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EFFECT OF DESMETHYLIMIPRAMINE, IPRINDOLE AND DL-ERYTHRO-α-(3,4-DICHLOROPHENYL)-β-(t-BUTYL AMINO) PROPANOL HCl* ON THE METABOLISM OF AMPHETAMINE

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Abstract—The ability of a compound to block norepinephrine accumulation or potentiate amphetamine action has been used in the past to evaluate antidepressant activity. The results presented here indicate the limitations of this approach. DL-erythro-α-(3,4)-dichlorophenyl)-β-(t-butyl amino) propanol HCl (B.W. 65-54), desmethylimipramine and iprindole, three compounds having antidepressant activity, all potentiate amphetamine action, but this effect is due to inhibition of amphetamine metabolism. Iprindole, a potent antidepressant in man, and B.W. 65-54 do not affect the levels of ^{3}H-norepinephrine in the rat heart while, as is well known, desmethylimipramine does.

RECENT studies in our laboratory have indicated that DL-erythro-α-(3,4-dichlorophenyl)-β-(t-butyl amino) propanol HCl (B.W. 65-54§), a compound structurally (Fig. 1) similar to dichloroisoproterenol, potentiates and prolongs the locomotor activity of amphetamine. Sulser *et al.*[1] and Consolo *et al.*[2] have shown that the potentiation of the CNS activity of amphetamine by desmethylimipramine results from the inhibition of amphetamine metabolism. Desmethylimipramine and imipramine

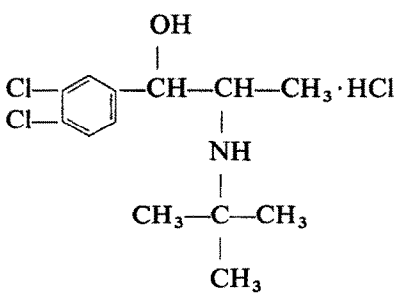

FIG. 1. Structural formula for DL-erythro-α-(3,4-dichlorophenyl)-β-(t-butyl amino) propanol HCl (B.W. 65-54).

* B. W. 65-54.

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§ B.W. 65-54 was synthesized and kindly supplied to us by Dr. N. Mehta of the Organic Chemistry Dept., Burroughs Wellcome & Company (USA), Inc. A manuscript by Dr. Mehta describing the synthesis of B.W. 65-54 is now in preparation.

are potent inhibitors of the metabolism of several drugs.[3–6] Iprindole, a compound similar in structure to desmethylimipramine, has also been found to enhance the pharmacological effects of amphetamine[7] and to possess potent antidepressant activity in man.[8–12] The present investigation describes the effect of B.W. 65-54, desmethylimipramine and iprindole on the metabolism of amphetamine, on the accumulation of ^{3}H-norepinephrine by the rat heart, and on the pharmacological effects of hexobarbital and zoxazolamine. The results also demonstrate that desmethylimipramine is a nonspecific inhibitor of microsomal hydroxylation, whereas iprindole and B.W. 65-54 are more specific inhibitors, since they inhibit amphetamine hydroxylation, but not hexobarbital and zoxazolamine metabolism.

METHODS

Adult male rats (Blue Spruce Farms) weighing 190–210 g were used in all the experiments described. ^{3}H-amphetamine (generally labeled; specific activity, 4·23 c/m-mole) was obtained from the New England Nuclear Corp. and was shown to be pure by chromatography in a butanol–acetic acid–water (4:1:1) ascending system.

^{3}H-amphetamine brain levels. Animals were injected with 2 mg/kg of ^{3}H-amphetamine intraperitoneally (10 μc/0·4 mg). The animals were killed, their brains were removed and immediately frozen on dry ice. The brains were homogenized in 5 vol. of 0·4 N perchloric acid and the radioactive amphetamine was extracted and assayed according to the method of Glowsinski *et al.*[13] An aliquot of the toluene–isoamyl alcohol (50:1) phase was added directly to scintillation vials having 10 ml of scintillation mixture containing 0·5 per cent PPO*, and 0·01 per cent POPOP* in toluene. The radioactivity was determined in a Packard Tri-Carb liquid scintillation counter.

