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Turnover rate of brain 5-hydroxytryptamine increased by D-amphetamine

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Summary

1. Administration of two doses of amphetamine HCl (5 mg/kg intraperitoneally) 45 min apart raised body temperature of rats by an average of 3·4° C and increased the turnover rate of brain 5-hydroxytryptamine (5-HT) by almost one-half.

2. Both effects were blocked by exposure to 4° C or by pretreatment with the β-blocker Kö 592 (1-(2-methylphenoxy)-3-isopropylamino-2-propanol), but not by the administration of the ganglionic blocker chlorisondamine combined with atropine.

3. Since it has previously been shown that hyperthermia *per se* increases the turnover rate of brain 5-HT, and that amphetamine does not directly affect the uptake and release of 5-HT in brain slices, it is concluded that the amphetamine-induced increase in 5-HT turnover may be secondary to the rise in temperature produced by the drug.

Introduction

Amphetamine releases biogenic amines from noradrenergic (McLean & McCartney, 1961 ; Carlsson, Lindquist, Dahlstrom, Fuxe & Masuoka, 1965 ; Carr & Moore, 1969) and dopaminergic (Besson, Cheramy, Feltz & Glowinski, 1969) neurones in brain, sympathetic nerves (Burn & Rand, 1958) and blood platelets (Buckingham & Maynert, 1964), and it is generally agreed that the drug produces many of its pharmacological effects by releasing catecholamines (Weissman, Koe & Tenen, 1966 ; Hanson, 1966 ; Dingell, Owens, Norrich & Sulser, 1967). In contrast, amphetamine administration does not consistently alter brain levels of 5-hydroxytryptamine (5-HT) (Paasonen & Vogt, 1956 ; McLean & McCartney, 1961 ; Pletscher, Bartholini, Bruderer, Burkard & Gey, 1964 ; Laverty & Sharman, 1965) even though para-chloro- analogues of amphetamine are potent 5-HT depletors (Pletscher *et al.*, 1964 ; Pletscher, DaPrada, Burkard & Tranzer, 1968). The failure of amphetamine to deplete brain 5-HT has discouraged speculation that this drug affects serotonergic neurones. However, tissue levels of 5-HT and other biogenic amines may remain virtually unchanged despite marked changes in their turnover rate (Reid, Volicer, Smookler, Beaven & Brodie, 1968 ; Volicer & Reid, 1969), and it has been suggested that the level of deaminated metabolites (5-hydroxyindoleacetic acid in the case of 5-HT) often provides a more sensitive screening index of neuronal activity or of drug action than does the concentration of amine (Reid *et al.*, 1968). The results of the present investigation disclose that amphetamine administration significantly

accelerates the turnover rate of brain 5-HT, and that this effect may be related to the increase in body temperature produced by the drug.

Methods

Male Sprague-Dawley rats weighing approximately 180 g were caged in groups of three at 21° C. Before administration of the drug and before killing, body temperature was measured with a telethermometer attached to a small animal probe inserted 6 cm into the rectum. The animals were killed by cervical fracture. The brain was removed, frozen on dry ice and later assayed for 5-hydroxyindoleacetic acid (5-HIAA) by the method of Udenfriend, Weissbach & Brodie (1958) and for 5-HT according to the procedure of Bogdanski, Pletscher, Brodie & Udenfriend (1956). The turnover rate (synthesis rate) of brain 5-HT was calculated from the product of the steady-state level of 5-HIAA and of the rate constant of 5-HIAA efflux from brain (Tozer, Neff & Brodie, 1966). The rate constant was determined from the slope of the exponential decline of brain 5-HIAA after inhibition of monoamine oxidase by administration of pargyline hydrochloride (50 mg/kg intraperitoneally) 30 min after the second of two doses of D-amphetamine HCl (5 mg/kg intraperitoneally) 45 min apart. The values for 5-HIAA following pargyline administration were logarithmically transformed for linearity of regression (Snedecor, 1956). In both control and amphetamine treated animals pargyline administration completely inhibited brain monoamine oxidase activity for the duration of the experiment, as determined by the method of McCaman (McCaman, Hunt & Smith, 1965). In animals not treated with pargyline the steady-state 5-HIAA level remained constant for the duration of the experiment.

Drug dosages were calculated as the salts and are given under **Results**. Brain levels of 5-HIAA were compared in drug treated and control animals by Student's *t* test (Snedecor, 1956).

