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Action of fenfluramine on monoamine stores of rat tissues

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Summary

1. Fenfluramine is an anorexogenic agent used clinically because it is devoid of central stimulatory effects.

2. In rats, fenfluramine causes a depletion of 5-hydroxytryptamine (5-HT) from the telencephalon + diencephalon which lasts longer than one might have expected from the biological half life of fenfluramine. The depleting effects of fenfluramine do not extend to brainstem, stomach and heart stores of 5-HT.

3. Fenfluramine causes an increase in the turnover rate of tel-diencephalic 5-HT but such an acceleration could not be detected in the 5-HT stores of the brainstem.

4. It is inferred that the effects of fenfluramine on brain 5-hydroxytryptamine may be related to the accumulation of a fenfluramine metabolite in 5-HT neurones.

5. High doses of fenfluramine cause a depletion of catecholamine stores in brain and heart but the time course of this depletion is shorter than the depletion of brain 5-HT.

Introduction

Fenfluramine is used as an anorexogenic agent in man because of its peculiar pharmacological characteristics. This drug can reduce the appetite without stimulating the central nervous system (CNS). The lack of overt central stimulant effects has suggested that fenfluramine may be devoid of many of the side effects elicited by (+)-amphetamine when used in man as an anorexogenic drug. In rats and mice, unlike (+)-amphetamine, fenfluramine depresses exploratory activity (Boissier, Simon, Fichelle & Hervouet, 1965), does not antagonize the CNS depression caused by reserpine or that caused by barbiturates, inhibits thirst, and its toxicity does not increase with animal aggregation (Le Douarec & Neveu, 1970). Similarly to (+)-amphetamine, fenfluramine can produce analgesia (Moorman & Opitz, 1967) and in rats made obese by lesions of the hypothalamus it exerts an anorexogenic effect stronger than that of (+)-amphetamine (Bernier, Sicot & Le Douarec, 1969).

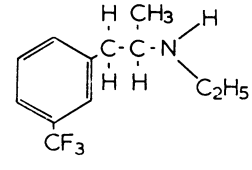
Using appropriate experimental conditions, rats can be shown to develop resistance to the anorexia and analgesia elicited by fenfluramine, and the time course of the development is similar to that reported for the development of resistance to the central action of (+)-amphetamine (Le Douarec & Neveu, 1970). Foxwell, Funderbuck & Ward (1969) studied the electroencephalogram of cats receiving (+)-amphet-

amine and fenfluramine ; these drugs exert a similar action on subcortical brain areas but they differ in their action on the cerebral cortex. Other reports show additional differences between these two drugs ; for instance fenfluramine, unlike (+)-amphetamine, reduces the 5-hydroxytryptamine (5-HT) concentration of brain tissue (Duhault & Verdavainne, 1967 ; Opitz, 1967).

This report shows that fenfluramine causes a longlasting depletion of the 5-HT stores of telencephalon + diencephalon but not of those of the brainstem, heart and stomach. Since fenfluramine inhibits neither tryptophan hydroxylase nor aromatic amino-acid decarboxylase *in vitro* (Le Douarec & Neveu, 1970) we decided to study the action of fenfluramine on the turnover rate of brain 5-HT *in vivo* in order to exclude inhibition of biosynthesis as the mechanism of depletion of brain 5-HT.

Methods

Sprague-Dawley male rats of 200–230 g body weight from Zivic Miller (Pittsburgh, Pa.) received various doses of fenfluramine (Robbins, Richmond, Virginia) intraperitoneally and were decapitated 4 h after the injections. In another experiment rats were injected intraperitoneally with 90 μmol/kg of fenfluramine and were decapitated at various times after the injection. Brain, heart and stomach were dissected immediately frozen and kept at −4° C until analysed for 5-hydroxytryptamine (5-HT), noradrenaline (NA) and dopamine (DM) according to a method previously described by Costa, Spano, Groppetti, Algeri & Neff (1968).



