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Hypothermic effects of apomorphine homologues in mice

M. K. MENON†, W. G. CLARK AND J. G. CANNON*

*Psychopharmacology Research Laboratory Veterans Administration Hospital, Sepulveda, California 91343, U.S.A. and *Division of Medicinal Chemistry and Natural Products, University of Iowa, College of Pharmacy, Iowa City, Iowa 52242, U.S.A.*

A single intraperitoneal (i.p.) injection in mice of apomorphine (I) and its analogues norapomorphine (II), *N*-ethylnorapomorphine (III), *N*-n-propylnorapomorphine (IV) and apocodeine (V), caused dose-related decreases in deep-core body temperature. The neuroleptic agent haloperidol blocked the hypothermia produced by these apomorphines but α-methyl-*p*-tyrosine failed to do so. This indicated a direct post-synaptic stimulation of dopamine receptors. Methysergide potentiated the hypothermic effect of the apomorphine analogues. Taking the amount of apomorphine to produce a 3 °C fall in temperature at 30 min as unity, the approximate relative potencies were: I 1·00, II 0·06, III 47·50, IV 85·00, V 0·340. The doses of the apomorphines needed to produce hypothermia were much less than those needed to cause stereotypy. The ratios of the minimal doses required to produce hypothermia, to those producing stereotypy were: I 8·82, II 4·00, III 125·00, IV 28·50, V 1·43.

Two of the major effects of apomorphine in rats and mice, are stereotypy (see review by Colpaert et al 1976 for references) and hypothermia (Lapin & Samsonova 1968; Barnett et al 1972; Fuxe & Sjöqvist 1972; Scheel-Krüger & Hasselager 1974; Schelkunov 1977). The stereotypy is known to be caused by a direct stimulation of the striatonigral dopamine areas by apomorphine (Ernst & Smelik 1966, 1967). As stereotypy-inducing properties of apomorphine are correlated with its antiparkinson effect, several studies were made on the structure-activity relationships of apomorphine and stereotypy (Koch et al 1968; Neumeyer et al 1973; Saari et al 1974; Atkinson et al 1975). Although apomorphine-induced hypothermia is associated with the dopamine system (see references above), the effect of apomorphine analogues on hypothermia has not been examined. This we have done.

MATERIALS AND METHODS

Male Swiss mice (23–28 G, Hilltop Laboratories, Scottdale, Pennsylvania) were used in experiments performed between 8 a.m. and 5 p.m. at a room temperature of 23 ± 1 °C. The animals were housed in groups until the time of the experiments.

The drugs used and their sources were: apomorphine HCl (M.S.D., Rahway, New Jersey), norapomorphine HCl, *N*-ethylnorapomorphine HCl, *N*-n-propylnorapomorphine HCl, apocodeine (the (−)-forms of these were used and were synthesized by one of us, J.G.C.), (±)-*N*-n-propylnorapomorphine HCl (gift from Dr J. L. Neumeyer), (±)-α-methyl-*p*-tyrosine methylester HCl (AMPT) (Astra Pharmaceuticals, Södertälje, Sweden), haloperidol (McNeil Laboratories, Fort Washington, Pa), and methysergide (Sandoz Pharmaceuticals, Hanover, N.J.).

The solutions of apomorphine and its analogues were freshly prepared in distilled water containing 0·1% ascorbic acid. AMPT and methysergide were dissolved in distilled water, and the haloperidol was dissolved in a minimal amount of glacial acetic acid and diluted with distilled water. Injections were given intraperitoneally in a volume of 0·01 ml g^{-1} weight.

Measurement of the deep-core body temperature. This was done on mice individually caged, by inserting thermistors rectally, 2 cm into the colon, and the temperature read on a digital thermometer (Technical Hardware, Inc., Fullerton, California). The animals were not restrained and the probes were inserted only at the time of taking a reading. After the normal temperature had been recorded, the animals were treated with drug(s) or distilled water and the temperature again recorded at 10, 30, 60, 90 and 120 min after the injections. For studying the effects of AMPT or haloperidol on the hypothermic effect of the apomorphines, the AMPT (280 mg kg^{-1} calculated as free base) was administered 3 h before, and the haloperidol (1 mg kg^{-1}) was given 10 min before the apomorphines or distilled water, the temperatures were noted, then the apomorphines or distilled water were given. Temperatures were then taken at the intervals mentioned. Similarly, the effect of methysergide pretreatment (3 mg kg^{-1} 10 min before) on the hypothermia caused by apomorphine and (−)-*N*-n-propylnorapomorphine was also studied.

