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ON THE CENTRAL MEDIATION OF ANOREXIGENIC DRUG EFFECTS*

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Abstract—The anorexigenic effects of *d*-amphetamine and of *d*-*p*-chloroamphetamine have been compared in male rats; the *p*-chloro compound proved to be 1·5–2 times more active than *d*-amphetamine. The suppression of food intake provoked by an ED_{95} of *d*-amphetamine could be antagonized by the following treatments: *p*-chlorophenylalanine (300 mg/kg orally, twice, 72 and 48 hr before the experiment), cyproheptadine (0·4–1·6 mg/kg i.p.), α-methyltryosine (5 and 10 mg/kg i.p.), disulfiram (200–600 mg/kg orally), phentolamine (0·5 and 1 mg/kg i.p.) and haloperidol (0·03–0·4 mg/kg i.p.). All these treatments either reduced the hypothalamic turnover of noradrenaline or blocked the receptors of this amine, which therefore is considered as the mediator of the anorexigenic effect of amphetamine. The effect of an ED_{95} of *d*-*p*-chloroamphetamine was antagonized by *p*-chlorophenylalanine, α-methyltyrosine and disulfiram in the doses mentioned above. Cyproheptadine had an effect only with the lowest dose used, and the antagonistic effects of phentolamine and especially of haloperidol were much weaker than in the case of amphetamine. These antagonistic effects and the alterations in levels and turnover of hypothalamic 5-HT, noradrenaline and dopamine suggest a participation of catecholamines and 5-HT in the mediation of the anorexigenic effect of *p*-chloroamphetamine.

AMPHETAMINE and its derivatives are usually regarded as indirectly acting sympathomimetics, and a mediation of the anorexigenic effect of this group of drugs by catecholamines appeared thus likely. However, especially the halogenated derivatives of amphetamine which, like fenfluramine and *p*-chloroamphetamine, also are known for their appetite-reducing action, have a strong effect on the central metabolism of 5-hydroxytryptamine (5-HT), too,[1–3] and such an effect, though somewhat less pronounced, has also been shown for amphetamine itself.[4–6] On the other hand, there are clinical reports on an appetite-enhancing action of the 5-HT antagonist cyproheptadine,[7–11] and this coincidence prompted the working hypothesis of a tryptaminergic mediation of drug-induced anorexia.

A role of 5-HT in the mediation of anorexigenic drug effects has been discussed repeatedly in the last years,[3,12–14] but the results of these authors have not been unequivocal. Other studies point to a predominant role of catecholamines, especially of noradrenaline, as mediators of the anorexigenic effect of amphetamine.[14–18]

In order to gain insight into the question of the central mediation of anorexigenic drug effects, we have tried to antagonize the anorexigenic effect of *d*-amphetamine and *d*-*p*-chloroamphetamine in rats by pretreatment with drugs known to affect levels and turnover of the central monoamines 5-HT, noradrenaline and dopamine,

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or to block the receptors for these amines. Further, the influence of both amphetamines and the antagonistic drugs on level and turnover of the monoamines in the hypothalamus, where the feeding and satiety centers are located, was studied.

MATERIAL AND METHODS

Male rats of Wistar II strain, weighing 250–350 g were used throughout (Tierzüchterei Brünger, D-4801 Bokel).

The anorexigenic experiments were done following the procedure of Spengler and Waser.[19] The rats were kept singly in Makrolon cages and trained for 1 week only to have access to food (Altromin M 6) from 9 a.m. to 4 p.m. The food intake from 9 to 11 a.m. was measured and used for the calculation of the control intake. On the experimental day, the rats were treated orally with the anorexigenic drug at 8 a.m. and food intake from 9 to 11 a.m. was again measured. Each treatment was given to at least 10 rats. The rats were only used once since *p*-chloroamphetamine especially is known to have long-lasting after-effects on central 5-HT.[20,21]

With the 2 amphetamines, at first the ED_{50} for suppression of food intake to below 5 g/2 hr was determined using the method of Litchfield and Wilcoxon.[22] In the later experiments in which we tried to antagonize the anorexigenic effect, the ED_{95} of both drugs was given. Using these doses, the distribution of food intake was in the vicinity of zero and correspondingly skew, so the logarithms of food intake were used for the calculation of the geometrical means and the range for one S.D. A food intake of zero was set to 0·1 g in order to make the calculation of the geometrical means possible.