The tissues were homogenized in 0·4 M perchloric acid and centrifuged. The supernatants were then passed through Dowex 50 × 4 (200–400) columns. The amines and their amino-acid precursors were selectively eluted and assayed spectrofluorimetrically.

To compare the conversion of ^{3}H-L-tryptophan and 3,5-^{3}H-L-tyrosine into tissue monoamines the rats were given 1·25 mCi/kg of 3,5-^{3}H-L-tyrosine (31·8 Ci/mmol, New England Nuclear) and 1·25 mCi/kg of ^{3}H-L-tryptophan (1·3 Ci/mmol, New England Nuclear) intravenously 2 h after saline or 90 μmol/kg of fenfluramine intraperitoneally. The rats were decapitated 15 min after the injection of the radioactive amino-acid. The brains were immediately removed, and the cerebellum discarded. The remaining brain tissue was placed on its dorsal surface and cut with a scalpel blade which was inserted at the anterior edge of the pons so that it passed in front of the superior colliculus. We have designated the brain tissue posterior to the section as brainstem and the remaining tissue as tel-diencephalon. The specific activity (SA) of tryptophan and tyrosine in heart, stomach, brainstem, tel-diencephalon, plasma and the SA of other monoamines were determined in the same tissue by the method described by Neff, Spano, Groppetti, Wang & Costa (1970). The SA of amino-acids and amines in tissues of fenfluramine treated and control rats was compared. Such a comparison is possible, because, as shown in Fig. 2, between 2 and 5 h after the injection of fenfluramine the tissue concentrations of various amines although depleted can be considered to have attained a new steady state.

The conversion rate of 3,5-^{3}H-tyrosine and ^{3}H-tryptophan into ^{3}H-NA, ^{3}H-DM and ^{3}H-5-HT was compared assuming the existence of a two compartment closed system. In this system the quantity of amine formed can be calculated from the SA of the precursor amino-acid and the radioactivity incorporated in the amine:

$$\text{mol amine/g of tissue} = \frac{\text{d.p.m. of labelled amine/g of tissue}}{\text{SA of substrate of rate limiting enzyme}} \quad \ldots\ldots\ldots\ldots(1)$$

Although this model fails to correct for the efflux of the amine from stores, if the measurements are taken shortly after the injection of the labelled amino-acid, it can be used for comparative studies of the incorporation rates of radioactive amino-acids into amines. In this report the quotient of equation (1) is designated 'conversion index' to indicate that this value is not an absolute measurement of incorporation rates. The conversion index was calculated for each animal and the mean and standard error for each group were obtained. The conversion indices of NA and DM were obtained from the SA of plasma tyrosine and that of 5-HT from the SA of tryptophan in each tissue.

Results

Tissue concentrations of monoamines

The concentrations of NA and 5-HT in brain tissue 4 h after the intraperitoneal injection of various doses of fenfluramine are shown in Fig. 1. After 10 μmol/kg the brain concentration of these amines was not significantly different from controls. Brain 5-HT concentrations were reduced when rats received 30 μmol/kg. In contrast, the brain NA content was not significantly lower than that of control rats. The concentrations of NA and 5-HT in brain tissue were reduced 4 h after 90 μmol/kg of fenfluramine (Fig. 1). Figure 2 shows the concentrations of 5-HT, NA and DM in brain tissue of rats receiving 90 μmol/kg of fenfluramine at various times before decapitation. These data, plotted as a percentage of controls, show that the 5-HT concentrations of brain tissue were reduced to a greater extent than the concentrations of brain NA and DM. The 5-HT depletion reached a maximum of 60% between 2 and 5 h after the intraperitoneal injection of fenfluramine. The brain 5-HT content recovered slowly; 48 h after the fenfluramine administration it was reduced to 80% of the control value. In contrast, brain DM and NA concentrations were slightly reduced between 2 and 5 h after the fenfluramine injection, but after 24 h they were not significantly different from those of control rats. The cardiac NA content of rats receiving 90 μmol/kg of fenfluramine intraperitoneally also changed significantly 1 h after injection (Fig. 2). Table 1 lists the concentrations of NA, DM, 5-HT, tyrosine and tryptophan in brain parts, heart, plasma and stomach 135 min after 90 μmol/kg of fenfluramine. These data show that fenfluramine reduced the 5-HT content in the tel-diencephalon to an extent comparable with that shown in Fig. 1 and 2 for the brain tissue. The 5-HT concentrations in the brainstem, however, were unaffected by this dose of fenfluramine. Moreover, the 5-HT content of stomach and heart of rats receiving 90 μmol/kg of fenfluramine intraperitoneally was the same as that of saline treated rats.

