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p-Chloroamphetamine-induced hyperthermia pharmacologically distinct from fenfluramine-induced hyperthermia

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The influence of various drug pretreatments upon the responses of rabbits to the putative indirect 5-hydroxytryptaminergic agonists *p*-chloroamphetamine (PCA) and fenfluramine were examined. In naive rabbits PCA evoked hyperthermia, behavioural excitation and prominent forepaw clonic activity, while fenfluramine produced only hyperthermia and behavioural stimulation. The hyperthermic and behavioural responses of both agents were reduced by the 5-hydroxytryptamine (5-HT) uptake inhibitor, fluoxetine, potentiated by the monoamine oxidase inhibitor, pheniprazine, and unaltered by the dopaminergic antagonist, haloperidol. Pretreatment with the 5-hydroxytryptaminergic receptor blockers cinanserin, cyproheptadine or D-2-bromolysergic acid diethylamide markedly attenuated the effects of fenfluramine but only slightly influenced the responses to PCA. Depeletion of central 5-HT stores with *p*-chlorophenylalanine also affected responses to fenfluramine more than responses to PCA. The tryptaminergic receptor blocker methergoline abolished both PCA-induced hyperthermia and forepaw clonus—but not behavioural stimulation—while the effects of fenfluramine were only partly reduced. We interpret these data to mean that PCA- and fenfluramine-induced drug effects have different underlying mechanisms, the PCA responses relying possibly upon tryptamine while the fenfluramine responses are 5-hydroxytryptaminergic.

p-Chloroamphetamine (PCA) is one of a number of halogenated arylalkylamines which have attracted much attention. One prominent neurochemical effect of such compounds is the ability to markedly reduce brain concentrations of 5-HT and its main metabolite 5-hydroxyindoleacetic acid (Pletscher et al 1963; 1964). PCA itself has been reported to lower brain 5-HT content in rats for up to 4 months following a single administration (Sanders-Bush et al 1972). There have been numerous hypotheses to explain this action of PCA (Fuller & Molloy 1974). One of these suggests that PCA can effect a rapid and long-lasting release of 5-HT from 5-hydroxytryptaminergic nerve terminals in the central nervous system (Pletscher et al 1965). This same mechanism of action has been attributed to a distant structural analogue, fenfluramine (Clineschmidt 1973; Clineschmidt et al 1975).

Systemic administration of PCA to rats produces an elevation in the body temperature (Mantegazza et al 1970; Sheard 1974; Frey 1975). These and other studies indicate that PCA may owe some of its acute pharmacological properties to the release of 5-HT from nerve endings with subsequent activation of 5-hydroxytryptaminergic receptors (Trulson & Jacobs 1976). In preliminary experiments we have found that intravenous administration of PCA to rabbits evokes a rise in the colonic temperature reminiscent of the hyperthermic response of rabbits to lysergic acid diethylamide (LSD) (Horita & Dille 1954) or the dopaminergic agonist apomorphine (Hill & Horita 1972). A previous investigation also demonstrates that fenfluramine evoked dose-dependent hyperthermia in rabbits, apparently through an indirect action upon thermoregulatory 5-hydroxytryptaminergic mechanisms (Quock & Beal 1976). In this paper, we compare the hyperthermic response of rabbits to PCA and fenfluramine and present data suggesting that PCA does not exert its temperature effect through the same neuroaminergic mechanism as fenfluramine.

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METHODS

Animals. Male New Zealand rabbits, 1·8 to 2·5 kg, on the day before experimentation, were conditioned to restraint in open, wooden stanchions (Shellenberger & Elder 1967) for 6–8 h at 23·0° ± 1·0° C. Colonic temperatures were electronically

monitored with flexible thermistor probes inserted 12 cm into the rectum and affixed to the base of the tail with adhesive tape. The probes were attached to a Model 47 telethermometer (Yellow Springs Instrument Company, Yellow Springs, Ohio). On the day of the experiment, the animals were placed into the stanchions 90 min before initial drug administration.