Pretreatments. *p*-Chlorophenylalanine (Labkemi, Göteborg, Sweden) was given orally as a suspension in 5 per cent gum acacia once, 48 hr, or twice, 72 and 48 hr, before the experiment. Cyproheptadine·HCl (Sharp and Dohme GmbH, München) was given i.p. 30 min before the feeding period. α-Methyltyrosine (Labkemi) was brought into solution with the aid of diluted hydrochloric acid and injected i.p., 2 hr before the feeding period. Disulfiram (A/S Dumex, Copenhagen, Denmark) was given orally as a suspension in 5 per cent gum acacia, 3 hr before the feeding period. Catecholamine anatagonists were injected i.p.: phentolamine (Regitin®) at the beginning of the feeding period, chlorpromazine (Megaphen®) and propranolol (Dociton®) 30 min, and haloperidol (Haloperidol®, Janssen) 3 hr before.

Monoamine determinations. In parallel experiments, levels and turnover of 5-HT, noradrenaline and dopamine were determined in the hypothalamus of rats that were kept under conditions identical to those of the feeding experiments. At least six determinations were done for each treatment or point of time. The hypothalamus was dissected according to Miller *et al.*[23]; 5-HT and 5-HIAA were determined by the method of Curzon and Green,[24] the catecholamines by that of Chang.[25] The turnover of 5-HT was determined by the method of Neff *et al.*[26] during the first 90 min of the feeding period in the anorexigenic experiments, the turnover of the catecholamines by the method of Brodie *et al.*[27] during the 2 hr of the feeding period. In the latter method which is based upon inhibition of catecholamine biosynthesis by α-methyltyrosine, i.p. injection of 100 mg/kg of this drug proved to be sufficient.

Anorexigenic drugs. *d*-Amphetamine sulfate (Merck, Darmstadt) and *d*-*p*-chloroamphetamine hydrogentartrate (Leo Pharmaceutical Products, Ballerup, Denmark) were used. Doses are given in terms of the base.

Statistics. The significance of differences was calculated by Student's *t*-test.

RESULTS

Anorexigenic effect of d-*amphetamine and* d-p-*chloroamphetamine.* After 1 week on the feeding schedule, food intake during the first 2 hr of the daily feeding period had stabilized to 11·7 ± 1·5 g (means ± S.D.) in a total of 180 rats.

Taking depression of food intake to below 5 g within these 2 hr as criterion, an oral ED_{50} of 1·05 mg/kg (0·72–1·5) *d*-amphetamine (= 7·8 μmoles/kg) and of 0·92 mg/kg (0·72–1·2) *d-p*-chloroamphetamine (= 5·4 μmoles/kg) was determined. On a molar base, the *p*-chloro compound has thus 1·44 times the anorexigenic potency of *d*-amphetamine.

The ED_{95} of *d*-amphetamine was 2·85 mg/kg (21 μmoles/kg), that of *d-p*-chloroamphetamine 1·95 mg/kg (11·5 μmoles/kg), corresponding to a molar potency ratio of 1·83 in favor of the halogenated compound. The ED_{95} of both drugs reduced food intake within the first 2 hr of the feeding period to 0·5 g (0·1–2·5). This figure is based on 94 rats in the case of *d*-amphetamine and on 85 rats in the case of the *p*-chloro compound.

Antagonism to the anorexigenic effect of d-*amphetamine and* d-p-*chloroamphetamine.* In these experiments, the ED_{95} of both amphetamines was used. The results of the experiments are depicted in Figs. 1 and 2.