Four hours after 90 μmol/kg of fenfluramine, the NA concentrations in the brain (Fig. 1) of rats injected were significantly reduced, although the NA content in the brainstem, tel-diencephalon, and other tissues was still unaltered 135 min after the drug injections. The data reported in Table 1 and Fig. 2 show that the cardiac

concentration of NA is lowered by fenfluramine but this drug effect appears to be shortlasting (Fig. 2). Tel-diencephalon DM is not reduced by fenfluramine. The plasma tyrosine and the tryptophan concentrations in various tissues were also unchanged by this drug.

Specific activity of NA, DM, 5-HT, tryptophan and tyrosine

Table 2 lists the SA of DM, NA and 5-HT in various tissues 15 min after intravenous injections of ^{3}H-L-tryptophan and 3,5-^{3}H-L-tyrosine at the doses indicated

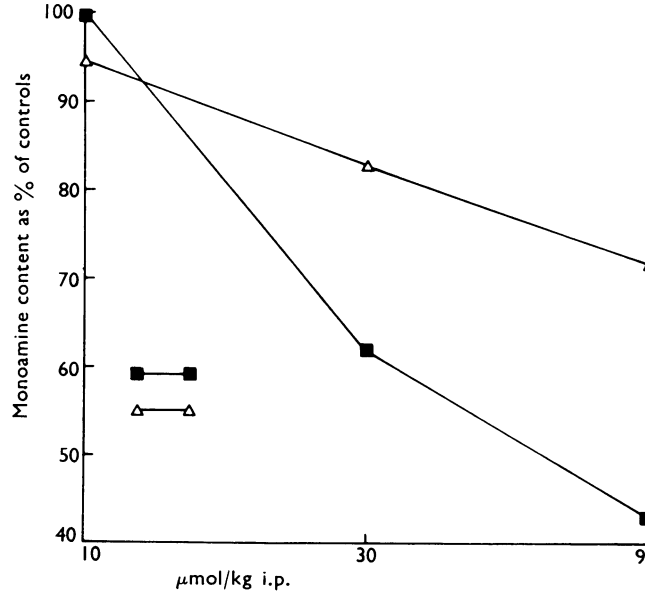


FIG. 1. Effect of increasing doses of fenfluramine on brain 5-HT (■) and NA (△) concentrations. Values are displayed as % of controls. The amine content was determined 4 h after the drug injection. The brain NA content after 10 and 30 μmol/kg intraperitoneally was not significantly different from controls, that of 5-HT was lower than controls after 30 μmol/kg intraperitoneally. Each point is the average of four rats. Amine concentrations (nmol/g ± S.E.) in brains of untreated rats: NA (1·9 ± 0·019); 5-HT (2·1 ± 0·12).

