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EFFECT OF DIETHYLAMINOETHYL DIPHENYLPROPYLACETATE HYDROCHLORIDE (SKF-525A) ON THE NOREPINEPHRINE-DEPLETING ACTIONS OF *d*-AMPHETAMINE

G. A. CLAY,* A. K. CHO† and M. ROBERFROID‡

Laboratory of Chemical Pharmacology, National Heart and Lung Institute, National Institutes of Health, Bethesda, Md. 20014, U.S.A.

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Abstract—The effect of SKF-525A (diethylaminoethyl diphenylpropylacetate hydrochloride) on norepinephrine depletion caused by amphetamine was examined. SKF-525A substantially reduced the depletion of cardiac norepinephrine caused by *d*- and *l*-amphetamine but had no effect on the depletion in the brain caused by *d*-amphetamine. The levels of *d*-amphetamine in both tissues were substantially elevated by SKF-525A while metabolite levels were reduced. Since SKF-525A was found to have no direct action on the nerve ending, the results suggest that the depleting actions of *d*-amphetamine on the heart and brain are different, and that the effect on the heart is due to one or more of the metabolites of *d*-amphetamine.

IT IS KNOWN that large doses of *d*-amphetamine cause a substantial depletion of the norepinephrine (NE) stores in various tissues.[1–3] In this laboratory Brodie *et al.*[4] showed that doses of 5–10 mg/kg of *d*- or *l*-amphetamine caused a progressive depletion of the stores of NE in the heart of the rat, with maximum depletion occurring approximately 6 hr after the administration of the drug. Since two *d*-amphetamine metabolites, α-methyltryamine (*p*-hydroxyamphetamine) and α-methyloctopamine (*p*-hydroxynorephedrine) can also deplete NE stores, they have been implicated in this pharmacological action of *d*-amphetamine.[3,5,6]

In the present study SKF-525A (diethylaminoethyl diphenylpropylacetate hydrochloride) was used to examine the role of amphetamine metabolism in the depletion of NE stores in the rat heart and brain. Tissue levels of *d*-amphetamine and two of its metabolites, *p*-hydroxyamphetamine and *p*-hydroxynorephedrine, were determined in an attempt to relate tissue levels of these amines with pharmacological events.

METHODS AND MATERIALS

Norepinephrine assays. Male Sprague–Dawley rats, 180–200 g, were used in this study. The animals were killed by cervical dislocation and the tissues removed and frozen before analysis. Tissue levels of NE were determined by the procedure of Laverty and Taylor.[7] The uptake of [^{3}H]Ne by the heart was determined in animals

* Present address: Department of Pharmacology, Bowman Gray School of Medicine, Winston-Salem, N.C., U.S.A.

† Present address: Department of Pharmacology, School of Medicine, University of California, Los Angeles, Calif., U.S.A.

‡ Present address: Institut de Pharmacie, Universite Catholique de Louvain, 4, Van Evenstraat, Louvain, Belgium

given an i.v. injection of 1 μc (169 ng) of [^{3}H]NE 20 min before killing. The [^{3}H]NE was assayed by the method of Bogdanski and Brodie[8] using liquid scintillation spectrometry.

Assay for d-*amphetamine and its basic metabolites.* Tissue levels of *d*-amphetamine and its metabolites were determined in animals given *d*-amphetamine-^{3}H (20 μc/animal). The tissues were homogenized in 9 vol. of 0·1 N HCl and the protein was precipitated with 0·2 ml of 70% $HClO_4$. The mixture was centrifuged at 12,000 *g* for 30 min and an aliquot of 3–5 ml of supernatant was adjusted to pH 6·5 with 0·5 M sodium phosphate (pH 6·5) and in sodium hydroxide. The neutralized aliquot was then passed through a 1-ml column of Dowex 50 × 4 previously adjusted to pH 6·5 with the sodium phosphate buffer. After washing the column with 5 ml of the phosphate buffer, then 5 ml of water, the bases were eluted with 6 ml of 6 M ammonium hydroxide. Amphetamine was extracted by shaking the eluate with 30 ml of benzene. A 10-ml aliquot of the benzene extract was counted as amphetamine.

