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# Differential effects of 5-hydroxytryptaminergic antagonists upon apomorphine- and lergotrile-induced hypothermia and stereotyped behaviour in rats

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The dopaminergic stimulants apomorphine and lergotrile both evoked hypothermia and stereotyped behaviour in rats. These drug effects were sensitive to antagonism by haloperidol, a dopaminergic receptor blocker. In rats pretreated with 5-hydroxytryptaminergic receptor blockers, cinanserin reduced apomorphine-induced hypothermia but cyproheptadine did not. Both cinanserin and cyproheptadine significantly potentiated lergotrile-induced hypothermia. Similarly, the stereotypic effects of apomorphine were partly reduced by cyproheptadine, although higher doses of cyproheptadine did potentiate lergotrile-induced stereotyped behaviour. These findings of different influences of 5-HT antagonists upon the effects of apomorphine and lergotrile indicate that these two dopaminergic stimulants may not work in identical manner to produce outwardly similar drug effects.

The naturally occurring ergot alkaloids of the fungus *Claviceps purpurea* have been of pharmacological interest for many years because of their ability to affect peripheral and central adrenergic and 5-hydroxytryptaminergic systems. One such derivative, lergotrile (2-chloro-6-methylergoline-8β-acetonitrile), has drawn much attention because of its therapeutic application in Parkinson's disease (Lieberman et al 1975; Teychenne et al 1978). It is now thought that lergotrile directly activates brain dopaminergic receptors (Corrodi et al 1973). Because of previous reports of a possible role for 5-hydroxytryptamine (5-HT) in mediation of dopaminergic drug effects (Grabowska et al 1973; Quock & Horita 1974; Cox & Lee 1979), the present study was conducted to compare the influence of 5-HT antagonists upon the hypothermia and stereotyped behaviour induced in rats by lergotrile and the standard dopaminergic stimulant apomorphine.

## MATERIALS AND METHODS

Male Wistar rats (Simonsen Laboratories, Gilroy, California), 150–250 g, were housed in community cages on a standard light-dark schedule (light, 0700–1400 h) with free access to food and water. Experiments were conducted at 22 ± 1 °C and always at the same time each day (dopaminergic drug challenges consistently administered at 1100 h).

In the body temperature experiments, animals were individually housed in cages (24 cm × 30 cm × 15 cm) and permitted to acclimatize for 60 min before the start of the experiment. Body temperatures were then monitored at 15-min intervals for 1 h before drug injection by insertion of a rectal thermistor probe connected to an electronic telethermometer (Yellow Springs Instruments, Yellow Springs, Ohio). The temperatures of apomorphine-challenged rats and their vehicle controls were measured every 15 min for 90 min after injection. Because of the longer duration of action of lergotrile, the temperatures of lergotrile-challenged animals were monitored every 30 min for up to 5 h.

In the stereotyped behaviour experiments, animals were acclimatized to individual cages for 60 min before drug injection. The intensity of drug-induced stereotyped behaviour was quantified using a rating scale (Table 1). The stereotyped behaviour score for each animal consisted of the cumulative number of points assigned to the animal by a trained observer every 5 min over a 90-min test period.

Drugs used included: apomorphine hydrochloride (Merck); lergotrile mesylate (Lilly), haloperidol ((Haldol ampoules) McNeil), cyproheptadine hydrochloride (Merck Sharp & Dohme), and cinanserin hydrochloride (Squibb). The haloperidol preparation was diluted to final concentration in double

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Table 1. Stereotyped behaviour rating scale

| Points | Description of behaviour |
|---|---|
| 0 | Experimental rats exhibit no stereotyped behaviour and are indistinguishable from control rats. |
| 1 | Experimental rats exhibit periodic sniffing and repetitive head and limb movements. |
| 2 | Experimental rats exhibit continuous sniffing and repetitive head and limb movements. Exploratory activity is present. |
| 3 | Experimental rats exhibit occasional licking, biting or gnawing. Exploratory activity is present. |
| 4 | Experimental rats exhibit persistent and intense licking, biting or gnawing. Locomotor activity is confined to a very limited area. |
| 5 | Experimental rats exhibit persistent and intense licking, biting or gnawing at one location without changing position. |