^{3}H-amphetamine levels in the whole rat. Amphetamine was administered to rats as described above. The animals were killed 3 hr later by a blow on the head and were immediately homogenized in a Waring blendor in 3 vol. of distilled water. The equivalent of 1 g of rat tissue (4 ml homogenate) was transferred to a 50-ml shaking tube containing 1 ml of 2 N NaOH and extracted for 15 min with 25 ml benzene–isoamyl alcohol (1·5%) according to the method of Maickel.[14] The organic phase was transferred to another 50-ml shaking tube and washed with 5 ml of 0·5 N NaOH. A 20-ml aliquot of the washed organic phase was then extracted for 15 min with 1 ml of 1 N HCl. The organic phase was discarded. The aqueous phase was alkalinized with 1·5 ml of 2 N NaOH, and extracted with 6 ml toluene–isoamyl alcohol (50:1). A 4-ml aliquot of the organic phase was transferred directly to vials containing scintillation mixture, and the radioactivity was determined as described above.

^{3}H-norepinephrine heart levels. ^{3}H-norepinephrine (50 μc/kg; specific activity, 7·07 c/m-mole) was administered intravenously to rats. The rats were killed 4 hr later by a blow on the head, their hearts were removed and homogenized in 9 vol. of methanol. The homogenate was centrifuged and a 1-ml aliquot of the supernatant was placed directly in a vial containing 10 ml of Bray's[15] liquid scintillation mixture. The radioactivity was determined in a Packard Tri-Carb liquid scintillation counter. The radioactivity in the heart at this time has been shown to be present exclusively as norepinephrine.[16]

* PPO = 2,5-diphenylopazole; POPOP = 1,4-*bis*-2-(4-methyl-5-phenylopazolyl) benzene.

Effect of desmethylimipramine, iprindole and B.W. 65-54 on ^{3}H-amphetamine brain levels. To determine the mechanism through which B.W. 65-54 potentiates the locomotor activity of amphetamine, rats were injected with either saline or B.W. 65-54 (15 mg/kg, i.p.) 30 min prior to the administration of ^{3}H-amphetamine (2 mg/kg; 10 μc/rat). Rats were killed at 30, 60, 120 and 240 min after the administration of ^{3}H-amphetamine and the amount of amphetamine in the brain was determined (Fig. 2).

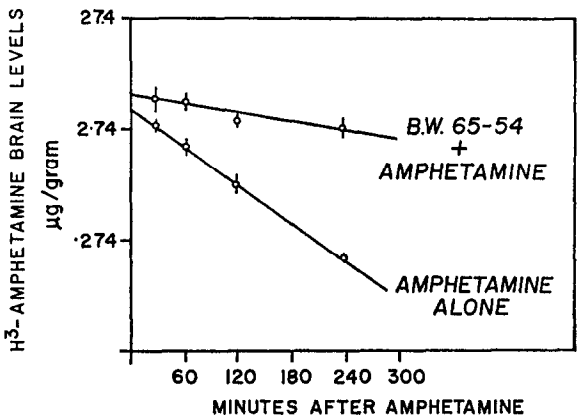

FIG. 2. Effect of B.W. 65-54 on ^{3}H-amphetamine levels in the rat brain. Adult male rats (200 g) were given either saline or B.W. 65-54 (15 mg/kg) 30 min prior to the administration of tritiated amphetamine (2 mg/kg; 10 μc/200 g rat). The animals were killed, their brains removed and amphetamine was determined by the method of Glowinski *et al.*[13] as described in Methods. Each point represents the average value for four rats. The range is also indicated.

When amphetamine was administered alone, the drug disappeared from the brain with a half-life of 50 min. In the group pretreated with B.W. 65-54, the decrease of amphetamine from the brain was much slower ($T_{1/2}$ about 210 min). The rats injected with amphetamine alone were huddled together and demonstrated marked ptosis 4 hr after amphetamine, whereas the animals pretreated with B.W. 65-54 prior to amphetamine administration were stimulated, showed excessive locomotor activity, and exhibited marked exophthalmos. The results in Table 1 show that the brain level of amphetamine was ten times greater in animals pretreated with B.W. 65-54, desmethylimipramine or iprindole than in rats given amphetamine alone. As little as 1–2 mg/kg of any one of these three compounds inhibited amphetamine metabolism (Table 1).