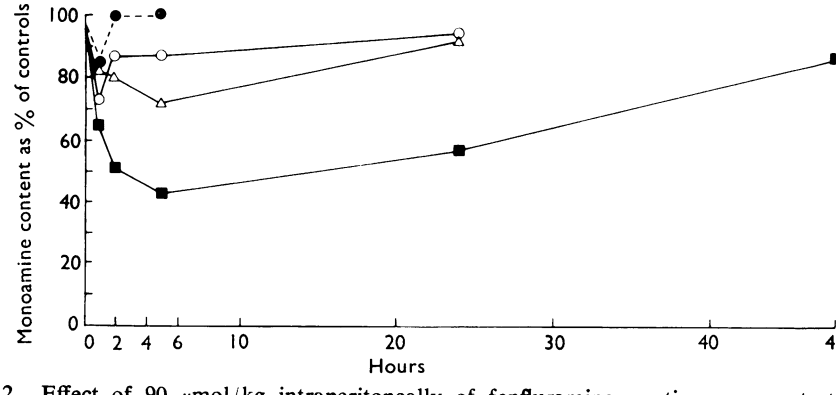


FIG. 2. Effect of 90 μmol/kg intraperitoneally of fenfluramine on tissue concentrations of NA, DM and 5-HT: ■——■, Brain 5-HT; ○——○, brain DM; △——△, brain NA; ●--●, heart NA. Values are displayed as % of controls. Each point is the average of four rats. Amine concentrations (nmol/g ± S.E.) in untreated rats: heart NA (9 ± 0·57); brain NA (1·8 ± 0·083); brain DM (8·2 ± 0·5); brain 5-HT (2·0 ± 0·065).

TABLE 1. *Effect of fenfluramine* on 5-hydroxytryptamine (5-HT), noradrenaline (NA), dopamine (DM), tyrosine (TY), and tryptophan (TP) concentrations of various tissues*

Tissue	Treatment	NA nmol/g±S.E.	5-HT nmol/g±S.E.	DM nmol/g±S.E.	TY nmol/g±S.E.	TP nmol/g±S.E.
Heart	Saline	9·0±0·57	2·5±0·28	—	—	32±1·5
	Fenfluramine	6·1±0·75†	2·3±0·22	—	—	28±1·6
Brainstem	Saline	4·3±0·14	1·9±0·17	—	—	21±0·57
	Fenfluramine	3·6±0·27	1·5±0·13	—	—	23±0·71
Tel-diencephalon	Saline	1·3±0·083	0·98±0·065	8·2±0·50	—	18±1·6
	Fenfluramine	1·1±0·10	0·36±0·026‡	7·2±0·24	—	18±1·3
Stomach	Saline	2·5±0·15	41±3·3	—	—	48±1·6
	Fenfluramine	2·2±0·15	50±4·2	—	—	49±1·1
Plasma	Saline	—	—	—	76±5	70±8
	Fenfluramine	—	—	—	97±9	61±5

* Rats received 90 μmol/kg of fenfluramine intraperitoneally 135 min before decapitation. Each value is the average of five determinations. Significance of difference from saline treated controls: † $P<0·05$; ‡ $P<0·1$.

TABLE 2. *Specific activity (SA) of 5-hydroxytryptamine (5-HT), noradrenaline (NA), dopamine (DM), tyrosine (TY) and tryptophan (TP) in various tissues of control and fenfluramine treated rats injected with labelled TP and TY**

Tissue	Treatment	NA d.p.m./nmol±S.E.	5-HT d.p.m./nmol±S.E.	DM d.p.m./nmol±S.E.	TY d.p.m./nmol±S.E.	TP d.p.m./nmol±S.E.
Heart	Saline	183±25	2,344±807	—	—	12,776±669
	Fenfluramine	784±255†	1,907±379	—	—	14,737±213
Brainstem	Saline	1,209±25	6,330±234	—	—	13,817±674
	Fenfluramine	2,412±332‡	11,138±1,797†	—	—	16,185±895†
Tel-diencephalon	Saline	1,118±45	3,262±106	1,828±66	—	16,047±764
	Fenfluramine	2,087±312‡	15,295±1,068‡	3,838±514‡	—	19,117±660
Stomach	Saline	352±34	2,897±105	—	—	7,675±595
	Fenfluramine	638±107‡	3,163±75	—	—	8,722±455
Plasma	Saline	—	—	—	14,343±932	13,996±702
	Fenfluramine	—	—	—	16,041±1,276	15,839±517