The aqueous phase was washed with benzene to remove traces of amphetamine and a 5-ml aliquot was treated with 0·25 ml of 2% sodium periodate to oxidize the *p*-hydroxynorephedrine to *p*-hydroxybenzaldehyde. The oxidation was stopped with 10% bisulfate (0·25 ml) and the reaction mixture made acidic (pH < 2) with concentrated HCl. The acid solution was then extracted with 20 ml of ether and the ether extract concentrated to ½ volume by evaporation with a stream of air in a counting vial and assayed.

The aqueous phase was again made basic with concentrated ammonium hydroxide and about 1 g each of sodium chloride and sodium carbonate were added. The resulting mixture was extracted with 15 ml of *n*-butanol and 10 ml of the butanol extract counted as *p*-hydroxyamphetamine. The recoveries in this procedure were 86–91 per cent when carrier amines (100 μg) were added to the $HClO_4$ supernatant just before absorption into the resin. The scintillation phosphor was a PPO–POPOP mixture containing 8 g PPO and 100 mg POPOP in 1 l. of toluene, and 10 ml of this mixture was added to each counting vial.

Assay for p-*hydroxynorephedrine-*^{3}H. In animals where *p*-hydroxynorephedrine-^{3}H was given directly, this compound was assayed essentially by the procedure described above except that the steps for the prior extraction of amphetamine and the subsequent extraction of *p*-hydroxyamphetamine were omitted. The tissues were homogenized and passed through the Dowex 50 column as described. The eluate was immediately oxidized, acidified, and the *p*-hydroxybenzaldehyde extracted into ether and counted.

Chemicals. Both *d* and *l*-amphetamine were purchased as HCl and H_2SO_4 salts from K & K Laboratories. *d*-Amphetamine-^{3}H (g. l., 4·9 c/m-mole) and *d, l-p*-hydroxynorephedrine-^{3}H (g. l., 6 c/m-mole), were obtained from New England Nuclear Corp. *d-p*-Hydroxyamphetamine (Paredrine) and SKF-525A (diethylaminoethyl diphenylpropylacetate hydrochloride) were kindly supplied by Dr. Glenn Ullyot of Smith, Kline & French Laboratories. All doses are expressed as the free base except for SKF-525A which is expressed as the hydrochloride. The drugs were given in saline by i.p. injection.

RESULTS

Depletion of norepinephrine caused by amphetamine and its metabolites. The effect of various doses of *d*-amphetamine on the NE levels in the heart and brain 6 hr after

administration of the drug is shown in Fig. 1. The dose of *d*-amphetamine required for 40 per cent depletion of NE in the heart was 5 mg/kg. The depletion curve for brain NE stores differed from the heart and had a plateau at 5 mg/kg. *l*-Amphetamine and *d*-amphetamine were equally potent in the rat heart (Table 1), but unlike *d*-amphetamine, *l*-amphetamine did not cause significant depletion of brain NE in doses up to 10 mg/kg.

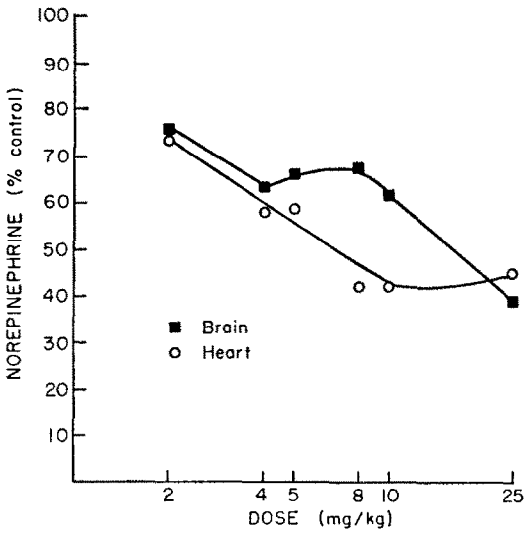

FIG. 1. Reduction in tissue levels of norepinephrine 6 hr after varying doses of *d*-amphetamine. Each point is based on experiments performed with at least six animals per point. The standard error of the absolute NE levels ranged from 3 to 10 per cent of the determined level.