Results

A single dose of D-amphetamine hydrochloride (5 mg/kg intraperitoneally) produced variable increments in body temperature and in the level of brain 5-HIAA. However, repeating this dose after 45 min and killing the animals 45 min later consistently raised body temperature by an average of 3·4° C and increased brain 5-HIAA by about 50% without altering the level of brain 5-HT (Table 1). Figure 1 illustrates the responses of 101 consecutive animals treated with the above regimen and reveals that the higher values of 5-HIAA tended to be observed in the most hyperthermic animals. However the correlation between the severity of hyperthermia and the extent of increase in 5-HIAA was not statistically significant. As

TABLE 1. *Effect of amphetamine on body temperature and on levels of 5-HIAA and 5-HT in brain*

	Change in body temperature °C±S.E.	(*n*)	5-HIAA level μg/g±S.E.	(*n*)	5-HT level μg/g±S.E.	(*n*)
Control	0	(111)	0·48±0·007	(111)	0·55±0·04	(15)
Amphetamine	+3·4±0·1	(126)	0·77±0·02	(126)	0·54±0·05	(15)
Significance	$P<0·001$		$P<0·001$		NS	

shown in Fig. 2, treatment with amphetamine did not alter the rate constant of decline in brain 5-HIAA following inhibition of monoamine oxidase with pargyline. The data in Table 2 (typical of three experiments) indicate that in amphetamine treated rats the turnover rate of brain 5-HT was increased in direct proportion to the rise in brain 5-HIAA concentration.

We attempted to dissociate the effects of amphetamine on body temperature and on brain 5-HIAA either by keeping the animals in a 4° C environment from 2 h before drug administration until they were killed 45 min after the second dose or by pretreating them with the β-adrenoceptor blocking agent Kö 592 (1-(2-methylphenoxy)-3-isopropylamino-2-propanol, 50 mg/kg intraperitoneally) 15 min before each dose of amphetamine. Cold exposure raised the 5-HIAA level slightly, but administration of amphetamine produced no further increment in the acid level (Table 3). Similarly, administration of Kö 592 raised brain 5-HIAA by about 20% but prevented any further increase in the acid after treatment with amphetamine (Table 4). Cold exposure and blockade of β-receptors failed to dissociate amphetamine-induced hyperthermia from the change in brain 5-HIAA, since both treatments prevented the characteristic rise in body temperature. In fact, at 4° C amphetamine administration elicited a marked hypothermia (Table 3).

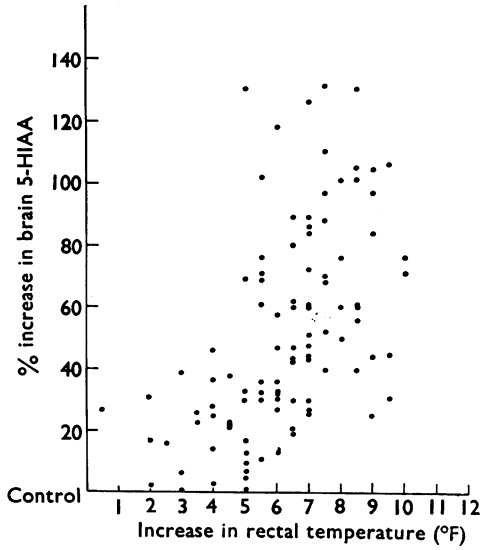

FIG. 1. Effect of D-amphetamine HCl (2 doses of 5 mg/kg intraperitoneally) on body temperature and brain 5-HIAA concentration. Rats were killed 45 min after the second dose. Rectal temperature was measured in each animal before amphetamine administration and just before killing.

TABLE 2. *Effect of amphetamine on turnover rate of 5-HT in brain*

Treatment	(*n*)	Steady-state level of 5-HIAA nmol/g±S.E.	Rate constant (*k*) of 5-HIAA efflux min^{-1}±S.E.	Turnover rate of 5-HT (nmol/g)/min	Change in turnover rate
Control	(15)	2·14±0·26	0·0066±0·001	0·014	—
Amphetamine	(17)	3·19±0·16	0·0062±0·001	0·020	+43%

To determine whether a peripheral action of amphetamine may be involved in raising body temperature, the drug was administered to rats 1 h after ganglionic blockade with chlorisondamine hydrochloride (10 mg/kg intraperitoneally) or after " complete autonomic blockade " (Trendelenburg, 1967 ; Steinberg & Hilton, 1967 ; Halmagyi, Neering, Varga & Pullin, 1969) by giving chlorisondamine (10 mg/kg intraperitoneally) and atropine sulphate (5 mg/kg intraperitoneally) 1 h and 0·5 h