Effect of desmethylimipramine and B.W. 65-54 on ^{3}H-amphetamine levels in the whole rat. Since several factors could theoretically be responsible for the increased brain levels of ^{3}H-amphetamine observed after treatment with various drugs, the effect of B.W. 65-54 and desmethylimipramine on the level of radioactive amphetamine in the whole animal was determined. Both desmethylimipramine and B.W. 65-54 inhibited ^{3}H-amphetamine metabolism in the whole rat (Table 2). An aliquot of the homogenate was extracted as described in Methods. The solvent was evaporated to dryness, and the residue was dissolved in methanol and chromatographed in a butanol–acetic acid–water (4:1:1) system (ascending). The extractable radioactivity had the same mobility as authentic amphetamine (R_f 0·83), while authentic *p*-hydroxyamphetamine, the

TABLE 1. EFFECT OF B.W. 65-54, DESMETHYLIMIPRAMINE AND IPRINDOLE ON BRAIN LEVELS OF AMPHETAMINE IN THE ADULT MALE RAT*

Pretreatment	Dose (mg/kg, i.p.)	Brain amphetamine level (μg/g ± S.E.)
Saline	—	0·28 ± 0·02
Desmethyl imipramine	2	1·86 ± 0·20
	15	3·58 ± 0·26
Iprindole	2	2·86 ± 0·19
	15	2·81 ± 0·27
B.W. 65-54	1	1·82 ± 0·16
	2	2·61 ± 0·17
	10	3·19 ± 0·20
	15	3·12 ± 0·18

* Saline, desmethylimipramine, iprindole or B.W. 65-54 was administered to adult male rats (200 g) in the specified doses 30 min prior to the administration of tritiated amphetamine (2 mg/kg; 10 μc/rat). The animals were killed after 4 hr, their brains were removed, and the amphetamine levels were assayed according to the method of Glowinski *et al.*[13] as described in Methods. Each value represents the average of at least six animals.

TABLE 2. EFFECT OF DESMETHYLIMIPRAMINE AND B.W. 65-54 ON THE METABOLISM OF AMPHETAMINE IN THE RAT*

Pretreatment	Amphetamine level in whole rat (μg/g ± S.E.)	% of injected dose metabolized†
Saline	0·39 ± 0·06	93
Desmethylimipramine	2·69 ± 0·24	52
B.W. 65-54	2·23 ± 0·31	60

* Saline, desmethylimipramine (15 mg/kg) or B.W. 65-54 (15 mg/kg) was administered to adult male rats (200 g) 30 min prior to the administration of tritiated amphetamine (2 mg/kg; 10 μc/rat). The animals were killed 3 hr later and the whole rat was homogenized in a Waring blendor and assayed for ^{3}H-amphetamine by a modification of the method of Maickel[14] as described in Methods. Each value represents the mean of four rats.

† The zero time value was obtained by injecting rats with the same dose of amphetamine and killing them immediately. This value was taken as 0% metabolism.

major metabolite of amphetamine in the rat, had an R_f of 0·67. No other radioactive peaks were present on the chromatogram.

Effect of desmethylimipramine and B.W. 65-54 *on hexobarbital sleeping time and zoxazolamine-induced loss of righting reflex.* Imipramine and desmethylimipramine are potent inhibitors of the liver microsomal enzyme systems which metabolize many

drugs.[3–6,17] Both compounds also potentiate the pharmacologic effects of hexobarbital.[3] To determine if B.W. 65-54 had any effect on hexobarbital sleeping time, adult male rats were pretreated with saline, desmethylimipramine (15 mg/kg) or B.W. 65-54 (15 mg/kg) 30 min prior to the administration of hexobarbital (175 mg/kg, i.p.). The results shown in Table 3 illustrate that the sleeping time in the B.W. 65-54 treated group did not differ significantly from that of the control group. In contrast, the hexobarbital sleeping time was prolonged an average of 53 min in the group pretreated

TABLE 3. EFFECT OF SEVERAL INHIBITORS OF AMPHETAMINE METABOLISM ON HEXOBARBITAL SLEEPING TIME AND ZOXAZOLAMINE MUSCLE PARALYSIS*

Pretreatment	Duration of action			
	Hexobarbital		Zoxazolamine	
	No. rats	Min (Mean ± S.E.)	No. rats	Min (Mean ± S.E.)
Saline	15	80 ± 6	7	235 ± 18
Desmethylimipramine	6	123 ± 12	6	403 ± 20
B.W. 65-54	14	75 ± 6	5	240 ± 23
Iprindole			6	232 ± 31

