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EVIDENCE FOR AN ENDOGENOUS DOPAMINE-MEDIATED HYPOTHERMIA IN THE RAT

B. COX & T.F. LEE

Department of Pharmacology, Materia Medica and Therapeutics, Stopford Building, Manchester University, Manchester M13 9PT

1 Unilateral intrahypothalamic injection of either dopamine (10 μg) or amphetamine (10 μg) caused a fall in core temperature in the rat. Pimozide (0.5 mg/kg, i.p.) significantly reduced the hypothermic response, whereas pretreatment with phentolamine (1 mg/kg, i.p.) or methysergide (5 mg/kg, i.p.) was ineffective.

2 Systemic pretreatment with cocaine (20 mg/kg) abolished the hypothermic effect of amphetamine, but slightly enhanced the hypothermic response to dopamine.

3 Systemic pretreatment with tranylcypromine (10 mg/kg) had no significant effect on the fall in core temperature induced by either amphetamine or dopamine.

4 Intraperitoneal injection of cocaine and tranylcypromine, on their own, caused a fall in core temperature in the rat, which was significantly antagonized by either systemic or central pretreatment with pimozide. Phentolamine and methysergide failed to block the hypothermia.

5 Unilateral intrahypothalamic injection of cocaine (20 μg) or tranylcypromine (10 μg) also caused a significant fall in core temperature, which was reduced by intrahypothalamic pretreatment with pimozide (0.5 μg), but not significantly changed by pretreatment with phentolamine (25 μg) or methysergide (5 μg).

6 These results provide evidence for the presence of a dopaminergic system within the preoptic region, which mediates a lowering of core temperature in the rat.

Introduction

Since the introduction of specific dopamine agonists and antagonists, evidence has accumulated to suggest that stimulation of central dopamine receptors can bring about thermoregulatory changes in several species (for review see Cox, 1979). However, most of the evidence has come from studies involving direct injection of dopamine or dopamine agonists. A role for endogenous dopamine in thermoregulation has been suggested by only a few studies. Thus, amphetamine caused a fall in core temperature in both the rat (Yehuda & Wurtman, 1972) and the guinea-pig (Amiss, Calne & Klawans, 1976) when they were maintained at low ambient temperatures. The hypothermia could be blocked by both pimozide and haloperidol, suggesting that the hypothermic action of amphetamine was mediated through the release of endogenous dopamine. More convincing evidence for dopamine involvement came from the study of Hill (1973), who reported that amphetamine-induced hyperthermia in the rabbit was abolished by 6-hydroxydopamine only when the dose of 6-hydroxydopamine was sufficient to reduce brain dopamine concentration to less than 50% of controls.

In some previous experiments we have shown that, in the rat, the preoptic anterior hypothalamus was the most sensitive site for injection of dopamine agonists (Cox & Lee, 1977) and evidence for a population of dopamine receptors within this region has also been presented (Cox, Kerwin & Lee, 1978). In the present study, drugs which act indirectly were used to assess whether dopamine, endogenous to the preoptic anterior hypothalamus, could produce the same effect as exogenous dopamine.

Methods

Male Sprague-Dawley rats (supplied by Manchester University Medical School Animal Unit) weighing 250 to 350 g were used in all experiments. Within any one experimental group the weight range was never greather than 50 g. The ambient temperature was maintained at 17 ± 1°C throughout the study and rats were acclimatized at this temperature for at least 2 h before starting the experiment.

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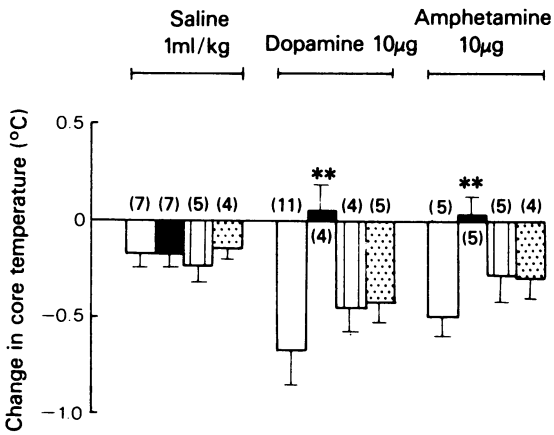

Figure 1 Change in core temperature after intraperitoneal injection of saline or intrahypothalamic injection of either dopamine 10 μg or amphetamine 10 μg alone (open columns) or after systemic pretreatment with pimozide 0.5 mg/kg (solid columns), phentolamine 1 mg/kg (striped columns) or methysergide 5 mg/kg (dotted columns). Each column represents the mean maximum fall in core temperature from the number of rats indicated in parentheses. Vertical bars indicate s.e. mean. Significantly different from appropriate agonist control, ** $P < 0.01$.