Drugs. The drugs used were gifts of: A. H. Robins (fenfluramine hydrochloride), Eli Lilly (fluoxetine hydrochloride), Merrell-National (pheniprazine hydrochloride), Merck Sharp and Dohme (cyproheptadine hydrochloride), Squibb (cinanserin hydrochloride), Farmitalia (methergoline), Sandoz (D-2-bromolysergic acid diethylamide, BOL), and McNeil (haloperidol). (±)-*p*-Chloroamphetamine hydrochloride (PCA) and *p*-chlorophenylalanine methyl ester hydrochloride were purchased from Regis and Nutritional Biochemical Chemical Companies, respectively. BOL and haloperidol were supplied in pre-prepared injection ampoules. All other drugs were prepared in aqueous solution. Methergoline was prepared in distilled water made acidic with a slight excess of ascorbic acid. Drug pretreatments were administered intravenously or intraperitoneally at various times before PCA or fenfluramine challenge. Intravenous injection volumes were at 1·0 ml kg^{-1}.

5-HT assay. Brainstem concentration of 5-HT in naive and PCPA-pretreated animals were determined spectrophotofluorimetrically by the method of Bogdanski et al (1956).

RESULTS

Control groups. Rabbits were administered increasing intravenous doses of PCA and responded with dose-related hyperthermia (Fig. 1). A dose of 0·5 mg kg^{-1} (not represented in Fig. 1) was without appreciable effect. The greatest dose attempted, 5·0 mg kg^{-1}, evoked a lethal hyperpyrexia in all cases. From this dose-response relationship, we adopted as our standard challenge dose 2·5 mg kg^{-1} of PCA. This dose produced a rise in colonic temperature that was maximal at +1·5° C 1 h after injection and lasted approximately 3–4 h. The hyperthermia was usually accompanied by behavioural excitation, increased respiration, mydriasis and constriction of the ear vasculature. Eight of 14 rabbits treated with this dose also exhibited extension of the fore-limbs with alternating up-and-down oscillations of the paws. A higher dose of PCA, 5·0 mg kg^{-1}, produced this forepaw clonic effect in 5 or 6 animals.

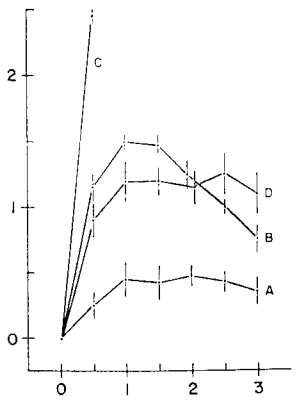

FIG. 1. PCA- and fenfluramine (FEN)-induced hyperthermia in the rabbit: A, PCA (1·0 mg kg^{-1}, n = 5); B, PCA (2·5 mg kg^{-1}, n = 14); C, PCA (5·0 mg kg^{-1}, n = 5); and D, FEN (10·0 mg kg^{-1}, n = 10). Vertical lines represent the s.e.m. Ordinate: change in colonic temperature (°C). Abscissa: time following challenge (h).

Experiments in fenfluramine-treated animals reaffirmed our earlier findings of its effects in rabbits. A standard challenge dose of 10·0 mg kg^{-1} evoked hyperthermia, mild behavioural stimulation, increased respiration, mydriasis and vasodilation of the ears. The hyperthermia reached a plateau at approximately +1·2° C and remained relatively stable for 90 min before declining (also Fig. 1). Animals receiving 15·0 mg kg^{-1} of fenfluramine, which proved to be a lethal dose, exhibited intense hyperthermia, pronounced locomotor activity, exopthalmus and mydriasis but no distinctive forepaw clonic activity.

Pretreatment with a 5-HT uptake blocker. In rabbits pretreated intravenously with 5·0 mg kg^{-1} of fluoxetine 60 min before PCA challenge, the hyperthermic, behavioural and autonomic responses to PCA were all significantly reduced (Fig. 2). Identical pretreatment with fluoxetine in another group of animals also resulted in abolition of the hyperthermia, behavioural and autonomic influences of fenfluramine (also Fig. 2).

Pretreatment with a monoamine oxidase inhibitor. Rabbits that had been pretreated 2 h earlier with intravenous injections of 5·0 mg kg^{-1} of pheniprazine responded wih lethal hyperpyrexia and enhanced behavioural excitation to either PCA or fenfluramine (Fig. 2).