* Adult male rats (200 g) were injected with saline, B.W. 65-54 (15mg/kg), desmethylimipramine (15 mg/kg) or iprindole (15 mg/kg) 30 min prior to the administration of either hexobarbital (175 mg/kg, i.p.) or zoxazolamine (80 mg/kg, i.p.). The return of the righting reflex was determined in each group.

with desmethylimipramine. The animals pretreated with saline or B.W. 65-54 were killed when they regained their righting reflex and their brain hexobarbital levels were determined.[18] The results indicated no significant difference in the brain hexobarbital levels between the saline and B.W. 65-54 pretreated rats. Conney *et al.*[19] have shown that the duration of the zoxazolamine-induced paralysis is dependent upon the metabolic degradation of zoxazolamine to an inactive metabolite. Since both amphetamine and zoxazolamine are metabolized by ring hydroxylation, we investigated the possibility that B.W. 65-54 and desmethylimipramine could also inhibit the hydroxylation of zoxazolamine. Zoxazolamine (80 mg/kg, i.p.) was administered to rats either alone, or after pretreatment with saline, B.W. 65-54 (15 mg/kg), desmethylimipramine (15 mg/kg) or iprindole (15 mg/kg). The results are shown in Table 3. Neither the B.W. 65-54 pretreatment nor the iprindole pretreatment had any significant effect on the time required for the righting reflex to return after zoxazolamine administration. In contrast, desmethylimipramine markedly prolonged the action of zoxazolamine.

Effect of B.W. 65-54 on the brain levels of ^{3}H-amphetamine in the guinea pig and rabbit. ^{3}H-amphetamine was administered to guinea pigs and rabbits either alone or after pretreatment with B.W. 65-54 (15 mg/kg). The animals were killed at 1, 2 and 4 hr after the dose of amphetamine and their brains were removed and assayed for

amphetamine. In contrast to the results obtained in the rat, the levels of ^{3}H-amphetamine in the guinea pig control group and B.W. 65-54 treated group were the same. The half-life was approximately 120 min in both groups. Similar results were obtained in rabbits; i.e. no differences from control were found in the brain amphetamine levels after pretreatment with B.W. 65-54.

Effect of desmethylimipramine, B.W. 65-54 and iprindole on the level of tritiated norepinephrine in the rat heart. After its intravenous administration, ^{3}H-norepinephrine is concentrated in the heart and other sympathetically innervated organs. Several compounds, such as imipramine and desmethylimipramine, markedly inhibit the uptake of ^{3}H-norepinephrine by the heart.[16] Rats were pretreated with B.W. 65-54 (15 mg/kg), iprindole (15 mg/kg) or desmethylimipramine (5 mg/kg and 15 mg/kg) 30 min prior to the administration of ^{3}H-norepinephrine (10 μc/rat). The hearts were removed 4 hr after the ^{3}H-norepinephrine was administered and the tritiated norepinephrine was determined as described in Methods. Desmethylimipramine, as expected,[16] markedly inhibited the accumulation of the radioactive norepinephrine by 83 and 87 per cent at doses of 5 and 15 mg/kg, respectively, whereas B.W. 65-54 and iprindole had no significant effect on ^{3}H-norepinephrine levels (Table 4).

TABLE 4. EFFECT OF DESMETHYLIMIPRAMINE, B.W. 65-54 AND IPRINDOLE ON THE LEVELS OF ^{3}H-NOREPINEPHRINE BY RAT HEART*

Pretreatment	No. rats	NE accumulation (ng/g heart ± S.E.)	% inhibition of NE accumulation
Saline	19	2·01 ± 0·17	
Desmethylimipramine			
(5 mg/kg)	4	0·36 ± 0·02	82†
(15 mg/kg)	17	0·26 ± 0·02	87†
B.W. 65-54			
(15 mg/kg)	15	2·24 ± 0·22	0‡
Iprindole			
(15 mg/kg)	8	1·82 ± 0·07	10‡

* Adult male rats (200 g) were pretreated with saline, desmethylimipramine, iprindole or B.W. 65-54 30 min prior to an intravenous injection of 10 μc ^{3}H-norepinephrine (NE). The rats were killed 4 hr later and the concentration of ^{3}H-norepinephrine in the heart was determined as described in Methods.

