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A central site for the hypothermic effects of (+)-amphetamine sulphate and *p*-hydroxyamphetamine hydrobromide in mice

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Summary

1. In unanaesthetized mice, weighing 20–30 g, the effect of (+)-amphetamine sulphate and *p*-hydroxyamphetamine hydrobromide on rectal temperature was examined. The drugs were injected intraperitoneally or into the cerebral ventricles.

2. Amphetamine produced hypothermia when injected intraperitoneally in doses of 1–5 mg/kg and intraventricularly in doses of 0·5 to 25 μg. Injections of larger doses—10 mg/kg intraperitoneally and 400 μg intraventricularly—resulted in hyperthermia followed by hypothermia.

3. Hydroxyamphetamine produced hypothermia only when given by the intraventricular route ; the effect was obtained with 0·5 to 25 μg. An intraventricular injection of 200 μg resulted in hyperthermia followed by hypothermia. When injected intraperitoneally the sole effect on temperature was hyperthermia, and this response was obtained with 5 and 10 mg/kg.

4. Hydroxyamphetamine injected intraperitoneally or intraventricularly in doses which produced hyperthermia reduced the noradrenaline but not the dopamine content of the brain. When injected intraventricularly in smaller doses which produced hypothermia no reduction in the noradrenaline content of the brain was obtained.

5. The hypothermia is attributed to an action on the anterior hypothalamus, and the possibility is discussed that it is brought about indirectly by the release of noradrenaline. The hyperthermia on the other hand is probably a peripheral effect.

Introduction

In determining the effect of intraperitoneal (+)-amphetamine sulphate on body temperature in mice, pronounced hypothermia was observed with doses between 1 and 5 mg/kg, while doses between 10 and 125 mg/kg resulted in hyperthermia (Robinson & Milberg, 1970). Other investigators have obtained hyperthermia with amphetamine given orally (Greenblatt & Osterberg, 1961) and intraperitoneally (Askew, 1962) to mice or intraperitoneally to rats (Jori & Garrattini, 1965 ; Morpurgo & Theobald, 1965). According to Gessa, Clay & Brodie (1969), the hyperthermia produced in rats by intraperitoneal amphetamine as well as by hydroxy-

amphetamine, a sympathomimetic amine with little central activity on systemic administration, is thought to be a peripheral effect related to the release of noradrenaline from sympathetic nerve endings in adipose tissue and the subsequent stimulation of free fatty acid metabolism.

In the present study, a hypothermic effect of central origin is described for the two drugs. Since in mice noradrenaline injected into the cerebral ventricles lowers body temperature (Brittain, 1966 ; Brittain & Handley, 1967) the hypothermia produced by these drugs might be mediated through the release of brain noradrenaline. However, no evidence was obtained in favour of this idea when determining the noradrenaline as well as the dopamine content of the brain after injections of hydroxyamphetamine.

Methods

Male Swiss albino mice (Charles River CF-1 strain), 20–30 g, were housed individually during test at a constant room temperature of 22° C. (+)-Amphetamine sulphate or *p*-hydroxyamphetamine hydrobromide were injected either intraperitoneally or into the cerebral ventricles, and body temperature was recorded for a 4 h period. Rectal thermistors (423 thermistors, Yellow Springs Instrument Co., Yellow Springs, Ohio), inserted to a depth of 2 cm and taped to the tail 30 min prior to drug injection, provided a means of measuring body temperature without handling the mice (Robinson & Milberg, 1970). Injections into the cerebral ventricles were made with a microlitre syringe (705-NCH syringe, Hamilton Co., Whittier, California) and a 28 gauge needle. A sheath of polyethylene tubing affixed to the needle limited its entry to 0·32 cm, the site selected for injection was as described by Brittain (1966). Dosage volume was kept constant at 0·02 ml for these injections, and 0·1 ml/10 g body weight for the intraperitoneal injections. Drug solutions were prepared in sterile, pyrogen-free saline. Food and water were not available to the mice during the test period.