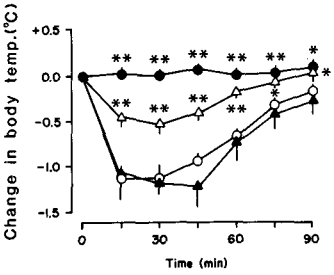


FIG. 1. Influence of various drug pretreatments upon apomorphine-induced hypothermia in rats: ○, apomorphine (2·0 mg kg$^{-1}$) (n = 17); ●, haloperidol (0·5 mg kg$^{-1}$) + apomorphine (n = 7); ▲, cyproheptadine (0·5 mg kg$^{-1}$) + apomorphine (n = 13); and △, cinanserin (5·0 mg kg$^{-1}$) + apomorphine (n = 12). Each point represents the mean change in body temperature and the vertical line its standard error of the mean. Time 0 temperatures of all groups were checked for homogeneity by Dunnett's *t*-test and were not found to be significantly different. Significance of difference: $*P < 0{\cdot}05$ and $**P < 0{\cdot}01$, compared to the apomorphine control (○).

distilled water. Other drugs were prepared in aqueous solution immediately before use. The doses represent the weights of the respective salts, except for haloperidol where doses are expressed in terms of the base. All drugs were administered intraperitoneally in volumes of 1·0 ml kg$^{-1}$. Haloperidol, cyproheptadine, cinanserin or vehicle (distilled water) was administered 30 min before time 0, at which time the apomorphine, lergotrile or distilled water challenge was made.

Differences among the various experimental and control groups of animals in the body temperature and stereotyped behaviour experiments were determined by analysis of variance and the multiple comparison test of Dunnett (1964).

## RESULTS

In preliminary experiments not shown, intraperitoneal administration of distilled water, haloperidol or cyproheptadine failed to significantly change the body temperature of test animals at the doses used in this study. Fig. 1 shows the findings of the apomorphine body temperature experiments. The standard challenge dose of 2·0 mg kg$^{-1}$ of apomorphine reduced body temperature by approximately 1·2 °C. This thermotropic effect was abolished by pretreatment with haloperidol, partly antagonized by cinanserin and not appreciably altered by cyproheptadine.

Fig. 2 shows the results of the lergotrile body temperature experiments. At a lower dose of 0·3 mg kg$^{-1}$, lergotrile induced a hypothermic effect of about 0·9 °C. This was abolished by haloperidol and potentiated by cyproheptadine. At the higher dose of 1·0 mg kg$^{-1}$, lergotrile reduced body temperatures by an average of 1·6 °C. The hypothermic effect was almost abolished by haloperidol, but significantly potentiated by cyproheptadine and cinanserin.

Table 2 shows the influence of various drug pretreatments upon apomorphine- and lergotrile-induced stereotyped behavioural responses in rats. Pretreatment with haloperidol abolished the stereotypic effects of both apomorphine and lergotrile. As the dose of cyproheptadine was increased, there appeared to be partial antagonism of apomorphine-induced stereotypy and potentiation of the lergotrile response.

## DISCUSSION

There is convincing evidence to link the activation of brain dopaminergic receptors with a hypothermic response (Cox 1979). The ability of haloperidol, a dopaminergic receptor blocker, to abolish apomorphine- and lergotrile-induced hypothermic effects in the present study reaffirms the dopaminergic nature of these thermotropic responses.

