Pharmacology Biochemistry & Behavior, Vol. 15, pp. 197–200, 1981. Printed in the U.S.A.

Mechanisms of PCA-Induced Hypothermia, Ejaculation, Salivation and Irritability in Rats

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Received 29 September 1980

HUMPHRIES, C. R., G. PAXINOS AND M. O'BRIEN. *Mechanisms of PCA-induced hypothermia, ejaculation, salivation and irritability in rats.* PHARMAC. BIOCHEM. BEHAV. 15(2) 197–200, 1981.—Injections of *p*-chloroamphetamine (PCA, 5 mg/kg) induced hypothermia, ejaculation, salivation and irritability in male rats kept at an ambient temperature of 20±1°C. PCA-induced hypothermia was attenuated by pretreatment with the 5-hydroxytryptamine (5-HT) uptake blockers Lundbeck 10-171 (Lu 10-171, 10 mg/kg) and chlorimipramine (CMI, 20 mg/kg) and the 5-HT synthesis inhibitor parachlorophenylalanine (PCPA, 150 mg/kg daily for 3 days); it was potentiated by pretreatment with the noradrenaline uptake blocker Lundbeck 5-003 (Lu 5-003, 10 mg/kg) and the catecholamine synthesis inhibitor alpha-methyl-*p*-tyrosine (AMPT, 50 mg/kg every 3 hr for 9 hr). PCA-induced ejaculation was attenuated by pretreatment with Lu 10-171 and CMI. PCA-induced salivation was attenuated by pretreatment with Lu 10-171 and CMI and potentiated by pretreatment with Lu 5-003. PCA-induced irritability was potentiated by pretreatment with PCPA. These results suggest that both 5-HT and the catecholamines play a role in PCA-induced hypothermia, ejaculation, and salivation.

PCA Thermoregulation Ejaculation Salivation Irritability Chlorimipramine
Parachlorophenylalanine Alpha-methyl-*p*-tyrosine Clonidine 5-Hydroxytryptamine Catecholamines

MALE rats within one hour after *p*-chloroamphetamine (PCA) injection have been reported to exhibit repetitive stereotyped movements [16], increased locomotor activity [4, 6, 11, 12, 13, 18], temperature change (hyperthermia [5, 8, 11, 12] and hypothermia [8,13]), ejaculation [7,8], salivation [7,8] and irritability [8]. Pharmacologically, PCA's main effects appear to be a short-term elevation of central 5-hydroxytryptamine (5-HT) and catecholamine levels [14] followed by a long-term depletion of central 5-HT [16]. However, the relative contributions of 5-HT and the catecholamines to various short-term PCA-induced behavioral changes are not clear.

Some researchers have attributed PCA's short-term behavioral effects to its releasing action on 5-HT [4, 6, 20], but others have attributed these effects to catecholamine release [18,19]. Opinion has also been divided on the extent to which some of the PCA-induced behavioral changes may depend on common neural substrates. For instance, Frey *et al.* [4,5] pretreated rats with the 5-HT synthesis inhibitor parachlorophenylalanine (PCPA) and obtained attenuation of both PCA-induced hypothermia and hyperactivity. They therefore attributed both of these effects to PCA-induced release of 5-HT. However, Messing *et al.* [13] pretreated rats with the 5-HT reuptake blocker Lilly 110140 to impede entry of PCA into 5-HT neurons, and blocked PCA-induced hyperactivity but not PCA-induced hypothermia. They therefore attributed the hyperactivity, but not the hypothermia, to PCA-induced release of 5-HT. Thus, there is some disagreement both on the neural substrate subserving PCA-induced temperature change and on the extent to which the temperature change and the PCA-induced hyperactivity depend on common neural systems.