Pretreatment with a dopaminergic receptor blocker. Pretreatment with intravenous haloperidol in a dose of 0·5 mg kg^{-1} 30 min before drug challenge had no appreciable influence upon the hyperthermic,

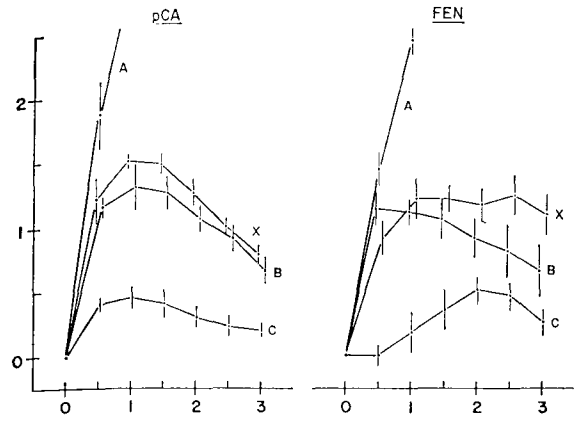

FIG. 2. Influence of various drug pretreatments upon PCA- and FEN-induced hyperthermia in the rabbit: X, PCA (2·5 mg kg^{-1}, n = 14) or FEN (10·0 mg kg^{-1}, n = 10); A, pheniprazine (5·0 mg kg^{-1}) + PCA (n = 7) or FEN (n = 5); B, haloperidol (0·5 mg kg^{-1}) + PCA (n = 5); and, C fluoxetine (5·0 mg kg^{-1}) + PCA (n = 10) or FEN (n = 6). Vertical lines represent the s.e.m. Ordinate: change in colonic temperature (°C). Abscissa: time following challenge (h).

behavioural or autonomic effects of either PCA or fenfluramine (Fig. 2).

Pretreatment with 5-hydroxytryptaminergic receptor blockers. Different groups of rabbits were pretreated intravenously with cyproheptadine (2·0 mg kg^{-1}, 30 min), cinanserin (5·0 mg kg^{-1}, 30 min) and BOL (1·0 mg kg^{-1}, 60 min), respectively. Of these pretreatments, cyproheptadine—but not cinanserin—reduced the intensity of PCA-induced hyperthermia (Fig. 3). BOL did delay the onset of the hyperthermic response for approximately 60 min but did not significantly change

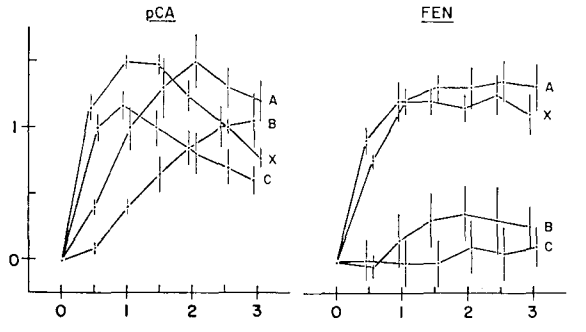

FIG. 3. Influence of various drug pretreatments upon PCA- and FEN-induced hyperthermia in the rabbit: X, PCA or FEN (refer to Fig. 2 for doses and numbers); A, BOL (1·0 mg kg^{-1}) + PCA (n = 5) or FEN (n = 5); B, cinanserin (5·0 mg kg^{-1}) + PCA (n = 5) or FEN (n = 5); and C, cyproheptadine (2·0 mg kg^{-1}) + PCA (n = 8) or FEN (n = 5). Vertical lines represent the s.e.m. Ordinate: change in colonic temperature (°C). Abscissa: time following challenge (h).

the magnitude of the temperature response once it had developed. In addition, none of these pretreatments was effective in influencing either the behavioural or forepaw clonic effects of PCA.

Similar pretreatment with cyproheptadine and cinanserin markedly attenuated fenfluramine-induced hyperthermia and behavioural stimulation (also Fig. 3). BOL, on the other hand, failed to reduce these effects of fenfluramine.

Pretreatment with a 5-HT synthesis inhibitor. Rabbits were administered 3 × 300 mg kg^{-1} of *p*-chlorophenylalanine, intraperitoneally, at 48, 24 and 12 h preceding PCA or fenfluramine injection. Those *p*-chlorophenylalanine-treated rabbits challenged with PCA responded with a slightly-reduced hyperthermia but behavioural excitation and forepaw clonus was unaltered, while those *p*-chlorophenylalanine-treated animals given fenfluramine demonstrated neither significant hyperthermic nor behavioural effects (Fig. 4). Additional rabbits