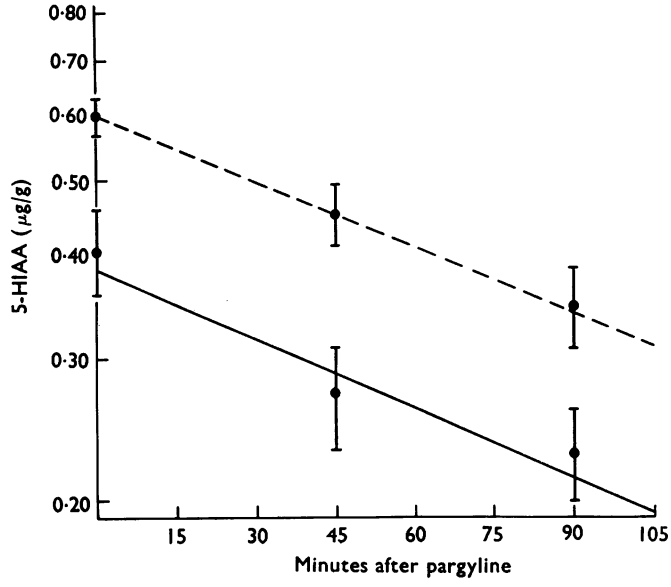

FIG. 2. Decline of brain 5-HIAA level after administration of pargyline HCl (50 mg/kg intraperitoneally). Each point represents a mean value of five or six animals and bars indicate standard error of the mean. Curves were drawn by the method of least squares. Solid line represents controls. Broken line represents animals given pargyline 30 min after the second of 2 doses of D-amphetamine HCl (5 mg/kg intraperitoneally) 45 min apart.

TABLE 3. *Effect of 2 h cold exposure (4° C) on the amphetamine induced rise in body temperature and in brain 5-HIAA level*

Environmental temperature °C	Control animals: Body temperature °C±S.E.	Control animals: 5-HIAA level μg/g±S.E.	(*n*)	Amphetamine treated: Change in body temperature °C±S.E.	Amphetamine treated: 5-HIAA level μg/g±S.E.	(*n*)	Significance* *P*
22°	36·8±0·07	0·40±0·01	(26)	(+) 3·0±0·29	0·65±0·04	(16)	<0·001
4°	36·6±0·15	0·50±0·03†	(11)	(−) 2·4±0·14	0·49±0·02‡	(22)	NS

* Comparison of amphetamine treated animals with controls at same environmental temperature.
† $P<0·01$ when compared with controls at 22° C.
‡ $P<0·001$ when compared with controls at 22° C.

TABLE 4. *Effect of the β-blocker Kö 592 on the amphetamine-induced rise in body temperature and in brain 5-HIAA leve*

Treatment	(*n*)	Change in body temperature °C±S.E.	5-HIAA level μg/g±S.E.	Significance* *P*
Control	(12)	0	0·54±0·02	—
Amphetamine	(12)	(+) 3·2±0·3	1·06±0·06	<0·001
Kö 592	(11)	(−) 1·4±0·3	0·66±0·03	<0·01
Kö 592+ amphetamine	(12)	(−) 0·1±0·2	0·64±0·03	<0·05

* Compared with control.

previously, respectively. Table 5 shows that chlorisondamine alone did not alter the effects of amphetamine on 5-HIAA levels and body temperature. "Complete autonomic blockade" by itself lowered body temperature an average of 1·4° C and minimally raised brain 5-HIAA levels, but only slightly reduced the effects of amphetamine on these parameters (Table 5). The data suggest that the hyperthermia is produced by an action of amphetamine distal to autonomic ganglia.

In contrast to amphetamine, whose effect on 5-HIAA was associated with an elevation of body temperature, various drugs which interact with central cholinergic neurones were found to produce relatively small but consistent rises in brain 5-HIAA without increasing body temperature (Table 6). In these experiments, Sprague-Dawley rats were obtained from Hormone Assay, Inc. (Chicago) and had slightly higher normal values of brain 5-HIAA than the N.I.H. strain used in the other studies. Within 45 min after administration of oxotremorine oxalate (1 mg/kg intraperitoneally) a variable drop in body temperature occurred and brain 5-HIAA rose by about one-third. Similar results were obtained 30 min after injection of physostigmine sulphate (0·2 mg/kg intraperitoneally).