It could be argued that the difference in the conclusions of Frey and Messing *et al.* is due to the fact that the former studied hyper- and the latter hypothermia. While this explanation is possible, it should be noted that the direction of PCA-induced temperature change is determined by the ambient temperature, and that PCA-induced changes in ejaculation, salivation and irritability occur both when rectal temperatures rise and when they fall [8]. The purpose of the present study was to examine the relative contributions of the indoleamine 5-HT and the catecholamines to PCA-induced hypothermia, ejaculation, salivation and irritability, and to determine whether the PCA-induced behavioral changes were correlated.

METHOD

Animals

Eighty-eight male Wistar rats weighing 372±57 (SD) g at the time of injection were used. They were individually

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housed in wire mesh cages kept in a room maintained at 20±1°C and on a 12:12 hr light/dark cycle. Food and water were given ad lib.

Drug Injections

All drugs were administered intraperitoneally at a volume of 1 ml/kg of body weight. Injections were given at approximately the seventh hour of the light cycle, or so that the PCA injection occurred at this time when more than one injection was given. PCA injections were administered at a dose of 5 mg/kg throughout. Thirteen rats were injected with DL-PCA HCl (Regis Chemical Co.) dissolved in saline. Six rats were injected with saline. Eight rats were injected with Lundbeck 10-171 (Lu 10-171, Lundbeck & Co.), 10 mg/kg. Another eight rats were injected with Lu 10-171, 10 mg/kg, followed after 30 min by PCA. Six rats were injected with chlorimipramine (CMI, CIBA Geigy Aust. Ltd.), 20 mg/kg. Eight rats were injected with CMI, 20 mg/kg, followed after 60 min by PCA. Seven rats were injected with PCPA (Sigma Chemical Co.), 450 mg/kg, administered in 150 mg/kg doses daily for 3 days followed 2 days later by PCA. Eight rats were injected with Lundbeck 5-003 (Lu 5-003, Lundbeck & Co.), 10 mg/kg. Another eight rats were injected with Lu 5-003, 10 mg/kg, followed after 30 min by PCA. Eight rats were injected with alpha-methyl-*p*-tyrosine (AMPT, Sigma Chemical Co.), administered in 50 mg/kg doses every 3 hr for 9 hr, followed 4 hr after the last injection by PCA. Eight rats were injected with AMPT as above, followed after 3 3/4 hr by clonidine (Boehringer-Ingelheim), 0.2 mg/kg, followed after another 15 min by PCA. The vehicle for all pretreatment drugs was saline except in the case of PCPA where an isotonic 1% solution of Tween 80 was used.

Procedure

Measurements of temperature and irritability were taken twice in the hour prior to drug injection and the second measurement was used as a pre-injection baseline. When drugs were used as pretreatments, a further temperature reading was taken between pretreatment and PCA injection. After PCA injection, measurements of temperature, ejaculation, salivation and irritability were taken once an hour for a minimum of 3 hr on a blind basis with the experimenter unaware of the prior treatment of the rats.

To measure temperature, an STC thermistor probe was inserted 3–5 cm into the rectum and held there until the dial reading stabilised.

Ejaculation was scored by counting the pieces of seminal material collected each hour from paper towelling placed beneath the cages. A fully formed gelatinous plug was taken as evidence of one ejaculation, while fragments were scored 0.5 or 0.25 according to size.

Salivation was scored according to the extent of wetness under the body: 0 (dry), 1 (wet under chin), 2 (wet under chin and abdomen).

Irritability scores were obtained by using the following three-category scale: (a) biting reaction to a gloved hand that pushed the rat against the cage wall; (b) resistance to capture; (c) vocalisation during the preceding two tests. Ratings of 0 (no response), 1 (weak response) or 2 (intense response) were given for each category.

Three days after PCA injections (5 mg/kg) forebrain 5-HT levels were assayed in eight rats (matched with experimental rats and kept in similar conditions) as described elsewhere [8].