The effect of SKF-525A (40 mg/kg) on the time course of NE depletion in heart and brain by 5 mg/kg *d*-amphetamine is shown in Fig. 2. The release of cardiac stores of NE by *d*-amphetamine was initially blocked by SKF-525A while the release of brain NE by *d*-amphetamine was unaffected. Since the depletion of brain NE did not

TABLE 1. DEPLETION OF NOREPINEPHRINE (NE) BY *d*- AND *l*-AMPHETAMINE*

Dose (mg/kg)	Heart NE levels (ng/g)		Brain NE levels (ng/g)	
	d-amphetamine	*l*-amphetamine	*d*-amphetamine	*l*-amphetamine
0	1164 ± 13		489 ± 15	
2·5	970 ± 32	983 ± 39	399 ± 18	489 ± 17
5	774 ± 31	833 ± 81	312 ± 11	462 ± 14
10	635 ± 35	605 ± 28	324 ± 13	518 ± 14

* Groups of five animals were killed 6 hr after the indicated doses of amphetamine isomers. The tissues were frozen overnight, then analyzed for norepinephrine as described in Methods. All values are given ± S.E.

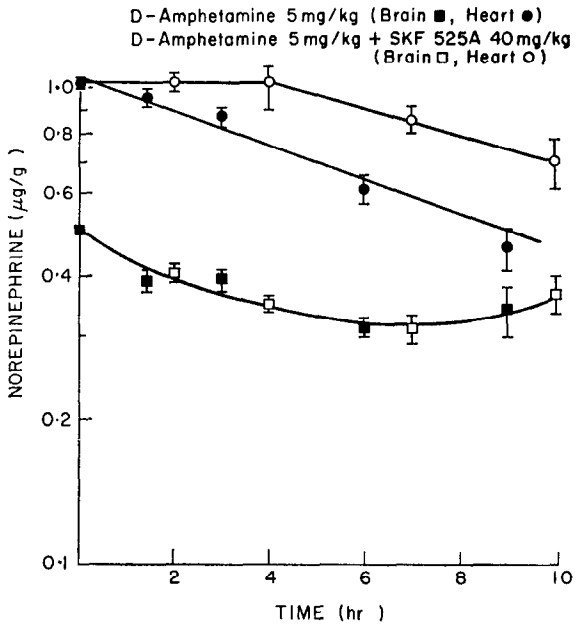

FIG. 2. Effect of diethylaminoethyl diphenylpropylacetate hydrochloride (SKF-525A) (40 mg/kg, 1 hr before *d*-amphetamine) on the rate of depletion of norepinephrine after *d*-amphetamine (5 mg/kg, i.p.). Each point is the mean of at least four animals. Vertical bars represent standard error.