Discussion

The present data clearly show that amphetamine has an effect on serotonergic neurones in brain. Since administration of the drug raises the level of 5-HIAA without significantly altering the rate constant of the removal of the acid from brain,

TABLE 5. *Effect of ganglionic blockade on the amphetamine induced rise in body temperature and in brain 5-HIAA level*

Treatment	(*n*)	Change in body temperature °C±S.E.	5-HIAA level μg/g±S.E.	Significance *P*
Control	(6)	0	0·44±0·03	—
Amphetamine	(6)	+2·7±0·4	0·92±0·04	<0·001
Chlorisondamine	(6)	−0·9±0·2	0·54±0·02	<0·05<0·1
Chlorisondamine + amphetamine	(6)	+2·6±0·2	0·85±0·04*	<0·001
Control	(31)	0	0·48±0·01	—
Amphetamine	(29)	+3·7±0·2	0·81±0·04	<0·001
Chlorisondamine + atropine	(28)	−1·4±0·1	0·53±0·01	<0·001
Chlorisondamine + atropine + amphetamine	(35)	+2·1±0·3	0·70±0·01†	<0·001

* $P>0·05$ compared with animals given amphetamine alone.
† $P<0·05$ compared with animals given amphetamine alone.

TABLE 6. *Effect of cholinergic drugs on 5-HIAA level in brain*

Drug	Change in body temperature °C	5-HIAA concentration: Control μg/g±S.E.	(*n*)	5-HIAA concentration: Drug treated μg/g±S.E.	(*n*)	Change in 5-HIAA %	Significance *P* value
Oxotremorine (1 mg/kg, intraperitoneally)	−0·64	0·59±0·05	(10)	0·77±0·06	(10)	+30	<0·05
Physostigmine (0·2 mg/kg, intraperitoneally)	−0·28	0·67±0·03	(18)	0·93±0·05	(18)	+39	<0·001

it may be concluded that the turnover rate of 5-HT is increased (Tozer *et al.*, 1966). Furthermore, the increased concentration of 5-HIAA following amphetamine administration makes it unlikely that the drug significantly inhibited monoamine oxidase in serotonergic neurones in brain, in contrast to the results obtained *in vitro* by Glowinski, Axelrod & Iversen (1966). Our data are also at variance with those of Laverty & Sharman (1965), who observed no change in brain 5-HIAA in cats and dogs 4 h after a subcutaneous injection of amphetamine, and with the negative results of Pletscher *et al.* (1964) in rats. It is likely that this discrepancy results from differences in species, dosage, route of administration and time of killing the animals, and from the possibility that Laverty and Sharman's animals were not hyperthermic when killed.

The mechanism whereby amphetamine increases the turnover rate of brain 5-HT is not entirely evident. Since the drug readily crosses the blood-brain barrier and exerts many biochemical and behavioural effects on the brain, it might seem probable that amphetamine releases the neurotransmitter by a direct action on serotonergic neurones, and this possibility has not been excluded by the present study. However, the data of Pletscher & Bartholini (1967) showing that amphetamine neither blocks the uptake of ^{14}C-5-HT nor releases the labelled amine in brain slices cast some doubt on this interpretation.

Previous work (Reid, Volicer, Beaven & Brodie, 1966 ; Reid *et al.*, 1968 ; Corrodi, Fuxe & Hokfelt, 1967) has demonstrated that the hyperthermia of animals exposed to a hot environment is associated with an increased turnover rate of brain 5-HT. The present results are compatible with the possibility that the increased turnover of serotonin may be secondary to the hyperthermia produced by the drug. In accord with this view are the data in Tables 3 and 4 showing that when the rise in temperature is prevented by pretreatment with Kö 592 (Gessa, Clay & Brodie, 1969) or by exposure to a cold environment (Dolfine, Garattini & Valzelli, 1969) the amphetamine induced increase in brain 5-HIAA is also blocked. However, it is possible that Kö 592 and cold exposure block the amphetamine induced increase in 5-HIAA by some mechanism other than by preventing the rise in body temperature.