Determination of the minimum stereotypy-producing dose. All the apomorphines produced stereotyped

† Correspondence.

biting responses in mice. Lower doses produced only stereotyped sniffing and blind studies were made to determine the doses of each of the apomorphine that produced weak sniffing behaviour in 90–100% of the animals. This was achieved by administering increasing doses of the drugs to groups of mice. Each animal was placed in an individual chamber (12 × 12 × 15 cm) with wire-meshed walls for 60 min at the end of which it was inactive and did not show any exploratory sniffing. After the drug injections, the animals behaviour was observed for 60 min. Those animals that showed intermittent sniffing behaviour, for not more than 10–15 s, were considered as showing a weak sniffing response. An experienced observer could easily differentiate this compulsive sniffing from exploratory behaviour.

RESULTS

Deep-core temperature. Apomorphine and its homologues as well as apocodeine produced hypothermia in mice, which was evident 10 min after injection, and this peaked at 30 min. Recovery occurred between 90 and 120 min. Since the maximum hypothermic effect occurred 30 min after treatment, the responses seen at this time were used for the dose-response curves. The minimum dose of apomorphine required to produce a significant decrease in temperature was 1 mg kg^{-1}. A dose-dependent fall in temperature was produced by apomorphine from 1–10 mg kg^{-1} (Fig. 1). Norapomorphine caused a dose-dependent fall from 10–100 mg kg^{-1} (Fig. 2). With *N*-n-ethylnorapomorphine, a dose-dependent temperature decrease was seen at 0·03–0·5 mg kg^{-1} (Fig. 2). A 0·06 mg kg^{-1} dose of (±)-*N*-n-propylnorapomorphine produced weak hypothermia, and a dose-related effect was seen up to a dose of 0·2 mg kg^{-1}, and the corresponding response produced by its (−)-isomer was significantly greater than the racemate mixture (Fig. 1). At 5 mg kg^{-1}, apocodeine caused a weak hypothermic effect, but a pronounced hypothermia was seen at higher doses (Fig. 2).

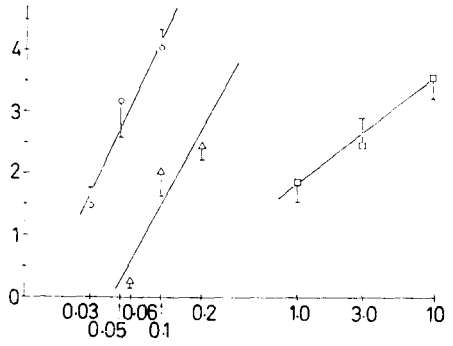

FIG. 1. Dose-response relationship of apomorphine (□——□), (—)-n-propylnorapomorphine (○——○) and (±)-n-propylnorapomorphine (△—△). The hypothermic response seen at 30 min (peak effect) was used for evaluation. Each point represents the mean ± s.e. of the difference between the drug-treated group and the water-treated controls. Ordinate: decrease in temperature (°C). Abscissa: dose (mg kg^{-1}).

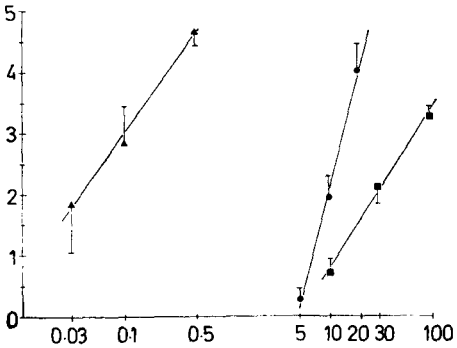

FIG. 2. Dose-response relationship of norapomorphine (■—■), ethylnorapomorphine (▲—▲) and apocodeine (●—●). For other details see legend for Fig. 1. Ordinate: decrease in temperature (°C). Abscissa: dose (mg kg^{-1}).

The doses of each of these apomorphines required to produce a 3 °C fall in temperature at 30 min was calculated from Figs 1 and 2 and are given in Table 1.