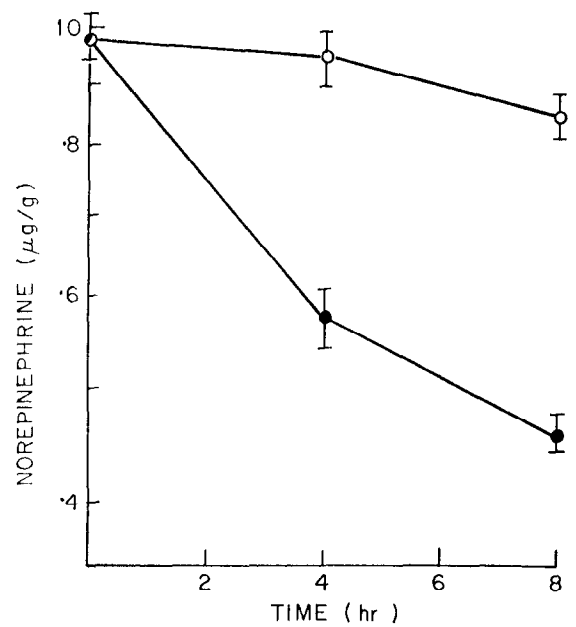

FIG. 3. Effect of diethylaminoethyl diphenylpropylacetate hydrochloride (SKF-525A) (40 mg/kg, 1 hr before *l*-amphetamine) on the rate of depletion of norepinephrine after *l*-amphetamine (5 mg/kg, i.p.). Open circles represent SKF-525A pretreated animals and closed circles represent values from animals treated with *l*-amphetamine alone. Each value is the mean of at least four animals.

appear to change with *d*-amphetamine dose between 5 and 10 mg/kg, the action of SKF-525A on a 2 mg/kg dose of *d*-amphetamine was also examined. This dose caused a 20 per cent depletion of brain NE stores and again SKF-525A did not alter the response.

Changes caused by SKF-525A (40 mg/kg) on the time course of depletion of NE by *l*-amphetamine (5 mg/kg) are shown in Fig. 3. The release of cardiac NE was again blocked by SKF-525A. Brain NE was not depleted by *l*-amphetamine and was unaffected by pretreatment with SKF-525A.

Figure 4 illustrates the effect of *p*-hydroxyamphetamine (5 mg/kg) on NE levels in rat heart and brain. *p*-Hydroxyamphetamine depleted NE more rapidly and completely than did *d*-amphetamine at comparable doses. At 6 hr the *p*-hydroxyamphetamine-treated animals had lost about 65 per cent of heart and brain NE, while *d*- and *l*-amphetamine treated animals had lost about 40 per cent of their NE stores. While SKF-525A blocked the NE depletion caused by *d*- or *l*-amphetamine in the rat heart, it had no effect on the depletion caused by *p*-hydroxyamphetamine.

The depletion of cardiac stores of NE after different doses of *p*-hydroxyamphetamine and *p*-hydroxynorephedrine is compared in Table 2. A dose of 0·5 mg/kg of *p*-hydroxyamphetamine depleted cardiac NE stores by about 26 per cent. In contrast, a comparable dose of *p*-hydroxynorephedrine depleted them about 50 per cent while the level of the drug was 113 ng/g.

SKF-525A (40 mg/kg) given alone had no effect on heart and brain NE levels. This compound also had no effect on the uptake *in vivo* of tracer amounts of [^{3}H]NE.

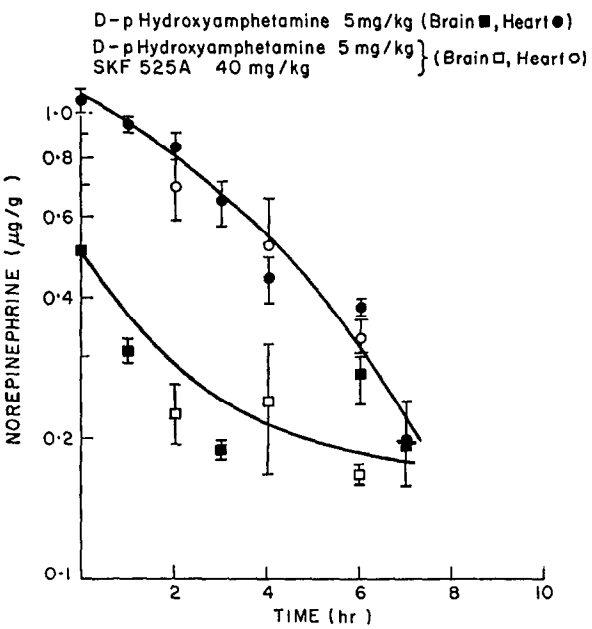

FIG. 4. Effect of diethylaminoethyl diphenylpropylacetate hydrochloride (SKF-525A) (40 mg/kg, 1 hr before *p*-hydroxyamphetamine) on norepinephrine depletion caused by *p*-hydroxyamphetamine (5 mg/kg, i.p.). Each value is the mean of at least four animals.