Whether amphetamine produces hyperthermia by a peripheral or central action is still unsettled, although there is evidence that the rise in body temperature is related to the central stimulant effects of the drug and depends on the release of catecholamines (Morpurgo & Theobald, 1967). That uncertainty exists is not surprising, since even the calorigenic action of catecholamines is poorly understood (see Himms-Hagen, 1967). Gessa *et al.* (1969) conclude that hyperthermia is primarily a peripheral metabolic effect of amphetamine because the rise in temperature is blocked by pretreatment with Kö 592, nicotinic acid, or prostaglandin E_1 but not by ganglionic blockade. Furthermore, pretreatment with *p*-hydroxyamphetamine which lacks significant central action, depletes peripheral stores of norepinephrine, raises body temperature and 6 h later, when noradrenaline stores are still depleted, blocks the hyperthermic action of amphetamine (Gessa *et al.*, 1969). It is also possible that the action of amphetamine on peripheral vasculature may play a role in raising body temperature. Our results (Table 5) showing that " complete autonomic blockade " does not prevent hyperthermia support the conclusion of Gessa *et al.* (1969) that the rise in temperature is primarily a peripheral effect of noradrenaline released by amphetamine from sympathetic nerve endings.

Investigators who hold the opposite view, namely that hyperthermia is a central action of amphetamine (Valzelli, Dolfine, Tanzella & Garattini, 1968), frequently cite the work of Belenky & Vitolina (1962) showing that cats decerebrated at the level between the inferior and superior quadrigeminal bodies maintain a virtually normal rectal temperature for 4–6 h after administration of amphetamine. It is unfortunate that these experiments, which were performed at 17° to 20° C, did not include untreated decerebrated animals as controls, because the constant temperature observed may have been the result of a thermogenic action of amphetamine preventing the marked decline in temperature which would be expected in this essentially poikilothermic preparation (Martin, 1930). If indeed there is a central component to amphetamine induced hyperthermia, it may well be a direct calorigenic effect on brain tissue rather than a selective stimulation of the heat production centre in the posterior hypothalamus, since most of the effects of the latter would be prevented by autonomic blockade (Sawyer & Schlossberg, 1933 ; Hsieh, Carlson & Gray, 1957 ; Benzinger, Pratt & Kitzinger, 1961). Pick & Feitelberg (1948) have demonstrated that amphetamine raises the temperature of the brain relative to that in the internal carotid artery, a drug effect which can be selectively blocked by depressing cerebral metabolism with dihydro-β-erythroidine (Pick & Richards, 1947). The rise in brain temperature could be important in stimulating 5-HT synthesis, since in animals exposed to a warm environment the 5-HT turnover rate increases only if the core temperature (and presumably brain temperature) is elevated (Reid *et al.*, 1968). It is likely that amphetamine induced hyperthermia also stimulates the turnover rate of brain catecholamines (Reid *et al.*, 1968) but this action would be difficult to evaluate, since the drug depletes cerebral catecholamine stores (McLean & McCartney, 1961 ; Carlsson *et al.*, 1965).

The rise in 5-HIAA following administration of oxotremorine or physostigmine (Table 6) makes it evident that hyperthermia is not the only mechanism by which drugs may release 5-HT in the brain, since these drugs do not raise body temperature. A possible explanation for the increase in 5-HIAA is that these cholinergic drugs raise the level of free acetylcholine which in turn depolarizes serotonergic nerve terminals by an action analogous to the release of catecholamines in adrenal medulla (Douglas & Poisner, 1962) and of noradrenaline from sympathetic neurones in heart (Richardson & Woods, 1959 ; Haeusler, Thoenen, Haefely & Hurlimann, 1968) and spleen (Kopin, 1966). The data in Table 6 are in accord with this speculation, since the drugs which raised 5-HIAA might be expected also to increase the concentration of acetylcholine in brain. For example, physostigmine raises the concentration of acetylcholine by preventing its degradation by cholinesterase, and oxotremorine produces a marked increase in the brain acetylcholine level by an unknown mechanism (Pepeu, 1963 ; Holmstedt, Lundgren & Sundwall, 1963). These studies provide an alternative explanation for the amphetamine induced increase in 5-HT turnover, since Beani, Bianchi, Santinoceto & Marcheti (1968) have shown that amphetamine can release acetylcholine in brain. It is possible that the acetylcholine released could depolarize serotonergic neurones and thereby accelerate the turnover rate of serotonin. It is interesting in this regard that amphetamine and oxotremorine also increase the concentration of the dopamine metabolite, homovanyllic acid, in the corpus striatum (Laverty & Sharman, 1965).

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