Interactions with AMPT and haloperidol. Pretreatment with AMPT failed to reduce the hypothermic effect of the apomorphines. However, haloperidol significantly reduced their effects (Table 2). In methysergide pretreated mice, both apomorphine and (−)-*N*-n-propylnorapomorphine caused significantly greater falls in temperature than in controls.

Stereotypy. The minimum doses of the apomorphines to produce stereotypy are given in Table 3 and are compared with doses of each required to produce 1 °C fall. All these drugs produced hypothermia in doses below those required to produce stereotypy.

Table 1. Doses required to produce a 3 °C fall in temperature at 30 min after drug administration[1].

Compound	Dose (mg kg^{-1})
Apomorphine	4·85
Norapomorphine	76·0
N-Ethylnorapomorphine	0·102
N-n-Propylnorapomorphine	0·057
(±)-*N*-n-Propylnorapomorphine	0·24
Apocodeine	14·4

[1] Calculated from Figs 1 and 2. Because of lack of parallelism between the dose-response curves, these values are estimates.

Table 2. Effects of various pretreatments on the hypothermic effects of apomorphine and its homologues

Treatment (dose mg kg^{-1})	Temperature (°C ± s.e.) at 30 min after pretreatment with			
	Water	AMPT	Haloperidol	Methysergide
Water	37·85 ± 0·21 (Control)	37·42 ± 0·08[1] (Control)	36·42 ± 0·03[1] (Control)	37·47 ± 0·14[1] (Control)
Apomorphine, 10	34·90 ± 0·33[2]	34·67 ± 0·36[2]	35·97 ± 0·40[3]	33·22 ± 0·34[2]
Norapomorphine, 30	35·74 ± 0·14[2]	35·63 ± 0·39[2]	36·11 ± 0·01[3]	
Ethylnorapomorphine, 0·6	34·17 ± 0·54[2]	32·95 ± 0·29[2]	36·00 ± 0·13[3]	
N-n-Propylnorapomorphine, 0·3	34·22 ± 0·32[2]	33·55 ± 0·26[2]	35·99 ± 0·10[3]	32·82 ± 0·33[2]
Apocodeine, 10	36·05 ± 0·38[2]	33·18 ± 0·47[2]	35·95 ± 0·26[3]	

Each value is the mean + s.e. of 6–12 mice. Probability was calculated using the Student's *t*-test.
1. Effects of AMPT, haloperidol and methysergide in control group are mean core temperatures at 3 h 30 min, 40 min and 40 min respectively. 2. $P < 0·001$ compared with respective controls. 3. N.S.

DISCUSSION

Body temperature changes induced by drugs could involve both central and peripheral components. However, the peripheral vasodilatation produced by DA receptor agonists is restricted to renal, superior mesenteric and coeliac vascular beds (for review, see Goldberg 1972). Moreover, intraventricular administration of apomorphine produces hypothermia in both mice and rats (for references, see Colpaert et al 1976). Thus it is evident that the apomorphine-induced hypothermia is of central in origin. Our unpublished findings showed that intraventricular injection of the apomorphine homologues produced hypothermia in mice, but, due to variation in the response of individual animals, a dose-response relationship could not be obtained. However, it was clear that both the *N*-propyl and *N*-ethyl derivatives were much more active, and norapomorphine much less active, than apomorphine. Since these results were comparable with our present findings in which the apomorphines were administered intraperitoneally, we concluded that the hypothermic effects of apomorphine and its homologues were the result of a central effect.