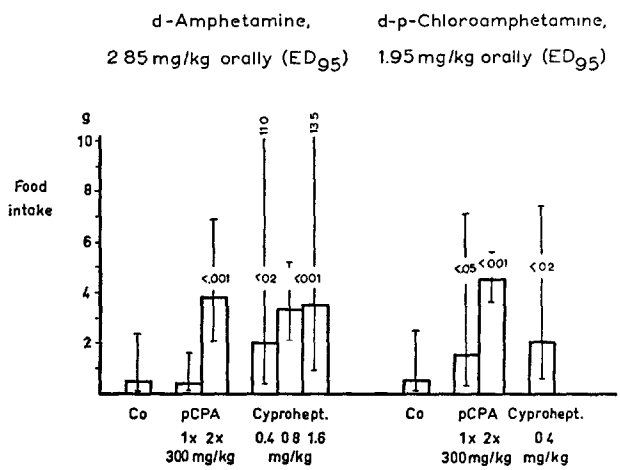

FIG. 1. Influence of *p*-chlorophenylalanine and cyproheptadine on the anorexia produced by an ED_{95} of *d*-amphetamine or *d-p*-chloroamphetamine in rats. The columns give the geometrical means of food intake with the range for one S.D. The significance of the difference to the food intake in rats only treated with the amphetamines is indicated above the columns. Food intake in untreated controls was 11·7 ± 1·5 g.

Pretreatment with *p*-chlorophenylalanine, 300 mg/kg orally once, 48 hr before the experiment, had no effect on the anorexigenic action of *d*-amphetamine and was only weakly antagonistic to that of the *p*-chloro compound, though it reduced the hypothalamic 5-HT by more than 70 per cent and the turnover of this amine to near zero (Table 1). When the drug was given twice, 72 and 48 hr before the experiment, a clear antagonism to both amphetamines was apparent. Food intake rose to about

4 g/2 hr. The 5-HT antagonist cyproheptadine displayed a rather weak, dose-dependent antagonism to *d*-amphetamine in the dose range from 0·4 to 1·6 mg/kg, but only the lowest of these doses antagonized the effect of *p*-chloroamphetamine.

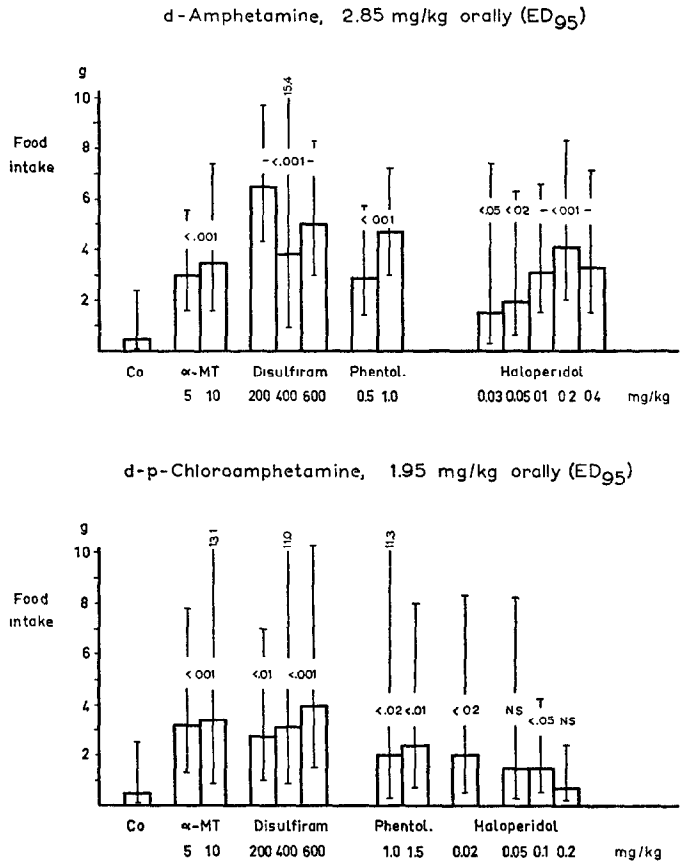

FIG. 2. Influence of drugs interfering with the biosynthesis of catecholamines or blocking catecholamine receptors on the anorexia produced by an ED_{95} of *d*-amphetamine or *d*-*p*-chloroamphetamine. For further explanation see Fig. 1. (N.S. = not significantly different from the controls.)