* Rats received intraperitoneally 90 μmol/kg of fenfluramine 2 h before injecting intravenously 1·25 mCi/kg of 3,5-^{3}H-L-tyrosine (31·8 Ci/mmol) and ^{3}H-L-tryptophan (1·3 Ci/mmol). Rats were decapitated 15 min after labelling. Values reported are averages from five animals. Significance of difference from saline treated controls: † $P<0·05$; ‡ $P<0·01$.

in **Methods**. The SA of DM and NA in the tissues assayed was greater in fenfluramine treated than control rats. The SA of 5-HT and tryptophan was also increased in brainstem and tel-diencephalon of the rats receiving fenfluramine but not in the heart or the stomach. The specific activities of the plasma tyrosine and those of tryptophan in plasma, stomach and heart were not changed by the 90 μmol/kg injection of fenfluramine. On ranking the concentrations (Table 1) and the SA (Table 2) of the tryptophan found in various tissues 15 min after labelling (Table 2) the highest SA was found to be present in those tissues with the lowest tryptophan concentrations. The plasma was an exception to this ranking order because the plasma had the highest concentration but not the lowest SA.

Conversion index of tryptophan into 5-HT and of tyrosine into NA and DM

Fenfluramine reduced the steady state concentrations of 5-HT only in the tel-diencephalon (Table 1) but increased its specific activity in the brainstem and the tel-diencephalon (Table 2). These discrepancies made it difficult to assess whether the conversion rate of the amino-acid into 5-HT was changed by the drug treatment simply by comparing the specific activities of the various amine stores. To obviate this difficulty we calculated the conversion index of the two amino-acids into the various monoamine stores as described in **Methods**.

Table 3 lists the conversion indices of 5-HT and catecholamines in various tissues of rats receiving the labelled amino-acids 2 h after pretreatment with either fenfluramine or saline. The conversion of plasma tyrosine into tel-diencephalic DM, brainstem and stomach NA is greater in fenfluramine treated than in control rats. The conversion indices for the other catecholamine stores were not increased by fenfluramine. The conversion index of tel-diencephalon tryptophan into 5-HT was increased whereas the conversion of this amino-acid into other tissue stores of 5-HT was not changed by fenfluramine.

Discussion

Our studies substantiate a qualitative difference in the action of (+)-amphetamine and fenfluramine on brain monoamine stores. We confirmed the findings of Duhault & Verdavainne (1967) and Opitz (1967) that fenfluramine depletes brain 5-HT

TABLE 3. *Conversion index of radioactive tryptophan or tyrosine into 5-hydroxytryptamine (5-HT), dopamine (DM) and noradrenaline (NA) in various tissues of control and fenfluramine treated rats.**

Tissue	Treatment	Conversion index at 15 min (nmol/g of tissue ± S.E.) DM§	NA§	5-HT¶
Heart	Saline		0·12 ±0·023	0·39±0·13
	Fenfluramine		0·28 ±0·11	0·29±0·047
Brainstem	Saline		0·36 ±0·222	0·86±0·10
	Fenfluramine		0·52 ±0·033‡	1·01±0·13
Tel-diencephalon	Saline	0·53±0·052	0·094±0·001	0·20±0·014
	Fenfluramine	0·89±0·15†	0·14 ±0·020	0·29±0·033†
Stomach	Saline		0·061±0·0026	2·8 ±0·34
	Fenfluramine		0·085±0·010†	3·1 ±0·35

* Rats received 90 μmol/kg of fenfluramine intraperitoneally 2 h before injecting intravenously 1.25 mCi/kg of 3,5-^{3}H-L-tyrosine (31·8 Ci/mmol) and ^{3}H-L-tryptophan (1·3 Ci/mmol). Rats were decapitated 15 min after labelling. Each value is the average from five animals.

