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Differential effects of amphetamine, fenfluramine and norfenfluramine stereoisomers on the increase of striatum homovanillic acid in rats

A. JORI, E. DOLFINI, G. TOGNONI AND S. GARATTINI

Istituto di Ricerche Farmacologiche "Mario Negri" Via Eritrea 62, 20157, Milan, Italy

(+)-Amphetamine is more effective in increasing the concentration of striatum homovanillic acid (HVA) in rats than the (−)-isomer while the (−)-isomers of fenfluramine and norfenfluramine are more active than their (+)-isomers. The concentrations of the drugs in the striatum do not explain the difference observed between the isomers on striatum HVA. Fenfluramine does not appear to act on striatum HVA through the formation of norfenfluramine.

The (+)- and (−)-isomers of amphetamine differ in several respects pharmacologically and biochemically. For instance, (−)-amphetamine lacks the stimulant (Prinzmetal & Alles, 1940) and the hyperthermic (Hajòs & Garattini, 1973) effects that are characteristic of the (+)-isomer and is less effective in eliciting stereotyped behaviour (Taylor & Snyder, 1970) and anorectic activity (Costa, Groppetti & Naimzada, 1972; Hajòs & Garattini, 1973) in rats. A finding consistent with the central effects is that (−)-amphetamine does not release brain noradrenaline (Taylor & Snyder, 1970, 1971; Clay, Cho & Roberfroid, 1971) being about 10 times less active than (+)-amphetamine in inhibiting catecholamine uptake *in vitro* (Coyle & Snyder, 1969) and *in vivo* (Taylor & Snyder, 1971) in several brain regions of the rat. However, the (−)- and (+)-forms are equipotent in this respect at the level of striatum (Taylor & Snyder, 1971), although the observations of Svensson (1971) and Scheel-Krüger (1972a,b) are at variance with these findings.

We have agreed with other authors (Laverty & Sharman, 1965; Fuentes & Del Rio, 1972) that (+)-amphetamine increases the level of homovanillic acid (HVA) (Jori & Bernardi, 1969; Bizzi, Bonaccorsi & others, 1970; Jori & Bernardi, 1972), the major metabolite of dopamine, in the striatum (Andén, Roos & Werdinius, 1963), we have now investigated the action of the (−)-isomer and also the effect on the level of striatum HVA of the (−)- and (+)-isomers of fenfluramine and norfenfluramine since previous observations had demonstrated that the racemic form of fenfluramine was also effective in increasing the striatum HVA content (Jori & Bernardi, 1969; Bizzi & others, 1970; Jori & Bernardi, 1972). In all instances, the level of the administered drug was also measured in the striatum.

MATERIALS AND METHODS

Charles River female rats (160 ± 10 g) were kept in Makrolon cages (40 × 25 × 18 cm), 4 per cage. Drugs were given intraperitoneally at doses of 3·75; 7·5 and 15 mg kg^{-1} at various times before decapitation. Brains were removed and the striata, after dissection, were immediately frozen on dry ice and kept in a freezer (−20°). HVA determinations were made on 4 pooled striata according to Korf, Ottema & Van der Veen (1971).

Drug concentrations were measured on the same samples, by using the formic acid elution fraction of G 10 Sephadex columns. Determinations were made according to the g.l.c. method of Änggård, Gunne & Niklasson (1970) for amphetamine and according to Belvedere, Tognoni & Morselli (1972) for fenfluramine and norfenfluramine. Drugs used were: (+)-amphetamine sulphate (kindly supplied by Recordati, Milan), (−)-amphetamine sulphate, (±)- (+)- and (−)-fenfluramine HCl; (±)-, (+)- and (−)-norfenfluramine HCl; (kindly supplied by Servier Labs, Orléans).

RESULTS

The effect of various doses of (+)-, (−)- and (±)-fenfluramine is shown in Table 1. At 15 mg kg^{-1} (i.p.) all the compounds cause a significant increase in HVA concentration, lasting more than 3 h. (−)-Fenfluramine is much more active than the (+)-isomer, the racemic compound showing intermediate activity. Striatal concentrations of fenfluramine and its de-ethylated metabolite, norfenfluramine, measured at 3 h after the administration of the isomers or the racemic form are comparable.

In Table 2, the effect of amphetamine and norfenfluramine isomers on striatum HVA levels are compared. (−)-Norfenfluramine appears to be more active than the (+)- and the (±)-compounds, while (+)-amphetamine increases HVA more than the (−)-form. The effect of (+)-amphetamine and (−)-fenfluramine on striatum HVA is similar and is longer lasting than that of (+)- or (−)-norfenfluramine or (+)-fenfluramine.

Concentrations of amphetamine or norfenfluramine in the striatum are similar after the administration of (−)- or the (+)-form (Table 3).

Table 1. *Effect of* (+)-, (−)- *or* (±)-*fenfluramine on striatal HVA in rats.*

Treatment	mg kg^{-1} i.p.	Striatum HVA (ng g^{-1} ± s.e.) 30 min	1 h	3 h	5 h	Fenfluramine (μg g^{-1} striatum ± s.e.) 3 h	Norfenfluramine 3 h
(±)-Fenfluramine	7·5	365±39*	—	—	—	—	—
	15	617±9*	477±19*	350± 4*	227±15	9.06±0·81	5·32±0·24
(−)-Fenfluramine	3·75	294±30*	—	—	—	—	—
	7·5	349±39*	—	—	—	—	—
	15	753±12*	734±19*	463±10*	325±11*	8·42±0·62	5·20±0·55
(+)-Fenfluramine	15	434±10*†	326±10*†	234±10†	—	10·92±0·36	4·72±0·28
Saline	—	—	204± 4	—	—	—	—

Each figure is the average of at least 4 determinations on 4 pooled striata of rats.
* $P<0·01$ *vs* saline group. † $P<0·01$ (+)-isomer *vs* the corresponding (−)-isomer.