RESULTS

PCA Alone, Saline and Other Drugs Used by Themselves

PCA alone induced hypothermia, ejaculation, salivation and irritability. All PCA-injected rats showed hypothermia, with a mean decrease of 1.9°C in rectal temperature at 1 hr after injection (Fig. 1). Additionally, they all ejaculated, with a mean score of 1.3 gelatinous plugs for the first hour after injection. Nine out of 13 rats in the PCA-alone group exhibited excessive salivation (mean score 0.5 1 hr after injection). Eleven out of 13 rats in the PCA-alone group exhibited irritability (mean irritability change score 0.9 1 hr after injection).

No rat in the vehicle-injected control group exhibited a significant change in temperature or irritability scores, salivated excessively or ejaculated.

None of the other drugs (Lu 10-171, CMI, PCPA, Lu 5-003, AMPT or AMPT/clonidine) induced ejaculation or excessive salivation in the absence of PCA, but some affected temperature and irritability. Lu 5-003 and the AMPT/clonidine combination induced some hypothermia (−0.9 and −1.4°C, respectively, at 1 hr after injection). PCPA induced marked irritability in three out of seven rats, with a mean score of 1.4.

Effects of Pretreatments on PCA-Induced Behavioral Changes

The effect of the combination of each pretreatment with PCA was compared with PCA alone at 1 hr after PCA injection for temperature change and ejaculation and over the first three hours after PCA injection for salivation and irritability using Dunnett tests (Fig. 1, see also [21]).

Temperature change. The 5-HT uptake blockers Lu 10-171 and CMI and the 5-HT synthesis inhibitor PCPA significantly reduced PCA-induced hypothermia ($p<0.005$). On the other hand, the NA uptake blocker Lu 5-003, the catecholamine synthesis inhibitor AMPT, and the combination of AMPT with clonidine significantly increased PCA-induced hypothermia ($p<0.005$).

Ejaculation. Lu 10-171 and CMI significantly reduced PCA-induced ejaculation ($p<0.005$ and $p<0.05$, respectively). No other drug had a significant effect on ejaculation, although there was a trend towards an enhancement following pretreatment with Lu 5-003 and AMPT.

Salivation. Lu 10-171 and CMI almost completely blocked PCA-induced salivation ($p<0.05$) while Lu 5-003 significantly increased it ($p<0.01$).

Irritability. Only PCPA significantly affected PCA-induced irritability ($p<0.05$). PCPA injections by themselves produced irritability in three out of seven rats. The subsequent injection of PCA increased the irritability of these rats and induced irritability in two more, yielding a mean irritability score for this group of 3.1 (compared to 1.9 for the PCA-alone group).

Factor analysis revealed that the effects on temperature, ejaculation, salivation and irritability were moderately correlated with one factor (possibly a general stimulation effect) which accounted for 41% of the variance. Changes in ejaculation, temperature and salivation were fairly strongly correlated with this factor ($r=0.7$) but changes in irritability only moderately ($r=0.4$).

Assays showed that forebrain 5-HT was depleted by approximately 60% at the time of decapitation, 3 days after PCA (alone) injection. While the assays do not indicate the effect of PCA on 5-HT levels at the time the rats were tested