Temperature measurement

Core temperature was measured in lightly restrained rats by a rectal thermistor probe (L. Light Ltd) inserted to a depth of 4 cm. Rats were tested immediately before and at 10 min intervals after drug or saline (0.9% w/v NaCl solution) injection throughout the whole experiment. In some experiments rats were pretreated systemically with either pimozide (2 h), phentolamine (1 h) or methysergide (1 h). When the antagonists were given centrally the pretreatment interval was 15 min.

Central injections

Stainless steel guide cannulae (0.5 mm external diameter) were implanted into the brains of rats anaesthetized with pentobarbitone (45 mg/kg i.p.) by use of a David Kopf stereotaxic frame according to the technique of Pellegrino & Cushman (1967). The coordinates used, with bregma as the reference point, were anterior–posterior 1.8 mm, lateral 1.2 mm and depth 5.0 mm. With these coordinates the tip of the guide cannula lay 3 mm above the desired point of injection in the preoptic region of the anterior hypothalamus. Drug injections were made a week later via an injection cannula which extended 3 mm past the cannula tip. Injection sites were subsequently verified histologically.

Statistics

Comparisons between groups were made by the non-parametric Mann-Whitney U test and unless otherwise stated a significant difference between groups was taken as $P < 0.05$. For ease of comparison mean ± s.e. is presented as the index of the response.

Drugs

The drugs used were (+)-amphetamine sulphate (Smith, Kline & French Ltd), cocaine hydrochloride (MacFarlan-Smith Ltd), dopamine hydrochloride (Koch-Light Ltd), methysergide bimaleate (Sandoz Ltd), phentolamine mesylate (Ciba Ltd), pimozide (Janssen Pharmaceuticals), and tranylcypromine sulphate (Smith, Kline & French Ltd). Drug solutions were prepared in sterile, pyrogen-free saline, except for pimozide. This was prepared by dilution from a stock solution of 10 mg/ml made by dissolving 100 mg of pimozide in 3 drops glacial acetic acid and 3 drops absolute ethanol before making up to a final volume of 10 ml with hot 5% w/v glucose solution. For the systemic pretreatments, drugs were injected intraperitoneally in a dose volume of 1 ml/kg. For central injections, drugs were injected unilaterally into the preoptic anterior hypothalamus in a volume of 1 μl. In each series, appropriate vehicle-injected controls were run simultaneously. All doses refer to the free base.

Results

Effects of central dopamine and amphetamine injections

None of the systemic antagonist pretreatments caused a significant change in core temperature although in the absence of central injections a small fall occurred (Figure 1). In contrast, unilateral intrahypothalamic injection of saline caused a slight rise in core temperature (+0.2 ± 0.1°C, $n = 7$). Dopamine and amphetamine (10 μg) caused a significant fall in core temperature when injected by the same route (Figure 1). The effects of dopamine and amphetamine were completely abolished by systemic pretreatment with pimozide (0.5 mg/kg) (Figure 1). Systemic pretreatment with either phentolamine (1 mg/kg) or methysergide (5 mg/kg) caused only a slight but statistically non-significant reduction in the response to central injections of dopamine and amphetamine (Figure 1).

The hypothermia following central dopamine injection was found not to be significantly changed by systemic pretreatment with either cocaine (20 mg/kg,