† $P = <0{\cdot}01$.

‡ Not significantly different from control ($P > 0{\cdot}05$).

The effect of tricyclic antidepressants such as desmethylimipramine and iprindole on the accumulation of ^{3}H-norepinephrine by heart has been studied by others.[16,20] These drugs do not effect the enzymes involved in catecholamine metabolism,[16,20] nor do they affect endogenous norepinephrine levels in the heart.[20,21] B.W. 65-54 does not inhibit monoamine oxidase nor does it affect endogenous heart norepinephrine.* This is suggestive evidence that the effects of these drugs on the accumulation

* L. Lemberger, E. Sernatinger and R. Kuntzman, unpublished observations.

of ^{3}H-norepinephrine are not due to changes in the rate of turnover in endogenous heart norepinephrine levels.

DISCUSSION

Tricyclic antidepressant drugs have been shown to potentiate the central nervous system effects of amphetamine. Recently, Sulser *et al.*[1] and Consolo *et al.*[2] have demonstrated that the potentiation of amphetamine by desmethylimipramine could be explained mainly by the ability of desmethylimipramine to inhibit the metabolism of amphetamine. We have investigated the effects of two tricyclic antidepressants, desmethylimipramine and iprindole, and B.W. 65-54, a congener of the β-adrenergic blocking agent, dichloroisoproterenol. It was found that these three compounds markedly potentiate the locomotor activity of amphetamine in rats. In an attempt to elucidate the mechanism of action of these compounds, total brain levels of amphetamine were determined. Four hr after the administration of ^{3}H-amphetamine, a 10-fold increase in brain amphetamine levels was found with animals that had been pretreated with 15 mg/kg of either desmethylimipramine, iprindole or B.W. 65-54. These compounds still increased the concentration of amphetamine in the brain at doses as small as 1–2 mg/kg.

The major pathway of amphetamine metabolism in rats is through aromatic hydroxylation to *p*-hydroxyamphetamine, while in rabbits and guinea pigs, aromatic hydroxylation plays a minor role and lactone formation is the major route of amphetamine degradation.[22] In the guinea pig and rabbit, treatment with B.W. 65-54 prior to the administration of labeled amphetamine did not significantly increase the brain levels of amphetamine. This would be consistent with the thesis that B.W. 65-54 inhibits the hydroxylation of amphetamine. In an attempt to determine whether the effect of desmethylimipramine and B.W. 65-54 was on the metabolism or distribution of amphetamine, total body levels of amphetamine were determined in rats pretreated with these compounds. Desmethylimipramine and B.W. 65-54 markedly increased the half-life of amphetamine in the whole rat, substantiating the idea that this was an effect on metabolism rather than an effect on the distribution of amphetamine into brain tissue.

Since these compounds inhibit the hydroxylation of amphetamine, it was of interest to see if they would inhibit the metabolism *in vivo* of zoxazolamine and hexobarbital. Zoxazolamine is metabolized by aromatic hydroxylation to a pharmacologically inactive metabolite,[19] while hexobarbital is hydroxylated on the side chain to a less active compound. Desmethylimipramine prolonged the hexobarbital sleeping time and the loss of righting reflex produced by zoxazolamine, whereas B.W. 65-54 was without effect on these pharmacological parameters. Like B.W. 65-54, iprindole did not potentiate the effects of zoxazolamine.

Two methods are widely used for screening antidepressant compounds. These utilize: (1) the ability of antidepressants to potentiate the pharmacological action of amphetamine, and (2) the ability of antidepressants to inhibit the accumulation of ^{3}H-norepinephrine by rat heart. The results presented here indicate that the potentiation of amphetamine activity may be secondary to a metabolic effect rather than a true potentiation and that levels of ^{3}H-norepinephrine in rat heart may not be a reliable screening method, since iprindole, a potent antidepressant in man,[8–12] had no effect

on this system. Similar results have been obtained by Gluckman and Baum,[20] who have recently shown that iprindole does not prevent the uptake of norepinephrine. Recently Sulser and Dingell[23] and Borella *et al.*[24] demonstrated that potentiation of amphetamine in the rat is not a valid index of antidepressant action. These authors showed that chlorpromazine, a tranquilizer, also markedly prolongs certain actions of amphetamine by inhibiting its metabolism.

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