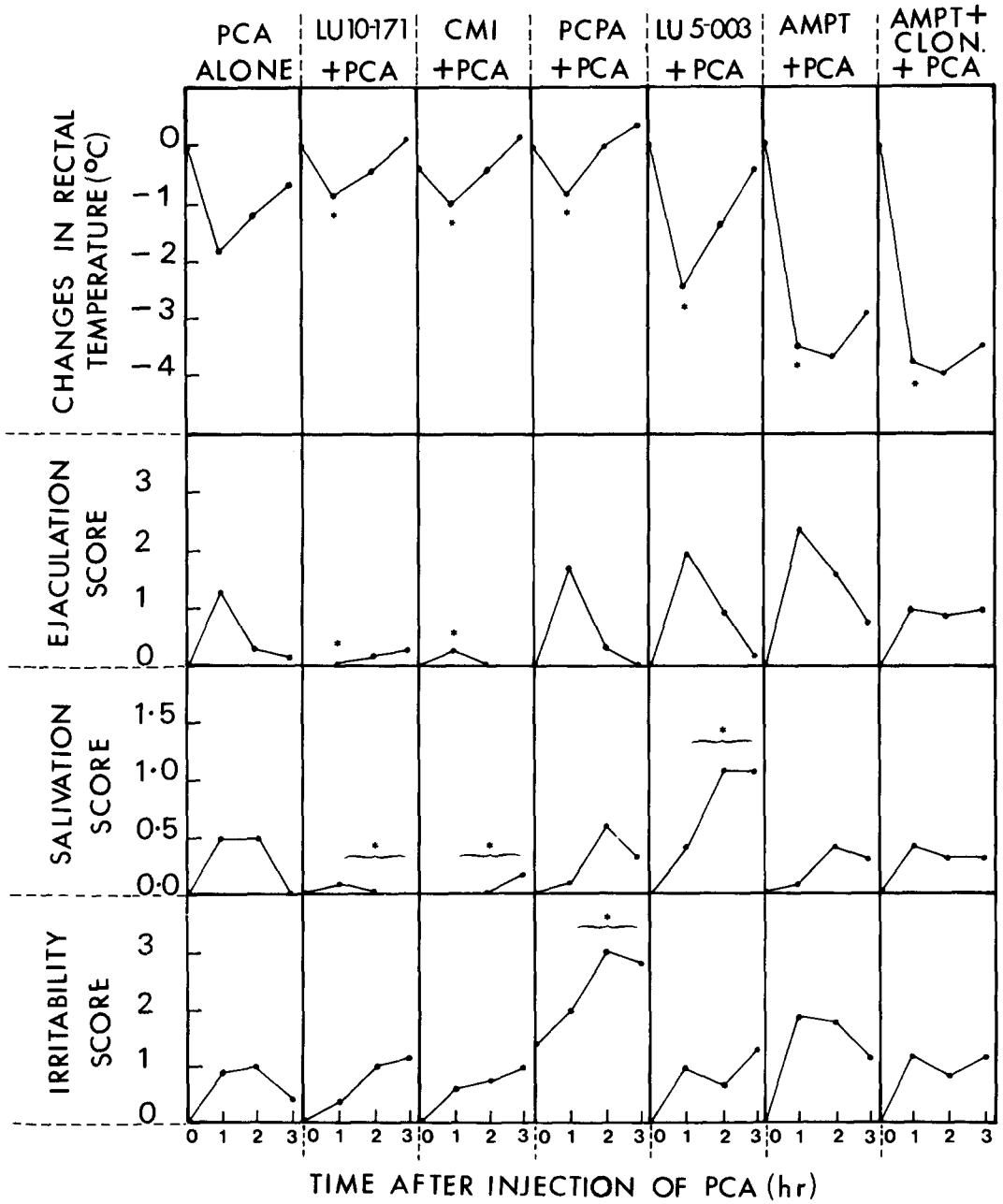

FIG. 1. The mean rectal temperature change, ejaculation, salivation and irritability scores for the PCA alone group and for pretreatment plus PCA groups, prior to and after PCA injection at the ambient temperature of 20±1°C. The effect of the combination of each pretreatment with PCA was compared with the effect of PCA alone at 1 hr after PCA injection for temperature change and ejaculation, and over the first 3 hr after PCA injection for salivation and irritability, using Dunnett tests. Asterisks denote significance at least at the 0.05 level.

(the first few hours after injection), they do show that PCA had the expected long term pharmacological effect.

DISCUSSION

In this study, at an ambient temperature of 20±1°C, PCA injections induced hypothermia, ejaculation, salivation and irritability in male rats.