For determination of the effects of selected doses of hydroxyamphetamine on brain catecholamines, the brains were excised and frozen in liquid nitrogen 30 min after the injection into the cerebral ventricles and 60 min after the intraperitoneal injection. Four brains were pooled for each sample, and homogenized in 0·4 N perchloric acid to yield a 14 ml supernatant. The catecholamines were extracted from alumina slurry with 0·05 N perchloric acid. Noradrenaline was then determined by the method of Maickel, Cox, Saillant & Miller (1968) and dopamine by the assay described by Chang (1964).

Results

The effects of intraperitoneal injections of different doses of amphetamine on rectal temperature are shown by the records on the left side in Fig. 1. With 10 mg/kg, a hyperthermia of 2° C occurred during the first 30 min, followed by a fall to about 3° C below the pre-injection level during the next 90 min, and temperature had not returned to normal 4 h after the injection. The injection of smaller doses resulted only in hypothermia. With 5 mg/kg temperature had fallen 3° C by the end of the second hour, with 2 mg/kg it had fallen over 4° C and with 1 mg/kg about 3° C at the end of the first hour. Injections of 0·5 and 0·1 mg/kg did not affect temperature.

The effects on rectal temperature of injections of amphetamine into the cerebral ventricles are shown on the right hand side of Fig. 1. The effects were similar to those obtained with intraperitoneal injections. The initial fall of 1° C during the first 15 min following the injection of 400 μg of amphetamine was probably not an effect of the drug since it was obtained also with control injections of saline solution as shown in the bottom record of Fig. 1. It was followed during the next 45 min by a rise of 2·5° C above the pre-injection level, and then temperature fell steadily to 1° C below this level over the next 3 hours. From a comparison of the upper two temperature records of Fig. 1, it is evident that the time course of the development of hyperthermia was slower following an intraventricular than an intraperitoneal