§ μmol/g of tissue DM (or NA) $= \frac{\text{d.p.m./g (DM or NA)}}{\text{SA plasma tyrosine}}$

¶ μmol/g of tissue 5-HT $= \frac{\text{d.p.m./g (5-HT)}}{\text{SA tissue TP}}$

Significance of difference from saline treated controls: †$P<0·05$; ‡ $P<0·01$.

stores. When rats are injected intraperitoneally with 90 μmol/kg of fenfluramine, the depletion of brain 5-HT is associated with a depletion of brain NA (Fig. 1). With 30 μmol/kg intraperitoneally, a selective depletion of brain 5-HT is present. Not only is the brain 5-HT depletion elicited by fenfluramine greater than that of the catecholamine stores but the fenfluramine action on 5-HT stores is also longer lasting (Fig. 2). Since the brain concentrations of fenfluramine in rats are very low 24 h after drug injections (Morgan, Groppetti, Revuelta, Cattabeni & Costa, 1970), the time course of brain 5-HT depletion after fenfluramine suggests that this effect may be unrelated to the tissue concentrations of the drug. From the data reported in Fig. 2, one might infer that a similar mechanism cannot be invoked for the depletion of catecholamine stores.

The effect of fenfluramine on tel-diencephalic 5-HT stores appears to be selective: none of the other tissues of rats receiving fenfluramine that we have assayed have shown a depletion of this monoamine (Table 1). Moreover, the tel-diencephalon of fenfluramine treated rats was the only tissue with a conversion index for ^{3}H-L-tryptophan into ^{3}H-5-HT different from that of rats injected with saline. Since the conversion index of ^{3}H-L-tryptophan into ^{3}H-5-HT was either the same or increased in tissues from rats receiving fenfluramine, we can exclude the possibility that fenfluramine either directly or indirectly reduces the biosynthesis of 5-HT in tel-diencephalon or other tissues. Rather, these data support the view that synthesis of tel-diencephalic 5-HT is accelerated by fenfluramine. Such a conclusion does not apply to brainstem 5-HT or to the other tissue stores of 5-HT we have assayed (Table 3).

The results presented in this report also indicate that fenfluramine shares several actions on brain amine stores with (+)-amphetamine: (*a*) both drugs can increase the incorporation of ^{3}H-tyrosine into tel-diencephalic DM (Table 3, and Costa & Groppetti, 1970); (*b*) they can deplete brain NA content (Figs. 1 and 2); and (*c*) they increase the turnover rate of brainstem and stomach NA if given in high doses (Table 3). The mode of brain NA depletion might be different for the two drugs: the effect of fenfluramine is readily reversible, that of (+)-amphetamine outlasts the presence of amphetamine in tissues (Groppetti & Costa, 1969) and seems related to the accumulation of *p*-hydroxynorephedrine, a metabolite of (+)-amphetamine (Costa & Groppetti, 1970). It is tempting by analogy to speculate on the possible mechanism of the longlasting depletion of brain 5-HT elicited by fenfluramine and to advance the working hypothesis that a metabolite of fenfluramine is present in the 5-HT-containing neurones and maintains the longlasting depletion of brain 5-HT described in this report.

Obviously the biochemical effects elicited by these high doses of fenfluramine cannot be extrapolated to explain the mechanism of the anorexogenic action elicited by small doses of fenfluramine. The results presented in this report suggest that fenfluramine in high doses causes a slowly reversible impairment of 5-HT binding in tel-diencephalon but not in brainstem. Our results also show that fenfluramine slightly increases the conversion index of NA in stomach and does not change that of heart, suggesting that fenfluramine is not a potent indirectly acting sympathomimetic drug in the periphery.