DISCUSSION

A stereospecific effect has been observed in the capacity of three anorectic agents to increase rat striatum HVA. For amphetamine the (+)-isomer is more effective than the (−)-form while for fenfluramine and norfenfluramine the reverse is true. The concentrations of the drugs in the striatum are not the reason for the observed difference because concentrations were similar for all the isomers. However, the availability of the isomers at the subcellular sites where they exert their effect on

Table 2. *Effect of norfenfluramine and amphetamine isomers on the striatum HVA in rats.*

Treatment	Striatum HVA (ng g^{-1} ± s.e.)			
	30 min	1 h	3 h	5 h
(±)-Norfenfluramine	476 ± 10*	347 ± 13*	274 ± 8*	—
(−)-Norfenfluramine	624 ± 15*	404 ± 10*	278 ± 1*	—
(+)-Norfenfluramine	377 ± 7*†	330 ± 7*†	269 ± 2*†	—
(−)-Amphetamine	241 ± 19	345 ± 9*	401 ± 20*	321 ± 13*
(+)-Amphetamine	318 ± 9*‡	416 ± 9*†	581 ± 18*†	333 ± 23*
Saline	—	211 ± 6	—	—

Drugs were given intraperitoneally at the dose of 15 mg kg^{-1}.
Each figure is the average of 4 determinations on 4 pooled striata of rats.
* $P<0.01$ *vs* saline group. † $P<0.01$ and ‡ $P<0.05$ (+)-isomer *vs* the corresponding (−)-isomer.

Table 3. *Concentrations of amphetamine and norfenfluramine in the striatum of rats*

Treatment	Drug striatum concentration (μg g^{-1} ± s.e.)			
	30 min	60 min	180 min	300 min
(−)-Norfenfluramine	16·05 ± 0·6	16·85 ± 0·7	12·87 ± 0·9	—
(+)-Norfenfluramine	14·43 ± 1·6	17·01 ± 0·4	12·36 ± 0·4	—
(−)-Amphetamine	—	17·18 ± 1·8	—	2·63 ± 0·4
(+)-Amphetamine	—	18·02 ± 1·1	—	3·98 ± 0·4

Norfenfluramine and amphetamine were measured on the same samples used for the HVA determinations reported in Table 2.

the dopaminergic system, may be different. Since (+) and (−)-amphetamine exhibit different pharmacological effects it may be interesting to speculate on the relation between these effects and the increase of striatum HVA. For example the stereotyped behaviour (compulsive gnawing syndrome) is more marked for the (+)- than for the (−)-form (Taylor & Snyder, 1970, 1971; Wallach, Angrist & Gershon, 1971) in agreement with the effect on striatum HVA. However, fenfluramine and norfenfluramine, although very effective in increasing striatum HVA in rats, do not elicit any gnawing syndrome according to Le Douarec, Schmitt & Laubie (1966) and Le Douarec & Neveu (1970). We have found (unpublished results) that in particular, the (−)-isomers are more effective than the (+)-forms on the striatum HVA, but only the (+)-forms show a poor activity in stereotyped behaviour. These data seem to be at variance with the suggested relation between stereotyped behaviour and the dopaminergic system (Taylor & Snyder, 1970, 1971; Coyle & Snyder, 1969; Wallach & others, 1971).

However, in the interpretation of our results it should be underlined that amphetamine and fenfluramine may act on striatum HVA by different mechanisms. This is suggested by the observation that fenfluramine can still increase the HVA in animals made resistant to amphetamine by repeated treatment with amphetamine (Jori & Bernardi, 1972).

Furthermore, fenfluramine and norfenfluramine, unlike amphetamine, lower the levels of brain 5-HT (Duhault & Verdavainne, 1967; Costa, Groppetti & Revuelta, 1971; Morgan, Cattabeni & Costa, 1972) and this may interfere with the action on the dopaminergic system. No difference between the (−)- and (+)-isomers of fenfluramine or norfenfluramine have been observed in their actions in lowering of brain 5-HT and 5-hydroxyindoleacetic acid (Kon, Tognoni, Valzelli & Garattini, unpublished observations).

Finally, amphetamine differs from fenfluramine in decreasing the level of brain noradrenaline (Costa & Garattini, 1970). Amphetamine, on one hand, and fenfluramine on the other, therefore exert a typical spectrum of biochemical effects on brain monoamines, which is quantitatively different according to the isomer considered. It is this spectrum of activity that may be more important than the action on one single amine, in explaining the characteristic behavioural effects.

An additional finding of the present investigation concerns the suggested importance of the formation of norfenfluramine after metabolic dealkylation of fenfluramine (Bruce & Maynard, 1968) to explain the effects of fenfluramine. It is evident that the concentrations of striatal norfenfluramine necessary to increase the level of HVA, are much higher than those present in the same area after administration of fenfluramine at doses that induce the same increase of striatal HVA. It is, therefore, unlikely that norfenfluramine is the active metabolite of fenfluramine in the dopaminergic system.

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