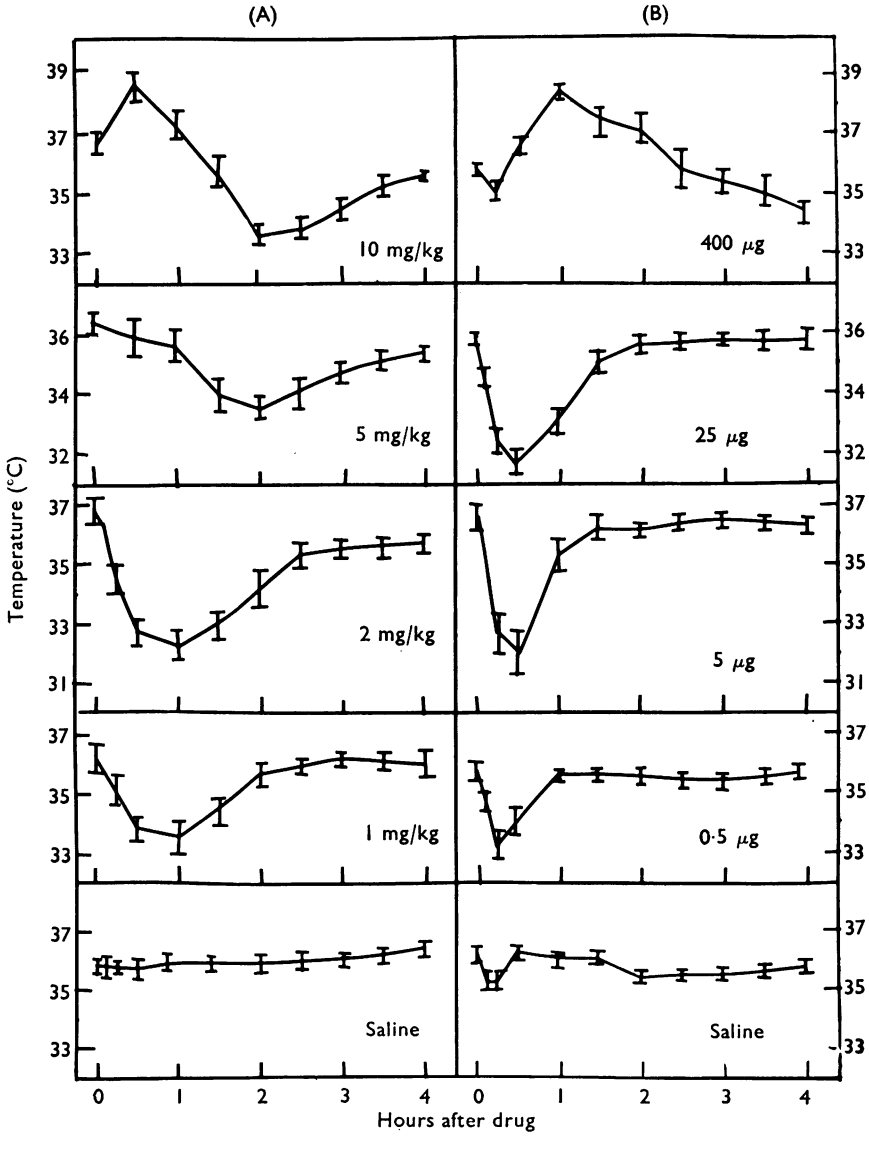

FIG. 1. Mean rectal temperatures of mice injected with intraperitoneal (A) and intracerebral (B) (+)-amphetamine sulphate. Each point represents the mean ± S.E.M. of eight mice.

injection. With 25 and 5 μg, temperature fell about 4° C during the first 30 min after the injection and then returned to normal within another 60 min ; with 0·5 μg, temperature fell about 2·5° C during the first 15 min and returned to normal during the next 45 min.

With intraperitoneal injections of hydroxyamphetamine (5 and 10 mg/kg), hyperthermia only was obtained as shown by the records on the left hand side of Fig. 2. In contrast, as shown by the records on the right hand side, the temperature effects produced by injections into the cerebral ventricles were similar to those obtained with amphetamine—a rise with 200 μg and a dose dependent fall with 25 to 0·5 μg. Again the time course of the development of hyperthermia was slower following the intraventricular injection than after the intraperitoneal injection.

With amphetamine signs of stimulation of the central nervous system were observed primarily with doses that caused a rise in temperature—that is, with 5 and