TABLE 2. EFFECTS OF *p*-HYDROXYAMPHETAMINE AND *p*-HYDROXYNOREPHEDRINE ON HEART NOREPINEPHRINE LEVELS*

p-Hydroxyamphetamine(HA)			*p*-Hydroxynorephedrine(HN)			
Dose of HA (mg/kg)	Norepinephrine levels	Per cent control	Dose of HN (mg/kg)	Norepinephrine levels	Per cent control	OH Norephedrine levels (ng/g)
0	1012 ± 56		0	1012 ± 56		
			0·25	851 ± 87	84	87 ± 15
0·5	752 ± 40	74	0·5	569 ± 46	56	113 ± 38
1·0	622 ± 82	61	1·0	393 ± 27	39	262 ± 31
2·5	569 ± 75	56	2·5	260 ± 19	26	336 ± 33
			5·0	218 ± 12	22	490 ± 12

* Groups of four animals were killed 4 hr after an i.p. injection of the drug at the doses indicated. The hearts were frozen overnight then analyzed for norephinephrine as described in Methods. All values are given ± S.E.

Under comparable conditions desipramine (20 mg/kg) causes a substantial reduction in NE uptake (Table 3).

Effect of diethylaminoethyl diphenylpropylacetate hydrochloride (SKF-525A) on d-amphetamine metabolism. The tissue levels of amphetamine and its basic metabolites 2 hr after administration of various doses of the drug are shown in Table 4. The brain had higher levels of amphetamine than the heart but lower levels of metabolites. The levels of *p*-hydroxynorephedrine in the heart reached maximum levels at a dose of 2·5 mg/kg, while the levels of amphetamine and *p*-hydroxyamphetamine continued to rise in proportion to dose. Brain levels of *p*-hydroxynorephedrine were also less than those found in the heart, but the levels of all three compounds rose in proportion to dose. SKF-525A pretreatment increased heart and brain levels of *d*-amphetamine 2- to 4-fold, but did not alter the rate of disappearance of amphetamine from either tissue (Figs. 5 and 6). Nevertheless, SKF-525A decreased the tissue levels of *p*-hydroxynorephedrine, the only metabolite detected in the SKF-525A-treated animals (Table 5).

TABLE 3. EFFECT OF DRUGS ON THE UPTAKE OF [^{3}H]-NOREPINEPHRINE*

Drug	Norepinephrine taken up (dis./min/g heart)
Control	6620 ± 345
SKF-525A (40 mg/kg, i.p.)	5908 ± 328
DMI (20 mg/kg, i.p.)	1484 ± 98

* Groups of six rats were given an iv. injection of 1 μc of norepinephrine (169 ng) 1 hr after the administration of the SKF-525A or DMI. Twenty min later the animals were killed and the hearts assayed. Values are given ± S.E.