Doses of 5 and 10 mg/kg α-methyltyrosine antagonized the anorexigenic effect of both amphetamines to about the same degree. With higher doses, this antagonism was lost, probably on account of the sedative effect. Disulfiram displayed a highly significant antagonism in the dose range from 200 to 600 mg/kg which was, however, more pronounced in the case of *d*-amphetamine. Also the catecholamine antagonistic drugs phentolamine and haloperidol proved to be most active against the anorexigenic effect of *d*-amphetamine. Phentolamine had a rather pronounced effect against amphetamine in doses of 0·5 and 1 mg/kg, but was only weakly antagonistic to *p*-chloroamphetamine in doses of 1 and 1·5 mg/kg, and haloperidol displayed a dose-dependent antagonism to *d*-amphetamine in the range of 0·03 to 0·2 mg/kg, whereas *p*-chloroamphetamine was weakly antagonized only by 0·02 mg/kg. Chlorpromazine (0·05–1·6 mg/kg) and propranolol (0·1–2 mg/kg) lacked an antagonistic effect and even led to a further depression of food intake.

TABLE 1. HYPOTHALAMIC LEVELS AND TURNOVER OF 5-HT, NORADRENALINE AND DOPAMINE

Treatment (dose and time before the experiment)	5-HT Level (μg/g)†	5-HT Turnover (μg/g/hr)‡	5-HIAA Level (μg/g)	Noradrenaline Level (μg/g)	Noradrenaline Turnover (μg/g/hr)	Dopamine Level (μg/g)	Dopamine Turnover (μg/g/hr)
Controls	1·11 ± 0·14 (18)¶	0·22 ± 0·047 (12)	0·47 ± 0·086 (18)	1·61 ± 0·17(18)	0·32 ± 0·033 (12)	0·42 ± 0·11 (18)	0·082 ± 0·028 (12)
d-Amphetamine	1 hr: 1·24 ± 0·065(*)	0·32 ± 0·048***§	1 hr 0·53 ± 0·084	1 hr: 1·23 ± 9·21***	0·33 ± 0·083 (12)	1 hr: 0·39 ± 0·14	0·12 ± 0·023**
2·85 mg/kg, 1 hr	3 hr: 1·2 ± 0·056		3 hr: 0·68 ± 0·076***	3 hr: 1·59 ± 0·27 (12)		3 hr: 0·36 ± 0·078 (11)	
d-p-Chloroamphetamine	1 hr: 1·15 ± 0·077	0·26 ± 0·033§	1 hr: 0·53 ± 0·042	1 hr: 1·29 ± 0·19***	0·05 ± 0·046 (12)***	1 hr: 0·59 ± 0·12**	0·033 ± 0·022 (12)**
1·95 mg/kg, 1 hr	3 hr: 0·87 ± 0·1***		3 hr: 0·4 ± 0·1	3 hr: 1·32 ± 0·2***		3 hr: 0·36 ± 0·098 (12)	
p-Chlorophenylalanine							
300 mg/kg, 48 hr	0·31 ± 0·097***	0·012***‖	0·13***‖	1·36 ± 0·24*	0·21 ± 0·07***	0·4 ± 0·18 (5)	0·11 ± 0·041
2 × 300 mg/kg, 72 + 48 hr	0·21 ± 0·017***	0·014***‖	0***	1·07 ± 0·06***	0·24 ± 0·024***	0·29 ± 0·11*	0·064 ± 0·025
