and a capacity to release a prostaglandin-like substance from guinea-pig lungs. Although the actual contribution of these effects of PCPA to the overall behavioural status of the animal is beyond the scope of this paper, these should be borne in mind in any evaluation of PCPA activity.

Appendix

Thin layer chromatography on the purity of p-*chlorophenylalanine ester*

These experiments were kindly performed by Sigma Chemical Co. on the sample of PCPA methyl ester used in our experiments. Chromatography was performed using thin-layers of MN-300 cellulose in three solvent systems. The following data were obtained. They indicate that the only contaminant present was free PCPA.

System I. *n*-Butanol : glacial acetic acid : distilled water (60 : 15 : 25)

R_F		R_F	*Markers*
0.96	Major ninhydrin spot	0.96	*p*-chlorophenethylamine
0.89	Minor ninhydrin spot (estimated at 1–2%)	0.89	*p*-chlorophenylalanine
		0.80	phenylalanine
		0.95	phenylalanine methyl ester

System II. Ethyl acetate : pyridine : distilled water : glacial acetic acid (36 : 36 : 21 : 7)

R_F		R_F	*Markers*
0.95	Major ninhydrin spot	0.84	*p*-chlorophenethylamine
0.72	Minor ninhydrin spot (estimated at 1–2%)	0.72	*p*-chlorophenylalanine
		0.61	phenylalanine
		0.92	phenylalanine methyl ester

System III. Methyl ethyl ketone : pyridine : distilled water : glacial acetic acid (70 : 15 : 15 : 2)

R_F		R_F	*Markers*
0.98	Major ninhydrin spot	0.89	*p*-chlorophenethylamine
0.78	Minor ninhydrin spot (estimated at 1–2%)	0.78	*p*-chlorophenylalanine
		0.60	phenylalanine
		0.94	phenylalanine methyl ester

We are indebted to Sigma Chemical Co. for performing the thin-layer chromatography on *p*-chlorophenylalanine and its congeners, and for allowing us to publish their results. We are grateful to the Medical Research Council and to the Bethlem Royal and Maudsley Hospitals Research Fund for financial support. We would like to thank Dr A. Bennett for tetrodotoxin, prostaglandin $F_{2\alpha}$ and polyphloretin; Mercke, Sharp & Dohme for indomethacin, Roche for Ro4-4602 and Sandoz for methysergide. Thanks are due also to Mr D. J. Allen for technical assistance and to Dr A. Brading for helpful discussion on smooth muscle physiology.

This work will form part of J.E.W.'s Ph.D. thesis, University of London. J.E.W. is an M.R.C. scholar.

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