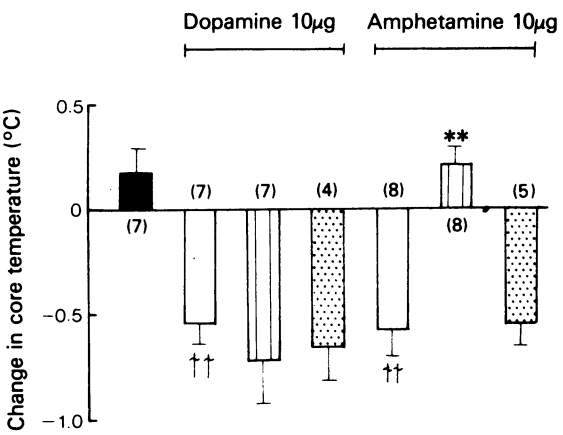

Figure 2 Change in core temperature after intrahypothalamic injection of either dopamine 10 μg or amphetamine 10 μg alone (open columns) or after systemic pretreatment with cocaine 20 mg/kg (striped columns) or tranylcypromine 10 mg/kg (dotted columns). The solid column represents the response to 1 μl intrahypothalamic saline injection. Each column represents the mean maximum fall in core temperature with vertical bars indicating s.e. mean. Figures in parentheses indicate the group size. Significantly different from saline control, †† $P < 0.01$; significantly different from appropriate agonist control, ** $P < 0.01$.

90 min previously) or tranylcypromine (10 mg/kg, 150 min previously) (Figure 2). Amphetamine-induced hypothermia was significantly antagonized by systemic pretreatment with cocaine ($P < 0.01$), but was unaffected by tranylcypromine pretreatment (Figure 2).

Effects of cocaine and tranylcypromine on the body temperature of rats

Following the intraperitoneal injection of cocaine (20 mg/kg) and tranylcypromine (10 mg/kg), the core temperature of the rat immediately began to fall and the peak effect occurred at 90 and 150 min after injection respectively (Figure 3a). The response to intraperitoneal injection of either cocaine or tranylcypromine was significantly inhibited by pimozide given either systemically (0.5 mg/kg (Figure 4) or centrally (0.5 μg bilateral intrahypothalamic injection) (Figure 5). Neither phentolamine (1 mg/kg i.p.; 25 μg intrahypothalamic) nor methysergide (5 mg/kg i.p.; 5 μg intrahypothalamic) significantly changed the responses to cocaine and tranylcypromine (Figures 4 & 5).

Unilateral intrahypothalamic injections of either cocaine (20 μg) or tranylcypromine (10 μg) caused a fall in core temperature with a peak which occurred at about 20 min after injection (Figure 3b). These responses were found to be significantly different from those seen in the controls ($P < 0.01$) and were significantly inhibited by unilateral intrahypothalamic pretreatment with pimozide (0.5 μg) ($P < 0.05$) (Figure 6). Intrahypothalamic pretreatment with either phentolamine (25 μg) or methysergide (5 μg) apparently reduced the hypothermic effects of cocaine and tranylcypromine, but the differences did not achieve significance (Figure 6).

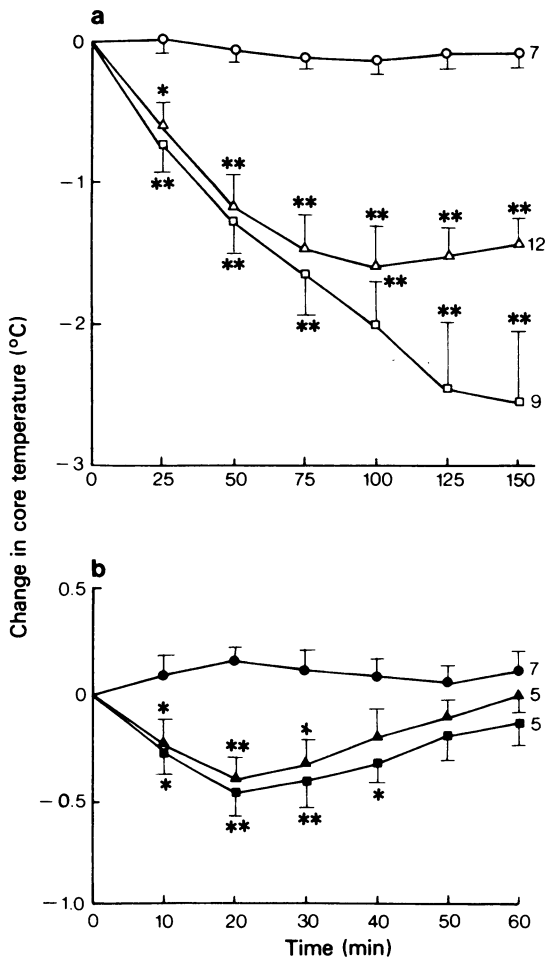

Figure 3 Time course of the core temperature response to intraperitoneal (a) or intrahypothalamic (b) injection of cocaine and tranylcypromine in rats. Each point represents the mean change in temperature from the number of rats shown beside each line after injection of saline (○, 0.1 ml/kg; ●, 1 μl), cocaine 20 mg/kg (△) or 20 μg (▲), tranylcypromine 10 mg/kg (□) or 10 μg (■). Vertical bars indicate s.e. mean. Significantly different from saline control, * $P < 0.05$, ** $P < 0.01$.