α-Methyltyrosine							
5 mg/kg, 2 hr	2 hr: 1·13 ± 0·094	0·22 ± 0·04 (12)	2 hr: 0·47 ± 0·031	2 hr: 1·39 ± 0·2*		2 hr: 0·21 ± 0·07***	
	4 hr: 1·15 ± 0·11		4 hr: 0·48 ± 0·041	4 hr: 1·09 ± 0·2***		4 hr: 0·19 ± 0·11***	
10 mg/kg, 2 hr	2 hr: 1·1 ± 0·13	0·23 ± 0·037 (12)	2 hr: 0·51 ± 0·04	2 hr: 1·22 ± 0·19***		2 hr: 0·26 ± 0·13**	
	4 hr: 1·04 ± 0·079		4 hr: 0·5 ± 0·036	4 hr: 1·26 ± 0·21***		4 hr: 0·17 ± 0·12***	
Disulfiram							
200 mg/kg, 3 hr	3 hr: 1·13 ± 0·13	0·23 ± 0·025	3 hr: 0·58 ± 0·057**	3 hr: 1·31 ± 0·19	0·19 ± 0·055***	3 hr: 0·32 ± 0·064	0·11 ± 0·025(*)
	5 hr: 1·04 ± 0·079		5 hr: 0·38 ± 0·052(*)	5 hr: 1·22 ± 0·26***		5 hr: 0·45 ± 0·097	
400 mg/kg, 3 hr		0·19 ± 0·037					
600 mg/kg, 3hr	3 hr: 1·15 ± 0·19	0·37 ± 0·038 (18)***	3 hr: 0·59 ± 0·073**	3 hr: 1·58 ± 0·21	0·32 ± 0·036	3 hr: 0·6 ± 0·14	0·17 ± 0·024***
	5 hr: 1·07 ± 0·084		5 hr: 0·47 ± 0·044	5 hr: 1·38 ± 0·27**		5 hr: 0·54 ± 0·1(*)	
Cyproheptadine · HCl							
0·4 mg/kg, 30 min		0·27 ± 0·039(*)			0·31 ± 0·033		0·16 ± 0·031***
1·6 mg/kg, 30 min		0·34 ± 0·037***			0·23 ± 0·063***		0·031 ± 0·031(*)
Phentolamine							
0.5 mg/kg		0·51 ± 0·07***			0·43 ± 0·046***		0·072 ± 0·025
1·5 mg/kg		0·33 ± 0·079**			0·3 ± 0·11		0·085 ± 0·029
Haloperidol							
0·02 mg/kg, 3 hr		0·38 ± 0·051***			0·28 ± 0·063		0·053 ± 0.043
0·2 mg/kg, 3 hr		0·33 ± 0·067***			0·17 ± 0·05***		0.077 ± 0.049
Chlorpromazine							
0·05 mg/kg, 30 min		0·17 ± 0·045(*)			0·29 ± 0·033		0· 1 ± 0.046
1·6 mg/kg, 30 min		0·34 ± 0·033***			0·21 ± 0·053***		0·083 ± 0·019
Propranolol							
0·1 mg/kg, 30 min		0·2 ± 0·033			0·26 ± 0·042**		0·14 ± 0·015*
2·0 mg/kg, 30 min		0·21 ± 0·055			0·28 ± 0·069		0·049 ± 0·031

(*) $P < 0.05$; * $P < 0.02$; ** $P < 0.01$; *** $P < 0.001$.
† Mean ± S.D.
‡ Mean ± S.E.
§ For further explanation, see text.
‖ Most of the values were zero.
¶ Figure in parentheses = number of determinations; if not mentioned, 6 determinations were done.

Influence of the amphetamines and of antagonists of their anorexigenic effect on level and turnover of biogenic amines in the hypothalamus. The results of the determinations of levels and turnover of 5-HT, noradrenaline and dopamine in the hypothalamus after the different treatments are summarized in Table 1.