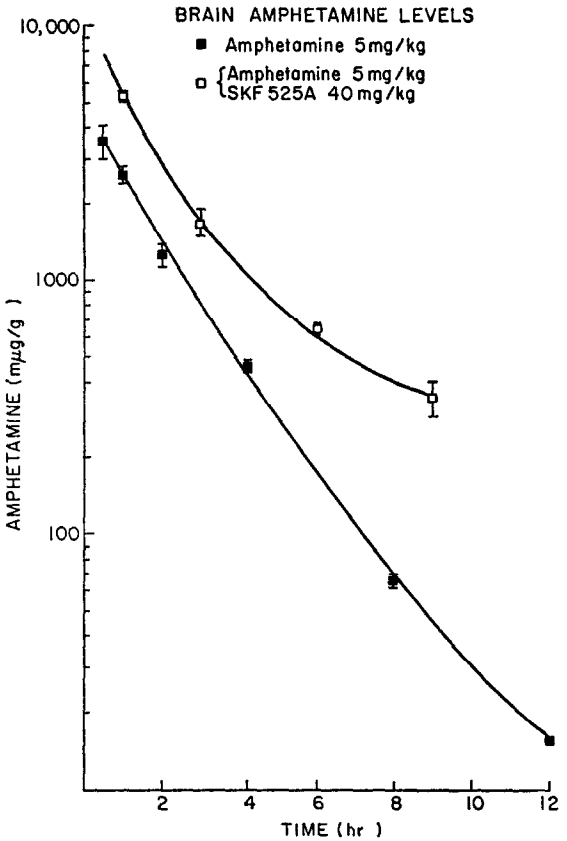

FIG. 5. Effect of diethylaminoethyl diphenylpropylacetate hydrochloride (SKF-525A) (40 mg/kg, 1 hr pretreatment) on brain levels of *d*-amphetamine after administration of *d*-amphetamine-^{3}H (5 mg/kg). Each value is the mean of six animals.

TABLE 4. LEVELS OF AMPHETAMINE AND ITS BASIC METABOLITES IN RAT HEART AND BRAIN*

Dose of amphetamine (mg/kg)	Amphetamine levels		*p*-Hydroxyamphetamine levels		*p*-Hydroxynorephedrine levels	
	Heart (ng/g)	Brain (ng/g)	Heart (ng/g)	Brain (ng/g)	Heart (ng/g)	Brain (ng/g)
0·5	42 ± 4	103 ± 6	10 ± 1	N.D. (<5)	53 ± 6	4 ± 0·4
1·0	66 ± 1	156 ± 26	12 ± 1	N.D. (<5)	65 ± 9	7·2 ± 1
2·5	172 ± 15	418 ± 39	27 ± 3	N.D. (<5)	122 ± 14	17 ± 1
5·0	375 ± 51	886 ± 100	31 ± 4	10 ± 1	112 ± 3	31 ± 2
10·0	1056 ± 125	2384 ± 298	68 ± 7	25 ± 3	109 ± 13	64 ± 5
15·0	2283 ± 202	4973 ± 997	113 ± 6	54 ± 5	88 ± 10	103 ± 2

* Groups of at least four animals were given i.p. injections of *d*-amphetamine containing 20 μc amphetamine-^{3}H. Two hr later the animals were killed by cervical dislocation and the tissues collected and assayed as described in Methods. N.D. = not detectable. All values are given ± S.E.

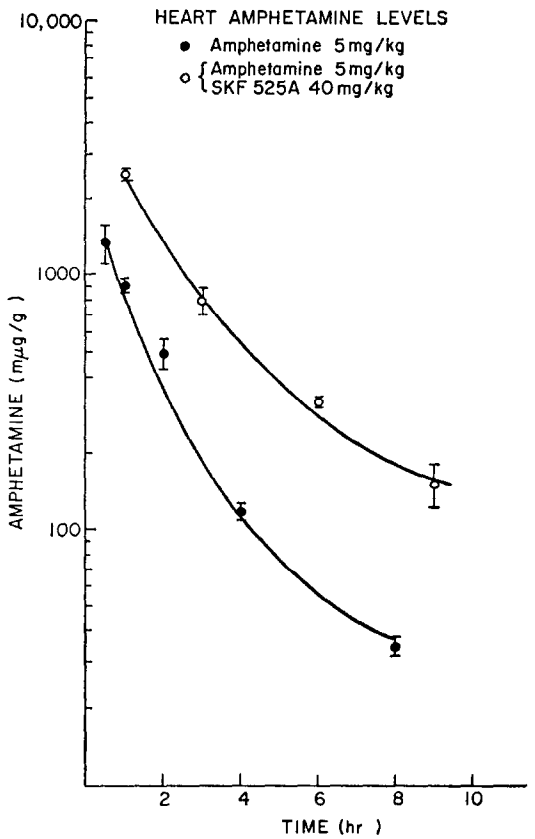

FIG. 6. Effect of diethylaminoethyl diphenylpropylacetate hydrochloride (SKF-525A) (40 mg/kg, 1 hr pretreatment) on heart levels of *d*-amphetamine after administration of *d*-amphetamine-^{3}H (5 mg/kg) Each value is the mean of six animals.