PCA-induced hypothermia was attenuated by pretreatment with Lu 10-171, CMI and PCPA. Lu 10-171 is reported to be a potent and relatively selective inhibitor of 5-HT uptake [2,10]; the inhibition is competitive and not associated with an increased efflux of 5-HT. Lu 10-171 does not change the endogenous levels of brain monoamines [10]. Lu 10-171 and CMI presumably blocked uptake of PCA into 5-HT neurons and protected them from the releasing action of PCA [5,17]. The 5-HT synthesis inhibitor PCPA presumably decreased the amount of 5-HT that could be released by

PCA. PCA-induced hypothermia was potentiated by pretreatment with Lu 5-003, AMPT and the combination of AMPT with the alpa-adrenergic agonist clonidine. Lu 5-003 was given to impede the entrance of PCA into NA neurons because it is reported to be a powerful and relatively selective NA uptake inhibitor [15]. The significant changes produced by injection of drugs that affect either 5-HT or the catecholamines suggest that these neurotransmitters have important and opposing roles in PCA-induced hypothermia.

The suggestion that 5-HT mediates PCA-induced hypothermia is not in agreement with Messing *et al.*'s [13] conclusions that 5-HT did not play a role in this effect. However in their study the 5-HT reuptake blocker Lilly 110140 was the only pretreatment used; in addition, examination of their graph suggests that although the pretreatment did not block PCA-induced hypothermia, it did substantially reduce it. Therefore the data of Messing *et al.* may not be incompatible with the present conclusions. The suggestion of a role for 5-HT in PCA-induced *hypothermia* is in agreement with other findings in regard to PCA-induced *hyperthermia* [5,17]. When considered together, Frey's study and ours show that both the hyperthermia and the hypothermia induced by PCA were attenuated by the same 5-HT uptake blocker (CMI) and the 5-HT synthesis inhibitor PCPA, and therefore suggest that 5-HT may play a significant role in PCA-induced rectal temperature changes, whether that change is a rise or a fall. The possibility that there may be an opposing catecholamine influence in PCA-induced hypothermia is compatible with findings regarding PCA-induced hyperthermia [5,17].

In the present study there was no significant potentiation of AMPT/PCA induced hypothermia by the addition of clonidine to AMPT pretreatment, possible because of a ceiling effect obtained in the AMPT/PCA combination. A synergistic interaction between clonidine and AMPT has been observed in the investigation of other behaviors [9].

It is possible that 5-HT plays some part in PCA-induced ejaculation since ejaculation was almost blocked by pretreatment with the 5-HT uptake blockers, though not by the 5-HT synthesis inhibitor. Since PCA-induced ejaculation was not significantly influenced by pretreatments affecting catecholamine metabolism, no role for the catecholamines in this effect is suggested by these results. The attenuation of PCA-induced ejaculation by pretreatment with 5-HT uptake blockers, but not by AMPT or a catecholamine uptake blocker, is in agreement with the results reported by Growdon [7].

In addition to PCA, amantidine has been reported to produce hypothermia in mice [3] and spontaneous ejaculation in rats [1]. It was suggested that amantadine's releasing influence on brain dopamine [3] or on both dopamine and noradrenaline [1] was responsible for these effects. Amantadine-induced ejaculation was observed following 'rearing on the hindlegs and arching of the back'. In the present study PCA-induced ejaculation was observed while the rats simply shifted about restlessly on all fours.

PCA-induced salivation was almost completely blocked by pretreatment with the 5-HT uptake blockers, and potentiated by a noradrenaline uptake blocker. Thus it seems possible that 5-HT and noradrenaline have opposing influences in this effect.

In the case of PCA-induced irritability, the role of 5-HT and of the catecholamines remains unclear. PCA-induced irritability was enhanced following depletion of 5-HT by the PCPA pretreatment, but although the PCPA/PCA group had high mean scores for irritability, the percentage of rats exhibiting irritability was relatively low in this group (71%).

Results of the present study indicate that PCA may act through both 5-HT and the catecholamines to produce some of its behavioral effects.

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