In the present study, haloperidol, but not AMPT, blocked the hypothermic effects of apomorphine and its homologues. Pimozide (0·5 mg kg^{-1}, 2 h before), a more specific DA receptor blocking agent than haloperidol, also blocked their hypothermic response (unpublished observation). Hence it was concluded that the hypothermic response of these compounds is the result of a direct post-synaptic effect of these drugs on the DA receptors and does not involve central noradrenaline and 5-HT receptors. This conclusion is in agreement with the report of Barnett et al (1972) Fuxe & Sjöqvist (1972) and Cox & Lee (1977), all of whom based their conclusions solely on the ability of neuroleptics to block the hypothermic response. The view that the hypothermic effect of apomorphine involves the indirect stimulation of 5-HT receptors (Grabowska et al 1973) is not supported by the present studies, since we found that methysergide failed to block the effects of either apomorphine or *N*-n-propylnorapomorphine. In fact, methysergide potentiated their responses, and recently we have found that such an effect was elicited by all 5-HT antagonists irrespective of their chemical structures (in preparation). Changes in the *N*-substituent caused marked alterations in the hypothermic response to apomorphine (Tables 1 and last column of Table 4). Absence of the *N*-methyl group weakens the response, as seen from the fact that norapomorphine is only 1/16 as active as apomorphine in its hypothermic effect. On the other hand, ethyl- and n-propyl substitution caused large increases in potency, the *N*-n-propyl derivative being 85 times more active than apomorphine. At all doses, the responses to the racemic *N*-n-propylnorapomorphine were much weaker than those to the (−)-isomer (Fig. 1). It is possible that the (+)-isomer either exerts an antagonistic effect at the receptor site, or that it has a hyperthermic effect, which partly neutralizes the hypothermic action of

Table 3. Minimum dose for stereotypy

Drug	Minimum dose of drug to produce stereotypy[1] (mg kg^{-1})	Dose required to produce 1 °C fall[2] (mg kg^{-1})	Ratio: Stereotypy dose / Hypothermia dose
Apomorphine	3	0·34	8·8
Norapomorphine	50	12·5	4·0
Ethylnorapomorphine	1	0·008	125·0
(−)-*N*-n-Propylnorapromorphine	0·6	0·021	28·6
Apocodeine	10	7	1·4

1. Dose required to produce weak stereotyped sniffing behaviour in most of the animals (N = 12) observed during the present studies. 2. Extrapolated from Figs 1 and 2.

Table 4. Comparison of the present data with the various reports on the relative potencies of apomorphine homologues and apocodeine with respect to apomorphine

Compound	Relative potencies using the following techniques: Postural asymmetries in 6-OH-DA-caudate-lesioned mice[1]	Minimum dose to produce behavioural changes in mice[2]	Minimum dose to produce behavioural changes in mice[3]	Produce locomotor stimulation in reserpinized mice[4]	Threshold to produce stereotypy in mice[5]	Hypothermia (Present studies)
Apomorphine	1	1	1	1	1	1
Norapomorphine	not studied	0·0032	0·04	0·07–0·2	0·06	0·06
N-Ethylnorapomorphine	10	1·38	3·2	2	3	47·5
N-n-Propylnorapomorphine	27	7·5	1·1	2	6	85·1
Apocodeine[6]	not studied	not studied	Inactive	not studied	0·3	0·34

1. Pearl, 1978. 2. Atkinson et al 1975 3. Koch et al 1968. 4. Menon et al 1976. 5. Calculated from Table 3. 6. Saari et al 1974, found apocodeine to be only 1/16 as active as apomorphine in producing postural asymmetries in caudate-lesioned mice.

the (−)-isomer. The requirement of a catechol group was illustrated by the fact that apocodeine was only 1/3 as active as apomorphine.

The dopaminergic potencies of the various apomorphines in mice reported by several investigators are compared in Table 4. Though the weaker effect of norapomorphine and apocodeine as well as the greater potency of the ethyl and n-propyl derivatives compared with apomorphine are evident in the behavioural effect, it may be seen that both the ethyl and n-propyl apomorphines show much greater effectiveness in producing the hypothermic effect than in producing behavioural effects. This has also been demonstrated in the data in Table 3 in which the minimum doses of each of these agents required to produce behavioural changes are compared with the doses required to produce minimum hypothermia. It is evident that all the drugs lowered deep-core temperature in doses well below the doses required to produce stereotyped behaviour, e.g. the ethyl derivative was 125 times more active in producing hypothermia than in producing stereotypy. The temperature-regulating centre is located in the preoptic anterior hypothalamus and the existence of dopamine receptors in this area has also been demonstrated (Cox & Lee 1977). Hence it is likely that the hypothermic effect of apomorphine and its derivatives is exerted in this area. Evidence for the existence of different types of dopamine receptors in the dopaminergic areas has been presented by several investigators (Cools & van Rossum 1976; Chiara et al 1976; Koc & Marley 1977; Titeler et al 1978). Whether the receptor sites mediating the hypothermic response of apomorphine belong to one of the already proposed classes of dopamine receptors has yet to be ascertained.

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