The turnover of 5-HT after both amphetamines deserves a special comment. The method of Neff *et al.*[26] is based on the rise of 5-HIAA after inhibition of its transport out of the central nervous system by probenecid. 5-HT concentrations were determined simultaneously, but showed usually only minor variations during the 90 min-interval of the turnover determination. The only exception were the amphetamines with which a considerable rise of 5-HT occurred. In the case of *d*-amphetamine, the average rise of 5-HIAA corresponded to 0·19 μg/g/hr, that of 5-HT to a further 0·13 μg/g/hr. With *d*-*p*-chloroamphetamine, the rise in 5-HIAA amounted to only 0·1 μg/g/hr, surely an expression of the inhibitory action of the drug on monoamine oxidase.[2,28] Besides this rise in 5-HIAA, a rise in 5-HT corresponding to 0·16 μg/g/hr occurred. We consider also the rise in the 5-HT concentration as an expression of an enhanced turnover of this amine which obviously occurs when monoamine oxidase is inhibited to a certain degree—a property which is known for both amphetamines—and have added the rises of 5-HT and 5-HIAA in the figures in Table 1. But, since the elimination of unmetabolized 5-HT from the central nervous system probably is not inhibited by probenecid to the same degree as that of 5-HIAA, these figures may be underestimates of the true turnover.

The conspicious rise in 5-HT turnover seen with the highest dose of disulfiram corresponds to findings of Johnson *et al.*[29] with other inhibitors of dopamine-β-hydroxylase.

DISCUSSION

The starting point of our study was the working hypothesis of a tryptaminergic mediation of drug-induced anorexia. This hypothesis, originating from the clinical finding of the appetite-enhancing effect of the 5-HT antagonist cyproheptadine, was in agreement with previous experimental findings of Jespersen and Scheel-Krüger,[12] who in dogs had the impression that methysergide antagonized the anorexigenic effect of fenfluramine. Also Funderburk *et al.*[13] were able to antagonize the anorexigenic effect of fenfluramine, but not of *d*-amphetamine and chlorphentermine, by pretreatment with a 5-HT antagonist in rats. On the other hand, *p*-chlorophenylalanine was without effect in these experiments as well as in those of Opitz.[3]

At first sight, our results with *p*-chlorophenylalanine and the 5-HT antagonist cyproheptadine seemed to corroborate the hypothesis of a tryptaminergic mediation of anorexigenic drug effects. A massive pretreatment with *p*-chlorophenylalanine, reducing hypothalamic 5-HT by more than 80 per cent and the turnover of this amine to near zero, produced a highly significant antagonism to the anorexigenic effect of both amphetamines. A certain antagonism was also seen with cyproheptadine, this was dose-dependent from 0·4–1·6 mg/kg in the case of *d*-amphetamine, but the effect of *p*-chloroamphetamine was only influenced weakly by the lowest of these doses. The dose range of cyproheptadine used by us was far below the doses that have been reported to enhance food intake in rats (12·5 mg/kg).[30] However, only the dose of 0·4 mg/kg can be considered as purely 5-HT antagonistic, higher doses of cyproheptadine may have an additional α-adrenolytic effect.[31] Our working hypothesis

was further weakened by the finding that *p*-chlorophenylalanine, besides its well known effects on level and turnover of 5-HT, also led to a highly significant reduction of level and turnover of noradrenaline.

We have studied therefore in further experiments the influence of treatments interfering with catecholamine metabolism on the anorexigenic drug effects. Low doses of α-methyltyrosine proved to antagonize the anorexigenic effect, a result which is in agreement with previous studies of Abdallah[16] and Holtzman and Jewett.[18] Disulfiram was about as active as α-methyltyrosine in antagonizing the effect of *p*-chloroamphetamine, and even more active than α-methyltyrosine in the case of *d*-amphetamine. These results speak in favor of a predominant role of noradrenaline and our results with the catecholamine antagonists phentolamine and haloperidol corroborate this conclusion, at least for amphetamine. The anorexigenic effect of this drug was antagonized by rather low doses of phentolamine as well as by higher doses of haloperidol for which a certain α-adrenolytic action might exist besides the dopamine-antagonistic effect. The antagonism of phentolamine and haloperidol against *p*-chloroamphetamine was weak and less convincing, especially since only the lowest dose of 0·02 mg/kg haloperidol, which must be considered as purely dopamine-antagonistic, had a significant effect. Chlorpromazine, which at least in the higher doses blocks the receptors for all three amines, and the β-adrenolytic drug propranolol even increased the drug-induced anorexia. Our results are thus at variance with studies of Leibowitz,[15] Berger *et al.*[17] and Goldman *et al.*[14] These authors saw an increased food intake after intraventricular or hypothalamic injections of noradrenaline, an effect that could be abolished by phentolamine, whereas propranolol increased food intake further or inhibited the anorexigenic effect of β-stimulating drugs. This discrepancy cannot be explained at present.