Our experiments also reveal differential influences of 5-HT receptor blockers upon apomorphine- and lergotrile-induced hypothermic responses. For 5-HT antagonists, we selected cinanserin (Rubin et al 1964) and cyproheptadine (Stone et al 1961) at doses previously reported to be effective in antagonizing the temperature effects of 5-HT and 5-hydroxytryptophan (Dooley & Quock 1976; Cox & Lee 1979). Cyproheptadine also possesses prominent antihistaminergic properties but generally resembles cinanserin in antagonism of 5-HT-induced temperature changes (Girault & Jacob 1979). In the present

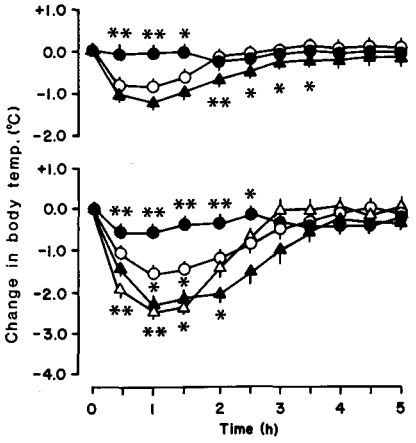


FIG. 2. Influence of various drug pretreatments upon lergotrile-induced hypothermia in rats. Upper graph: ○, lergotrile (0·3 mg kg$^{-1}$) (n = 9); ●, haloperidol (0·5 mg kg$^{-1}$) + lergotrile (n = 8); and ▲, cyproheptadine (0·5 mg kg$^{-1}$) + lergotrile (n = 9). Lower graph: ○, lergotrile (1·0 mg kg$^{-1}$) (n = 12); ●, haloperidol (0·5 mg kg$^{-1}$) + lergotrile (n = 11); ▲, cyproheptadine (0·5 mg kg$^{-1}$) + lergotrile (n = 9); and △, cinnanserin (5·0 mg kg$^{-1}$) + lergotrile (n = 10). Each point represents the mean change in body temperature and the vertical line its standard error of the mean. Time 0 temperatures of all groups were checked for homogeneity by Dunnett's *t*-test and were not found to be significantly different. Significance of difference: $*P < 0.05$ and $**P < 0.01$, compared with the lergotrile control (○).

study, cinanserin, but not cyproheptadine, reduced the magnitude of apomorphine-induced hypothermia yet both 5-HT antagonists significantly potentiated the hypothermic effect of lergotrile.

Over the years, there have been reports of a possible 5-HT involvement in dopaminergic receptor-mediated temperature effects (Grabowska et al 1973; Quock & Horita 1974; Cox & Lee 1979). Midbrain raphe lesions and pretreatment with lysergic acid diethylamide, but not methysergide or *p*-chlorophenylalanine (PCPA), have been reported to antagonize apomorphine-induced hypothermia in rats (Grabowska et al 1973: Grabowska 1974). Elsewhere the 5-HT receptor blockers cyproheptadine and methysergide were found to be effective antagonists of apomorphine- or dopamine-induced hypothermia in rats (Cox & Lee 1979). On the other hand, still another study reported that apomorphine-induced hypothermia was potentiated by methysergide, cinanserin, brom-lysergic acid diethylamide or PCPA (Menon & Vivonia 1981). A reasonable explanation for these discrepancies in experimental findings has not yet emerged.

That apomorphine and other dopaminergic stimulants induce stereotyped behaviour has long been known (Colpaert et al 1976; DiChiara & Gessa 1978). Our study shows that both apomorphine and lergotrile are able to evoke prominent stereotyped behaviour sensitive to reversal by haloperidol. We also demonstrate partial reversal of apomorphine-induced stereotypy as well as potentiation of lergotrile-induced stereotypy in rats treated with higher doses of cyproheptadine, thus suggesting opposing roles for 5-HT in the stereotypic responses to the two dopaminergic stimulants. The literature is filled with conflicting reports of the interaction between 5-HT antagonists and apomorphine-induced stereotyped behaviour. 5-HT receptor blockers have been reported to potentiate (Mogilnicka et al 1977), antagonize (Carter & Pycock 1978) or exert no influence (Rotrosen et al 1972; Baldessarini et al 1975) upon apomorphine-induced stereotyped behaviour in rats. Similar discrepancies have been reported on the influence of depletion of brain 5-HT by PCPA, neurotoxic tryptamine derivatives or midbrain raphe lesions upon stereotyped behaviour. Potentiation (Baldessarini & Griffith 1976; Mogilnicka et al 1977), antagonism (Costall & Naylor 1974; Grabowska 1974) and no effect (Ernst 1972; Rotrosen et al 1972; Baldessarini et al 1975; Hole et al 1976) have all been reported.