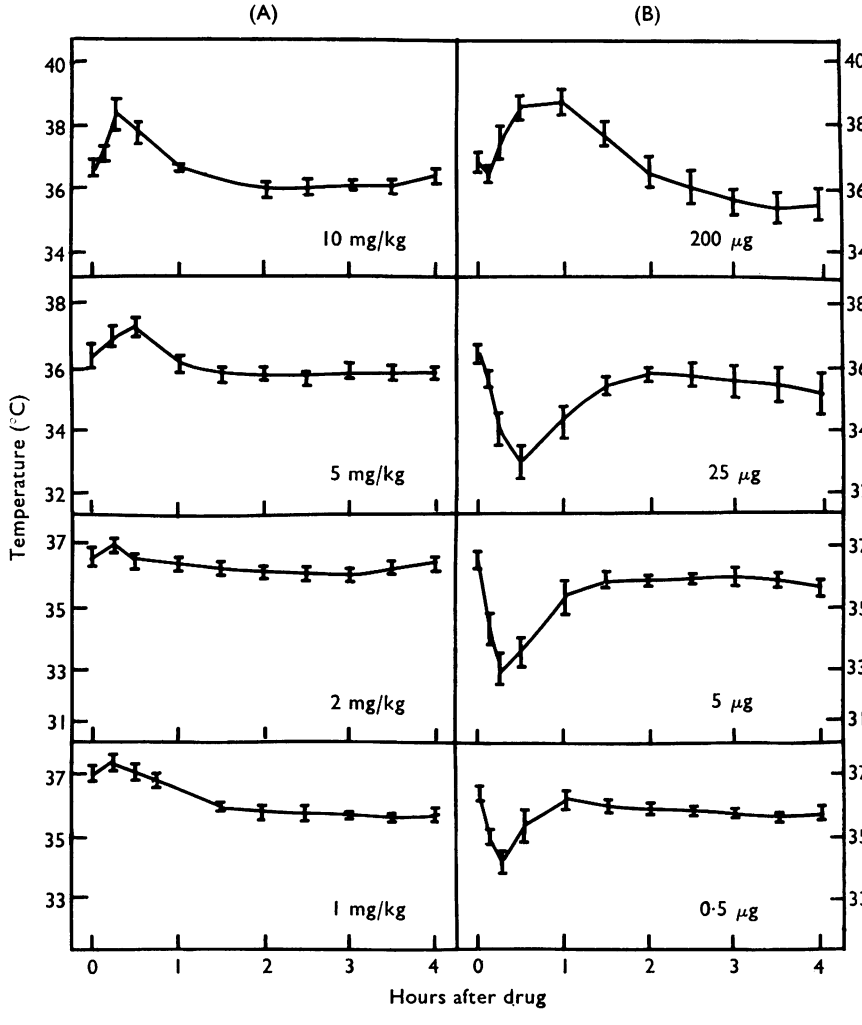

FIG. 2. Mean rectal temperatures of mice injected with intraperitoneal (A) and intracerebral (B) *p*-hydroxyamphetamine hydrobromide. Each point represents the mean±S.E.M. of eight mice.

10 mg/kg injected intraperitoneally and with 200 and 400 μg injected into the cerebral ventricles. The intraperitoneal injections produced increased locomotor activity and exploratory behaviour for about 30 min followed by a stereotype behaviour consisting of intense sniffing, licking and fixation. Within 20 s, the injections into the cerebral ventricles produced convulsions lasting 60 to 90 seconds. Thereafter the mice were hyperactive with intense circling movements, grooming activity and salivation persisting for about 30 min ; grooming activity continued during the next hour and there was fixation. The injections of smaller doses which caused hypothermia (1 and 2 mg/kg injected intraperitoneally, and 0·5 to 25 μg injected into the ventricles) produced no discernible alterations in behaviour.

With hydroxyamphetamine injected intraperitoneally, even in doses which caused hyperthermia (5 and 10 mg/kg) there were no behavioural signs of central stimulation ; on the contrary, the mice appeared to be sedated for about 30 min. The injection of 200 μg into the cerebral ventricles which also caused hyperthermia, produced initially also sedation, but only for about 15 min, followed by a period of increased locomotor activity with sniffing lasting for about an hour. The smaller doses of hydroxyamphetamine on intraperitoneal injection as well as on injection into the cerebral ventricles did not produce changes in behaviour.

Catecholamine content of brain after hydroxyamphetamine

The results are summarized in Table 1. The intraperitoneal injections of 10 and 50 mg/kg as well as the injections of 200 μg into the cerebral ventricles, reduced the noradrenaline content without affecting the content of dopamine. The injections of these doses would have produced a rise in temperature. The injections into the cerebral ventricles of 25 μg, which lowered temperature, affected neither the dopamine nor the noradrenaline content.

Discussion

Previous studies concerning the effect of amphetamine on body temperature in mice dealt mainly with hyperthermia, partly because the doses tested were too great to reveal the hypothermic effect obtained with smaller doses and partly because the method used for measuring temperature involved handling the animals. The latter, according to Brown & Julian (1968), may have accounted for 50% or more of the observed hyperthermia. In the present experiments, this source of error was excluded because temperature was measured with indwelling thermistors. The

TABLE 1. *Effect of* p-*hydroxyamphetamine hydrobromide on brain levels of noradrenaline and dopamine*

	μg/g in brain tissue ± S.E.M.	
Treatment	Noradrenaline	Dopamine
Control	0·53 ± 0·03	1·13 ± 0·13
Intraperitoneal		
10 mg/kg	0·31 ± 0·01	1·05 ± 0·07
50 mg/kg	0·33 ± 0·02	1·08 ± 0·05
Intracerebral		
25 μg	0·53 ± 0·01	1·03 ± 0·03
200 μg	0·26 ± 0·02	1·03 ± 0·04

n=four samples per treatment. Four brains were pooled for each sample.

observed rises with large doses of amphetamine and hydroxyamphetamine were therefore genuine effects of the drugs.

For the hyperthermia produced by large doses of amphetamine, the possibility cannot be excluded that it resulted from increased motor activity. The intraperitoneal injections produced increased locomotor activity and the injections into the cerebral ventricles resulted in convulsions followed by hyperactivity. Increased motor activity might also account for the hyperthermia produced by large doses of hydroxyamphetamine injected into the cerebral ventricles ; not, however, for the hyperthermia produced by large doses of this compound injected intraperitoneally because these injections were associated with sedation. It would therefore appear that the hyperthermias produced in conditions other than sedation were not at all, or only partly, the result of increased motor activity.