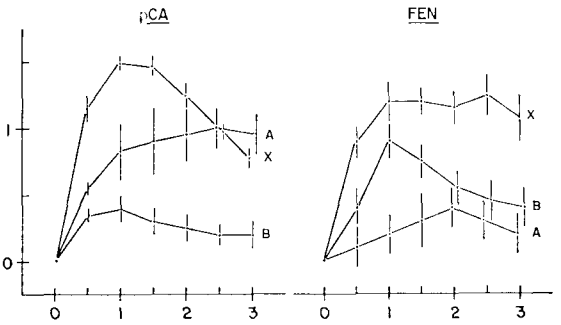

FIG. 4. Influence of various drug pretreatments upon PCA- and FEN-induced hyperthermia in the rabbit: X, PCA or FEN (refer to Fig. 2 for doses and numbers); A, *p*-chlorophenylalanine (3 × 300 mg kg^{-1}) + PCA (n = 6) or FEN (n = 7); and B, methergoline (1·0 mg kg^{-1}) + PCA (n = 10) or FEN (n = 5). Vertical lines represent the s.e.m. Ordinate: change in colonic temperature (°C). Abscissa: time following challenge (h).

treated with *p*-chlorophenylalanine were killed at time 0, and their brainstem had 5-HT concentrations of 0·13 ± 0·02 μg g^{-1} (mean ± s.e., n = 8), compared with normal values of 0·56 ± 0·04 μg g^{-1} (n = 6) ($P < 0·01$, Student's *t*-test).

Pretreatment with a tryptaminergic receptor blocker. Pretreatment with methergoline (1·0 mg kg^{-1}, 2 h) significantly lowered the magnitude of PCA-induced hyperthermia (Fig. 4). Though the behavioural excitatory component of the PCA response remained intact, none of the 10 methergoline-pretreated animals exhibited forepaw clonic activity

($P<0.025$, Chi-squared test). The same dose of methergoline was only partly effective in reducing fenfluramine-induced hyperthermia (also Fig. 4), but the stimulation due to fenfluramine was abolished by methergoline.

DISCUSSION

There is abundant evidence in the literature testifying to the ability of PCA to produce acute as well as chronic release of 5-HT from nerve terminals (Sanders-Bush et al 1974). While displacement of 5-HT is only one of several postulated actions of PCA, this particular mechanism presumably underlies many of the acute effects of the drug (Sheard 1974; Davis & Sheard 1976; Trulson & Jacobs 1976). Fenfluramine has also been postulated to be an indirect 5-hydroxytryptaminergic agonist (Clineschmidt 1973; Garattini et al 1975). The present study compared the effects of PCA and fenfluramine in the rabbit and revealed certain dissimilarities between these two putative indirect 5-hydroxytryptaminergic agonists following various pharmacological interventions.

Antagonism of both PCA- and fenfluramine-induced hyperthermia by fluoxetine, a known selective inhibitor of the 5-HT uptake process (Wong et al 1974), reaffirmed the concept that both drugs act indirectly rather than directly upon the target receptors mediating the temperature change. The marked potentiation of both drugs by the monoamine oxidase inhibitor, pheniprazine, is also consistent with this idea, further indicating the mediating substance released by both drugs to be subject to oxidative deamination.

The failure of haloperidol to reduce either PCA- or fenfluramine-induced hyperthermia demonstrated that the causative agent is not dopamine, which is the mediator of the hyperthermic effect caused by PCA's parent compound amphetamine (Horita & Hill 1973). The dose of haloperidol used was sufficient to block central dopaminergic receptors in the rabbit and has been reported to antagonize the effects of apomorphine in this species (Roszell & Horita 1975).

Antagonism of fenfluramine-induced hyperthermia by cinanserin and cyproheptadine is in agreement with previous observations that the temperature effects of fenfluramine have a 5-hydroxytryptaminergic basis (Jespersen & Scheel-Krüger 1970; Frey 1975; Quock & Beal 1976). The observed reversal by depletion of central 5-HT stores—as verified by brain assay of *p*-chlorophenylalanine-pretreated rabbits—indicates that this particular action of fenfluramine requires the integrity of transmitter stores in 5-hydroxytryptaminergic neurons; this has also been suggested by other investigators, employing other parameters of fenfluramine action (Duhault & Verdavainne 1967; Clineschmidt 1973; Mogilnicka et al 1975).