A comparison of the amphetamine-antagonistic effects in Figs. 1 and 2 and the corresponding biochemical effects on levels and turnover of hypothalamic monoamines in Table 1 shows that all treatments antagonizing the anorexigenic effect of *d*-amphetamine either reduced the turnover of noradrenaline significantly (*p*-chlorophenylalanine, the higher dose of cyproheptadine, α-methyltyrosine, disulfiram and even haloperidol) or blocked α-receptors (phentolamine). No such correlation can be seen for 5-HT or dopamine. The only weakness of this concept of a mediation of the amphetamine effect by noradrenaline is the apparent lack of a stimulation of noradrenaline turnover by amphetamine itself. However, such an effect might remain undiscovered by our method, if an enhanced turnover of this amine would be confined to minor parts of the hypothalamus, as for example the satiety center. A release of hypothalamic noradrenaline by relatively low doses or concentrations of amphetamine has recently been demonstrated by Strada and Sulser[32] and Ebstein *et al.*[33]

An interpretation of our results with *p*-chloroamphetamine is much more difficult. This drug strongly reduced the turnover of both catecholamines in the hypothalamus, while the turnover of 5-HT only was slightly increased. This may, however, be an underestimate of the true 5-HT turnover as explained under Results. The pronounced decline in hypothalamic noradrenaline during the first hour after administration shows in agreement with findings of Strada and Sulser[32] that the turnover of this amine must have been considerably high during the hour preceding the feeding period. Taking the inhibitory effect of *p*-chloroamphetamine on monoamine oxidase into

consideration,[2,28] our determination of the noradrenaline turnover might have been falsified by an accumulation of noradrenaline which could not be metabolized by this enzyme. Since the anorexigenic effect of the drug was antagonized by *p*-chlorophenylalanine and by the lowest dose of cyproheptadine which should only block tryptaminergic receptors, a participation of 5-HT seems likely in this case, but the effects of α-methyltyrosine and disulfiram cannot be explained by this mechanism. Treatment with these drugs eliminates the small functional pool of freshly synthetized noradrenaline which might be necessary even under conditions of a lowered turnover of this amine, and disulfiram enhanced the turnover of dopamine and might thus counteract the effects of *p*-chloroamphetamine on dopamine metabolism. The antagonistic effects of phentolamine and haloperidol were rather weak when compared to those against *d*-amphetamine, and might either point to a minor role of the catecholamines or even be unspecific. The results with *d*-*p*-chloroamphetamine seem thus to be explainable by a participation of both catecholaminergic and tryptaminergic mechanisms in the mediation of the anorexigenic effect. Similar conditions have been found in previous studies on the locomotor[34] and antinociceptive effects[35] of this drug.