DISCUSSION

Although *d*-amphetamine has been shown to cause depletion of NE when given in fairly large doses (see Fig. 1), the mechanism by which this depletion occurs is not known. Fischer *et al.*[5] postulated that the metabolites of *d*-amphetamine, α-methyltyramine and α-methyloctopamine might be important in causing this depletion. One approach to studying the role of the metabolites in the depletion has been to inhibit amphetamine metabolism with desipramine. In a series of experiments with desipramine, Lewander[2] has concluded that amphetamine metabolism is not necessary for depleting NE in heart and brain. However, Groppetti and Costa[3] have concluded from experiments with desmethylimipramine (DMI) and amphetamine that the metabolites of amphetamine are responsible for cardiac depletion. Since DMI has a direct action on the nerve ending[1] (Table 3), the interaction between these drugs at the nerve ending is difficult to interpret.

It was hoped that SKF-525A would be more appropriate for examining the role of metabolites since it has no action on the release of NE (Fig. 4) and has no effect on the uptake of [^{3}H]NE (Table 3). In addition, non-maximal depleting doses of

TABLE 5. LEVELS OF AMPHETAMINE AND METABOLITES IN HEART AND BRAIN AFTER AMPHETAMINE (5 mg/kg) AND SKF-525A (40 mg/kg)*

Time (hr)	Amphetamine levels		OH-Amphetamine levels		OH-Norephedrine levels	
	Control (ng/g ± S.E.)	SKF-525A (ng/g ± S.E.)	Control (ng/g ± S.E.)	SKF-525A	Control (ng/g ± S.E.)	SKF-525A (ng/g ± S.E.)
Heart						
1	911 ± 39	2528 ± 135	39 ± 5	N.D.	50 ± 4	6 ± 2
2	497 ± 66		31 ± 1		70 ± 6	
3	174 ± 28	796 ± 65		N.D.	66 ± 6	18 ± 2
4	118 ±		22 ± 3		82 ± 3	
6	42 ± 8	323 ± 19		N.D.	92 ± 11	22 ± 5
8	35 ± 6		38 ±		100 ± 20	
9		155 ± 32		N.D.		20 ± 5
12	8 ± 1		24 ±		92 ± 4	
Brain						
1	3120 ± 563	5301 ± 243	8·4 ± 1	N.D.	22 ± 2	3·2 ± 1
2	1258 ± 170		18 ± 2		30 ± 2	
3	421 ± 79	1835 ± 137		N.D.	21 ± 2	5 ± 1
4	461 ± 103		16 ± 2		28 ± 2	
6	118 ± 36	645 ± 33		N.D.	20 ± 2·4	5 ± 1
8	67 ± 34		N.D.		25 ± 3	
9		350 ± 56		N.D.		6 ± 1
12	16 ± 1				26 ± 2	

* The animals were killed at the indicated time after *d*-amphetamine-^{3}H (20 μc). The SKF-525A was given 1 hr before the amphetamine. Each value is the mean of at least six animals ± S.E. N.D. = not detectable.

d-amphetamine were employed for the study so that the depletion would be sensitive to changes in tissue levels of the amines resulting from altered metabolism. As shown in Figs. 5 and 6, SKF-525A pretreatment increased tissue levels of amphetamine by two to four times in heart and brain. However, the depletion of NE caused by *d*-amphetamine was prevented in the heart and unaffected in the brain. Because of the apparent plateau in the *d*-amphetamine dose depletion curve for the brain (Fig. 1), the actions of SKF-525A on brain NE depletion were also examined at a *d*-amphetamine dose of 2 mg/kg. Again SKF-525A did not alter the depletion of brain NE.