As a corollary we surmize that the lack of CNS stimulation attributed to fenfluramine does not exclude that this drug is devoid of other CNS effects. The action of fenfluramine on turnover of 5-HT in tel-diencephalon revealed by our

findings may contribute to CNS effects which are not apparent after acute administration but may be more important in chronic treatment with this drug. If this were the case, fenfluramine might not be devoid of potential risks in causing CNS disturbances when used as an anorexogenic drug in man.

REFERENCES

BERNIER, A., SICOT, N. & LE DOUAREC, J. C. (1969). Action comparée de la fenfluramine et de l'amphétamine chez le rats obèses hypothalamiques. *Rev. Franç. d'Etudes chim. biol.*, **14**, 762–772.

BOISSIER, J. R., SIMON, P., FICHELLE, J. & HERVOUET, E. (1965). Action psychoanalyeptique de quelques anorexigénes dérivés de la phényléthylamine. *Therapie*, **20**, 297–309.

COSTA, E. & GROPPETTI, A. (1970). Biosynthesis and storage of catecholamines in tissue of rats injected with various doses of *d*-amphetamine. In: *Amphetamines and Related Compounds*, Proceedings of the Mario Negri Institute for Pharmacological Research, Milan, Italy, ed. Costa, E. and Garrattini, S., pp. 231–255. New York: Raven Press.

COSTA, E., SPANO, P. F., GROPPETTI, A., ALGERI, S. & NEFF, N. H. (1968). Simultaneous determination of tryptophan, tyrosine, catecholamines and serotonin specific activity in rat brain. *Atti. Acad. Med. Lomb.*, **23**, 1100–1104.

DUHAULT, J. & VÈRDAVAINNE, C. (1967). Modification du taux de sérotonine cérébrale chez le rat par les trifluorométhyl-phényl-2-ethyl amino propane (Fenfluramine 768 S). *Archs int. Pharmacodyn. Thér.*, **170**, 276–286.

FOXWELL, M., FUNDERBUCK, W. H. & WARD, J. W. (1969). Studies on the site of action of a new anorectic agent, fenfluramine. *J. Pharmac. exp. Ther.*, **165**, 60–70.

GROPPETTI, A. & COSTA, E. (1969). Tissue concentrations of *p*-hydroxynorephedrine (*p*OHNE)-injected with *d*-amphetamine: effect of pretreatment with desipramine (DMI). *Life Sci., Oxford*, **8**, 653–665.

LE DOUAREC, J. C. & NEVEU, C. (1970). Pharmacology and biochemistry of fenfluramine. In: *Amphetamines and Related Compounds*, Proceedings of the Mario Negri Institute for Pharmacological Research, Milan, Italy, ed. Costa, E. and Garattini, S., pp. 75–105. New York: Raven Press.

MOORMAN, J. & OPITZ, K. (1967). Zur analgetischen Wirkung anorexigener substanzen. *Arzneitmittel-Forsch.*, **17**, 343–345.

MORGAN, C. D., GROPPETTI, A., REVUELTA, A., CATTABENI, F. & COSTA, E. (1970). Pharmacological- and biochemical effects of methamphetamine (M), fenfluramine (F) and 1-1-N-Benzyl-β-methoxy-3-trifluoromethylphenethylamine (1-SKF 39728), *Pharmacologist*, in the Press.

NEFF, N. H., SPANO, P. F., GROPPETTI, A., WANG, C. T. & COSTA, E. (1970). A simple procedure for calculating the synthesis rate of brain norepinephrine, dopamine and serotonin after a pulse injection of radioactive tyrosine and tryptophan. *J. Pharmac. exp. Ther.*, in the Press.

OPITZ, K. (1967). Anoroxigene phenylalkylamine and serotonistoffwechsel. *Naunyn-Schmiedebergs. Arch. Pharmak. exp. Path.*, **258**, 56–65.

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