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REFERENCES

1. R. W. FULLER, C. W. HINES and J. MILLS, *Biochem. Pharmac.* **14,** 483 (1965).
2. C. KAERGAARD NIELSEN, M. P. MAGNUSSEN, E. KAMPMANN and H.-H. FREY, *Arch. int. Pharmacodyn.* **170,** 428 (1967).
3. K. OPITZ, *Naunyn-Schmiedeberg's Arch. Pharmak. exp. Path.* **259,** 56 (1967).
4. W. E. FOOTE, M. H. SHEARD and G. K. AGHAJANIAN, *Nature, Lond.* **222,** 567 (1969).
5. W. D. REID, *Br. J. Pharmac.* **40,** 483 (1970).
6. J.-L. DIAZ and M. O. HUTTUNEN, *Psychopharmacologia, Berlin* **23,** 365 (1972).
7. A. F. LAVENSTEIN, E. P. DACANEY, L. LASAGNA and T. E. VAN METRE, *J. Am. med. Ass.* **180,** 912 (1962).
8. R. E. NOBLE, *J. Am. med. Ass.* **209,** 2054 (1969).
9. D. P. MERTZ, *Klin. Wschr.* **47,** 1185 (1969).
10. D. P. MERTZ and M. STELZER, *Klin. Wschr.* **47,** 1189 (1969).
11. D. P. MERTZ and M. STELZER, *Klin. Wschr.* **47,** 1194 (1969).
12. S. JESPERSEN and J. SCHEEL-KRÜGER, *J. Pharm. Pharmac.* **22,** 637 (1970).
13. W. H. FUNDERBURK, J. C. HAZELWOOD, R. T. RUCKART and J. W. WARD, *J. Pharm. Pharmac.* **23,** 468 (1971).
14. H. W. GOLDMAN, D. LEHR and E. FRIEDMAN, *Nature, Lond.* **231,** 453 (1971).
15. S. F. LEIBOWITZ, *Nature, Lond.* **226,** 963 (1970).
16. A. H. ABDALLAH, *Arch. int. Pharmacodyn.* **192,** 72 (1971).
17. B. D. BERGER, C. D. WISE and L. STEIN, *Science, N.Y.* **172,** 281 (1971).
18. S. G. HOLTZMAN and R. E. JEWETT, *Psychopharmacologia, Berlin* **22,** 151 (1971).
19. J. SPENGLER and P. WASER, *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.* **237,** 171 (1959).
20. H.-H. FREY, in *Amphetamines and Related Compounds*, p. 343. Raven Press, New York (1970).
21. E. SANDERS-BUSH, J. A. BUSHING and F. SULSER, *Eur. J. Pharmac.* **20,** 385 (1972).
22. J. T. LITCHFIELD and F. WILCOXON, *J. Pharmac. exp. Ther.* **96,** 99 (1949).
23. F. P. MILLER, R. H. COX, W. R. SNODGRASS and R. P. MAICKEL, *Biochem. Pharmac.* **19,** 435 (1970).
24. G. CURZON and A. R. GREEN, *Br. J. Pharmac.* **39,** 653 (1970).
25. C. C. CHANG, *Int. J. Neuropharmac.* **3,** 643 (1964).
26. N. H. NEFF, T. N. TOZER and B. B. BRODIE, *J. Pharmac. exp. Ther.* **158,** 214 (1967).
27. B. B. BRODIE, E. COSTA, A. DLABAC, N. H. NEFF and H. H. SMOOKLER, *J. Pharmac. exp. Ther.* **154,** 493 (1966).

28. R. W. Fuller and C. P. Walters, *Biochem. Pharmac.* **14,** 159 (1965).
29. G. A. Johnson, E. G. Kim and S. J. Boukma, *J. Pharmac. exp. Ther.* **180,** 539 (1972).
30. M. G. Baxter, A. A. Miller and F. E. Soroko, *Br. J. Pharmac.* **39,** 229 P (1970).
31. C. A. Stone, H. C. Wenger, C. T. Ludden, J. M. Stavorski and C. A. Roos, *J. Pharmac. exp. Ther.* **131,** 73 (1961).
32. S. J. Strada and F. Sulser, *Eur. J. Pharmac.* **15,** 45 (1971).
33. R. P. Ebstein, B. S. Ebstein, D. Samuel and B. D. Berger, *J. Neurochem.* **19,** 2703 (1972).
34. H.-H. Frey and M. P. Magnussen, *Biochem. Pharmac.* **17,** 1299 (1968).
35. B.-D. Görlitz and H.-H. Frey, *Eur. J. Pharmac.* **20,** 171 (1972).