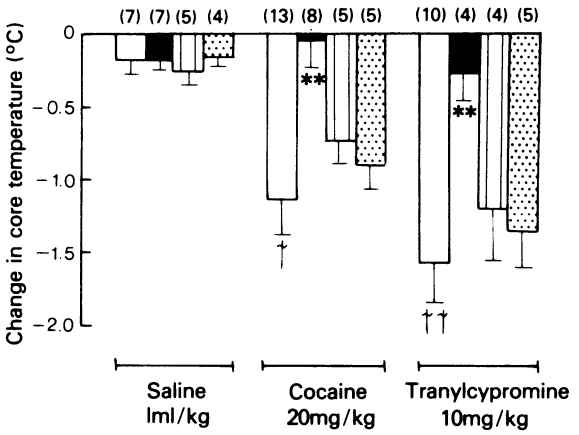

Figure 4 Change in core temperature of rats after intraperitoneal injection of saline 1 ml/kg, cocaine 20 mg/kg or tranylcypromine 10 mg/kg alone (open columns), or after intraperitoneal pretreatment with pimozide 0.5 mg/kg (solid columns), phentolamine 1 mg/kg (striped columns) or methysergide 5 mg/kg (dotted columns). Each column represents the mean maximum fall in core temperature with vertical bars indicating s.e. mean. Figures in parentheses indicate the group size. Significantly different from saline control. † $P < 0.05$; †† $P < 0.01$; significantly different from appropriate agonist control, ** $P < 0.01$.

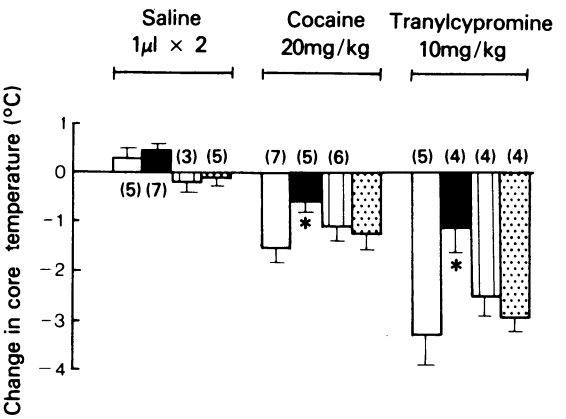

Figure 5 Change in core temperature after intraperitoneal injection of saline 1 ml/kg, cocaine 20 mg/kg or tranylcypromine 10 mg/kg (open columns) or after bilateral intrahypothalamic pretreatment with pimozide 0.5 μg per site (solid columns), phentolamine 25 μg per site (striped columns) or methysergide 5 μg per site (dotted columns). Each column represents the mean maximum fall in core temperature with vertical bars indicating s.e. mean. Figures in parentheses indicate the group size. Significantly different from appropriate agonist control, * $P < 0.05$.

Discussion

In this study, we have investigated the possibility that there is a role for endogenous dopamine in thermoregulatory processes in the rat. In order to achieve this objective, amphetamine, cocaine and tranylcypromine were used. These drugs are all reported to increase the concentration of dopamine at its receptors in the central nervous system, but in a number of different ways. Amphetamine is reported to cause the release of dopamine from dopaminergic nerve terminals (Besson, Cheramy, Feltz & Glowinski, 1971) and to inhibit its uptake (Taylor & Snyder, 1971). Cocaine is reported to inhibit uptake (Patrick & Barchas, 1977). Tranylcypromine is known to be a monoamine oxidase inhibitor (Koelle, 1975) and similarities between tranylcypromine and amphetamine have also been described (Glowinski, Axelrod & Iversen, 1966; Rutledge, 1970; Roffler-Tarlov, Sharman & Tegerdine, 1971). The doses of these drugs used in the present study were chosen on the basis of reports in the literature (Crow & Deakin, 1977; D'Mello & Stolerman, 1977).