The hyperthermias are unlikely to be due to an action on thermoregulatory structures in the anterior hypothalamus, otherwise the drugs would have been effective in smaller doses on intraventricular than on intraperitoneal injection. The 200 and 400 μg which were effective on intraventricular injection were of the same order or even slightly higher than the 5 and 10 mg/kg injected intraperitoneally into mice weighing 20 to 30 g. This also suggests that on intraventricular injection the two drugs had first to be absorbed into the blood stream before they produced their hyperthermic effect. Apparently there is no great difference in the rate of absorption from the liquor spaces and from the peritoneal cavity. In the rabbit the rate of absorption of adrenaline was found to be about the same after intraventricular and intraperitoneal injection (Hasselblatt & Sproull, 1961). No such comparisons have been made in other species, but in cats it was found that histamine was absorbed less than half as rapidly after intraventricular than after subcutaneous injection (Bhawe, 1958). In rats, Glowinski, Kopin & Axelrod (1965) estimated that about 25% of [^{3}H]-noradrenaline injected into the cerebral ventricles entered the circulation unchanged, and according to Cowell & Davey (1968) absorption of noradrenaline from the liquor spaces into the circulation accounts for the ability of nortriptyline to reverse the hypothermia produced in mice by an intraventricular injection of noradrenaline. On the assumption that the hyperthermias obtained with intraventricular injections of amphetamine and hydroxyamphetamine occurred only after these drugs have been absorbed into the blood stream, it would follow that their absorption from the liquor spaces was somewhat slower than from the peritoneal cavity, because for equivalent rises in temperature the time course of the development of the hyperthermia was slower on intraventricular than on intraperitoneal injection.

Gessa *et al.* (1969) have related the hyperthermia induced by intraperitoneal injection of amphetamine and hydroxyamphetamine to peripheral release of noradrenaline in adipose tissue. If this interpretation is correct, the same peripheral mechanism of action would apply for the hyperthermia produced by these drugs when given by the intraventricular route.

The hypothermic response, on the other hand, can readily be attributed to an action on the anterior hypothalamus, because with amphetamine it was obtained with much smaller doses on intraventricular than on intraperitoneal injection, and with hydroxyamphetamine hypothermia was obtained only when the intraventricular route was used. It is not clear why hypothermia did not occur when the hydroxyamphetamine was injected intraperitoneally in doses smaller than those required to

produce hyperthermia. One possibility would be that this compound does not readily pass the blood-brain barrier ; however, on intraperitoneal injection it produced sedation, a central effect.

The hypothermia may be a direct effect of amphetamine and hydroxyamphetamine on the anterior hypothalamus or it may be brought about indirectly by the release of noradrenaline in the brain. An indirect mechanism of action has been postulated for some central effects of amphetamine, particularly for the stereotype behaviour which has been related to an increased release and turnover of dopamine (Ernst, 1967 ; Scheel-Krüger & Randrup, 1967 ; Jonas & Scheel-Krüger, 1969). This idea was supported by the finding that intraperitoneal amphetamine reduces both the noradrenaline and dopamine stores (Lal & Chessick, 1964 ; Smith, 1965) whereas hydroxyamphetamine, which has scarcely any stimulating effects, primarily affects the noradrenaline store (Lewander, 1968). In the present experiments, too, it was found that the hydroxy compound which produced sedation reduced only the noradrenaline and not the dopamine content of the brain.

Since both the amphetamine and the hydroxyamphetamine reduce the noradrenaline content of the brain, and both drugs lower body temperature in mice when injected into the cerebral ventricles, it is suggested that the hypothermia produced by the two compounds is brought about indirectly by the release of this monoamine in the brain. However, in the present experiments a reduction in the noradrenaline content of the brain was obtained only with large doses of hydroxyamphetamine which produced hyperthermia, not with the smaller ones which caused hypothermia. It may be that the small doses affected primarily the noradrenaline stores in the anterior hypothalamus which would not have been revealed by determining the noradrenaline content of the whole brain. The possibility can therefore not be excluded that the hypothermia produced by amphetamine and hydroxyamphetamine is brought about indirectly by noradrenaline release.

In conducting the research described in this report, the investigators adhered to the *Guide for Laboratory Animal Facilities and Care,* as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences–National Research Council.

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