Because of its susceptibility to SKF-525A, the depleting actions of *d*-amphetamine on cardiac NE were examined further by comparing NE levels with tissue levels of the parent drug and the two basic metabolites. When the levels of amphetamine and its metabolites in the heart were examined 2 hr after different doses of amphetamine a plateau in the level of *p*-hydroxynorephedrine was noted. The plateau occurs at a dose of 2·5 mg/kg (Table 4), while levels of both amphetamine and *p*-hydroxyamphetamine continue to rise proportionally with dose. A comparison with the dose–response curve for depletion (Fig. 1) indicates that the heart level of *p*-hydroxynorephedrine is unchanged in the dose range of amphetamine causing 20–50 per cent depletion of NE. This would suggest that *p*-hydroxyamphetamine plays an important role in the depletion since it is the metabolite whose levels increased with dose.

Further evidence for the role of *p*-hydroxyamphetamine in depletion is provided by the apparent lack of stereospecificity in this action of amphetamine. The *d*- and

l-isomers of amphetamine are equipotent in their depletion of cardiac NE, but their metabolic pathways differ. Both *d*- and *l*-isomers are *p*-hydroxylated equally[9] but only the *d*-isomer is then β-hydroxylated to form *p*-hydroxynorephedrine.[10] Since *d*- and *l*-*p*-hydroxyamphetamine are formed from the two isomers, respectively, they seem to be the likely metabolites causing depletion of cardiac NE.

However, the heart NE levels in SKF-525A-*d*-amphetamine-treated animals show a slight but significant decline 10 hr after *d*-amphetamine (Fig. 2). At this time the only metabolite detected was *p*-hydroxynorephedrine (Table 5), but its level was too low to account for the 20 per cent depletion (Table 2). Animals treated with SKF-525A and *l*-amphetamine also show this decline in heart NE after 10 hr (Fig. 3). While these observations are at variance with the conclusions stated above, they could reflect an action of SKF-525A. One possibility is the resumption of *p*-hydroxylation, since the inhibition of drug metabolism caused by SKF-525A diminishes with time and 12–15 hr after injection there is an actual increase in drug metabolism rate.[11] However, the small amount of metabolite present at this time (Table 5) makes this unlikely. Alternatively, another metabolite capable of lowering endogeneous NE levels could be formed in larger amounts as a result of the inhibition of the normal pathways for amphetamine metabolism by SKF-525A.

These data indicate that the depletion of NE in the heart caused by amphetamine is due to its metabolite(s). In contrast, the mechanism of depletion of NE in the brain by this drug is totally different. Brain NE stores are depleted only by the *d*-isomer of amphetamine, while heart NE is depleted by both *d*- and *l*-amphetamine. Furthermore, the depletion of brain NE by *d*-amphetamine is not prevented by SKF-525A as is the depletion on the heart. This difference in the actions of SKF-525A is not due to a selective effect on metabolism in the heart, since the levels of metabolites in both tissues were decreased by a factor of 5 while amphetamine levels were increased two to four times.

Breese *et al.*[6] have recently reported that intracisternal injections of *d*-amphetamine do not deplete brain NE and have concluded that a metabolite is responsible for the depletion of brain NE by *d*-amphetamine. The results described here do not preclude the possibility that the amphetamine-induced depletion of brain NE is mediated by an active metabolite but suggest that the metabolite involved is neither *p*-hydroxyamphetamine nore *p*-hydroxynorephedrine. Dring *et al.*[12] have reported the isolation of small amounts of norephedrine in the urine of rats given *d*-amphetamine. Since *d*-amphetamine is a substrate for dopamine β-oxidase[13] *in vitro*, it is possible that this compound is formed in the brain and causes depletion. Initial experiments conducted in this laboratory to find norephedrine in the brain after amphetamine administration were negative,* but quantities less than 1 per cent of the level of amphetamine would not be detected by the technique used.

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