Intrahypothalamic injection of amphetamine into the preoptic anterior hypothalamus caused a fall in core temperature which was completely abolished by systemic pretreatment with the dopamine antagonist pimozide, but only slightly reduced by pretreatment with either phentolamine or methysergide. These antagonists were used in doses shown previously to be effective against the hypothermic response to central injections of dopamine, noradrenaline and 5-hydroxytryptamine (5-HT) respectively (Lee, 1978; Cox & Lee, 1979). Thus amphetamine hypothermia appears to be mediated via central dopamine receptors and it seems unlikely that either α-adrenoceptors or 5-HT receptors are involved. Similar findings with respect to pimozide and phentolamine have been reported previously (Yehuda & Wurtman, 1972). The hypothermic effect of central amphetamine was abolished by systemic pretreatment with cocaine, but the same dose of cocaine slightly increased the response to central dopamine. These results are to be expected since cocaine would prevent amphetamine from reaching its intraneuronal releasing sites, and by the same mechanism reduce inactivation of dopamine by the active uptake process. Therefore it would seem likely that amphetamine was acting indirectly via release of dopamine and that the dopamine itself was acting directly. Although tranylcypromine did not increase the magnitude of the response to either amphetamine or dopamine, it did increase the duration of the responses. This suggests that oxidative deamination is less important than uptake in the inactivation of dopamine.

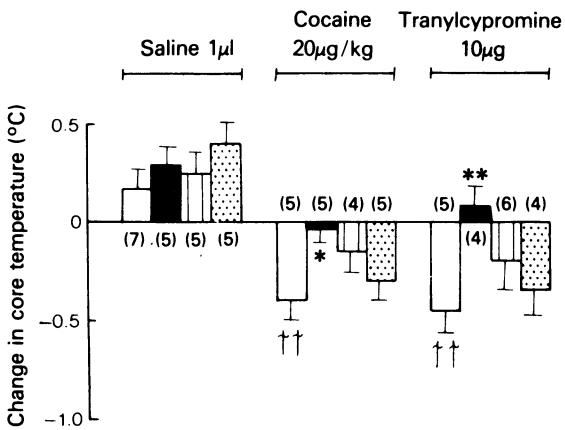

Figure 6 Change in core temperature after intrahypothalamic injection of either saline 1 μl, cocaine 20 μg or tranylcypromine 10 μg (open columns) or after intrahypothalamic pretreatment with pimozide 0.5 μg (solid columns), phentolamine 25 μg (striped columns) or methysergide 5 μg (dotted columns). Each column represents the mean maximum fall in core temperature with vertical bars indicating s.e. mean. Figures in parentheses indicate the group size. Significantly different from saline control, †† $P < 0.01$; significantly different from appropriate agonist control, * $P < 0.05$, ** $P < 0.01$.

Cocaine and tranylcypromine produced a significant fall in core temperature in rats when administered on their own by the systemic route. Similar findings have been reported previously (Feldberg & Lotti, 1967; Summers, 1974). Because of the fall in core temperature it was necessary to test the effects of amphetamine and dopamine when the core temperature had stabilized at a new lower level. However in spite of this change in the base line it was still possible to demonstrate a response to dopamine and, in the case of cocaine, to differentiate between dopamine and amphetamine. Since both cocaine and tranylcypromine would be expected to alter endogenous amine concentrations it seemed worthwhile determining whether there was an endogenous dopamine component in this response. The hypothermia following either cocaine or tranylcypromine, like that after amphetamine, was prevented by pimozide pretreatment but unaffected by either phentolamine or methysergide. Therefore once again, dopamine receptors are implicated. Summers (1974) found that the hypothermic response to tranylcypromine in the rat was blocked by haloperidol, which supports the idea that a dopamine receptor is involved. Although it is well documented that both cocaine and tranylcypromine can potentiate noradrenaline, 5-HT and dopamine (Brodie, Spector & Shore, 1959; Koelle, 1975), from the evidence above it seems unlikely that either adrenergic or tryptaminergic pathways are involved. Furthermore, the hypothermic response to systemic cocaine and tranylcypromine was significantly reduced by bilateral intrahypothalamic injection of pimozide, suggesting that their effects were being mediated through dopamine receptors within the hypothalamus. This hypothesis receives further support from the finding that cocaine and tranylcypromine lowered core temperature when they were injected directly into the hypothalamus.

Thus from the results of this study, it appears that there is an endogenous dopamine system within the preoptic anterior hypothalamus which mediates a fall in core temperature in the rat.

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