Identical doses of cinanserin and cyproheptadine —also reported effective in attentuating the hyperthermic action of the 5-hydroxytryptaminergic agonists quipazine (Quock et al 1976) and LSD (Rubin et al 1964)—only slightly altered PCA-induced hyperthermia. The classical 5-hydroxytryptaminergic blocker BOL was unexpectedly inactive against either PCA or fenfluramine. BOL did retard the development of the PCA response; however, once the hyperthermia was initiated, the ultimate magnitude of the effect was not diminished. While BOL has previously been reported to be an effective antagonist in this paradigm (Horita & Gogerty 1958), we are presently unable to explain why BOL failed to at least attenuate the fenfluramine response.

These observations prompted us to examine the possibility that a substance other than 5-HT was responsible for the effects of PCA. We found that PCA-induced forepaw clonic activity was not unlike that described in rats and rabbits following infusion of tryptamine (Tedeschi et al 1959). Methergoline was shown to be an effective antagonist of tryptamine-induced forepaw clonus in rats by Ferrini & Glasser (1965). More recently, Clineschmidt & Lotti (1974) demonstrated methergoline to be a potent inhibitor of tryptamine-induced forepaw clonus at doses which fail to block 5-hydroxytryptophan (5-HTP)-induced head twitching; methysergide proved to be somewhat less potent yet equiselective for both responses; both cinanserin and cyproheptadine were overwhelmingly selective against the 5-HTP response. Previous research in our laboratory (Quock & Weick 1978) has demonstrated that intravenous tryptamine treatment in rabbits induces certain temperature and forepaw clonic effects that are insensitive to antagonism by 5-hydroxytryptaminergic receptor blockers at doses which block 5-HTP. Conversely, these same tryptamine-evoked responses are antagonized by methergoline in doses that do not influence 5-HTP. We postulated that tryptamine-induced hyperthermia and forepaw clonus possibly resulted from the activation of specific tryptamine—rather than 5-HT-sensitive mechanisms.

In the present investigation, antagonism of PCA-evoked hyperthermia and forepaw clonus by

methergoline—but not by any of the 5-hydroxytryptaminergic receptor blockers—suggests that the thermotropic and clonic actions of PCA in rabbits might also involve a specific tryptaminergic mechanism. PCA-induced behavioural excitation was not influenced by methergoline or any of the 5-hydroxytryptaminergic or dopaminergic receptor blockers. Hence, the behavioural response is likely mediated by some other underlying mechanism in the same manner that the behavioural response to LSD is related to non-5-hydroxytryptaminergic neurons (Horita & Hamilton 1969).

Tryptamine has long been known to occur in the nervous tissues of a variety of species, including man, dog, cat, rabbit and rat (Hess et al 1959; Martin et al 1972; Snodgrass & Horn 1973; Sloan et al 1975). There have been investigations of the regional distribution of tryptamine in the brain (Knott et al 1974) and more recent research has identified specific tryptaminergic pathways in the central nervous system (Martin et al 1975; 1976). However, there is no evidence to date that PCA influences endogenous tryptamine concentrations nor is there definitive proof of neuronal uptake of tryptamine.

Our observed interaction between *p*-chlorophenylalanine and PCA is also consistent with the idea of a tryptaminergic neuronal substrate for PCA. The hyperthermic response was not abolished—though the time course of action was altered—by inhibition of 5-HT synthesis and reduction of brainstem 5-HT to less than 25% of the normal content. While we did not determine the influence of *p*-chlorophenylalanine pretreatment upon brain tryptamine concentrations, others have reported that it increases brain concentrations of tryptamine (Saavedra & Axelrod 1973; Marsden & Curzon 1974). Theoretically, this should result in an enhancement of PCA-induced hyperthermia; however, this was not observed.

In summary, our data show that fenfluramine and PCA are not identical in terms of how they are influenced by various drug pretreatments. We reaffirm that fenfluramine very likely acts indirectly through a 5-hydroxytryptaminergic mechanism in producing hyperthermia in the rabbit. However, drugs effective in abolishing fenfluramine-induced responses are either ineffective or only slightly effective in attenuating PCA-induced hyperthermia, behavioural stimulation or forepaw clonic activity. Only the putative tryptaminergic receptor blocker methergoline reduced the temperature and forepaw clonic effects of PCA, the hyperthermia being antagonized to a greater extent than the corresponding thermotropic effect of fenfluramine.

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