Our findings really do little to clarify the controversy about 5-HT participation in dopaminergic drug effects but they do clearly indicate that, whatever the nature of this involvement, it appears to differ for apomorphine- and lergotrile-induced drug effects. In other words, these two dopaminergic stimulants evoke thermotropic and stereotyped behavioural effects via stimulation of dopaminergic receptors in different pathways. That apomorphine

Table 2. Influence of various drug pretreatments upon apomorphine- and lergotrile-induced stereotyped behaviour in rats.

| Pretreatment | Apomorphine, 2·0 mg kg$^{-1}$ | Lergotrile, 1·0 mg kg$^{-1}$ |
|---|---|---|
| Vehicle control | 33·2 ± 3·1 (11) | 24·2 ± 2·7 (15) |
| Haloperidol, 0·5 mg kg$^{-1}$ | 0·9 ± 0·3 (9)** | 5·3 ± 1·1 (9)** |
| Cyproheptadine, 0·5 mg kg$^{-1}$ | 26·3 ± 4·7 (12) | ND |
| Cyproheptadine, 1·0 mg kg$^{-1}$ | 18·1 ± 4·9 (10)* | 29·9 ± 3·2 (10) |
| Cyproheptadine, 2·0 mg kg$^{-1}$ | ND | 35·6 ± 3·4 (9)* |

Figures represent the mean stereotyped behaviour scores ± standard errors of the mean for the number of rats indicated in parentheses. Significance of difference: $*P < 0.05$ and $**P < 0.01$, compared to the respective vehicle control groups. ND means that the experiment was not carried out.

and lergotrile might stimulate anatomically separate dopaminergic receptors has been suggested by studies of multiple dopaminergic receptors. One system for classifying dopaminergic receptors labels adenylate cyclase-linked entities as $D^{-1}$ receptors and adenylate cyclase-independent species as $D^{-2}$ receptors (Kebabian & Calne 1979). Apomorphine has been shown to be capable of stimulating adenylate cyclase enzyme activity while lergotrile is reportedly devoid of this property (Schmidt & Hill 1977; Kebabian & Calne 1979).

However, there is recent evidence to indicate that apomorphine-induced hypothermia in rats is unrelated to adenylate cyclase and may be mediated by $D^{-2}$ receptors (Baeyens & Moreno 1983; Colboc et al 1983). If this is indeed so, then neither apomorphine- nor lergotrile-induced hypothermic response is a $D^{-1}$ receptor-mediated drug effect, yet these qualitatively similar responses can still be pharmacologically differentiated by 5-HT antagonist drugs. One interpretaton of these findings is that 5-HT is a mediator between the dopaminergic receptor (which is not a $D^{-1}$ receptor) and the final hypothermic or stereotyped behavioural mechanism stimulated by apomorphine, while 5-HT is inhibitory upon the mechanism activated by lergotrile. Alternatively, the discrepancy between apomorphine and lergotrile might be due to an action of the ergot derivative upon 5-HT receptors (Silbergeld & Hruska 1979). For instance, the lergotrile-induced hypothermic response might represent a composite of a more dominant dopaminergic receptor-mediated hypothermia plus a weaker 5-HT receptor-mediated hyperthermia. Blockade of the 5-HT receptor with cyproheptadine or cinanserin might nullify the hyperthermic mechanism and unmask a more prominent hypothermic response to lergotrile.

These findings indicate the complexity of the neuropharmacology of central dopaminergic mechanisms. It also cautions us that, other than different subtypes of dopaminergic receptors, there may still be multiple brain mechanisms, stimulation of which can produce similar drug effects and that what might hold true for one dopaminergic stimulant might not be true for all dopaminergic stimulants.

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