Biochemical Pharmacology, Vol. 19, pp. 1709–1717. Pergamon Press. 1970. Printed in Great Britain

BLOCKADE OF NOREPINEPHRINE UPTAKE AND RELATED ACTIVITIES OF *CIS-* AND *TRANS-N,N'*-BIS-(1-NAPHTHYLMETHYL)-1,4-CYCLOHEXANE BIS-(METHYLAMINE) DIHYDROCHLORIDE*

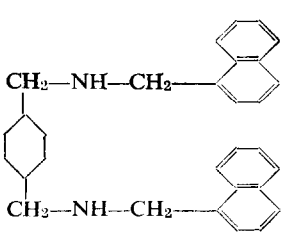
W. LIPPMANN

Biogenic Amines Laboratory, Ayerst Laboratories, Montreal, Quebec, Canada

(*Received* 15 *July* 1969; *accepted* 29 *September* 1969)

Abstract—The effects of *cis-* (C; AY-20,552) and *trans-* (T; AY-20,553) *N,N'*-bis (1-naphthylmethyl)-1,4-cyclohexane bis (methylamine) dihydrochloride on the uptake and storage of the monoamines and their related properties were determined. Both compounds reduced the ^{3}H-norepinephrine (^{3}H-NE) in the mouse and rat heart when given before, but not after, the ^{3}H-NE; T was more potent in its action. The uptake activity was largely recovered by 7 hr after C, 16 hr after T or imipramine, and 24 hr after desmethylimipramine. C and T reduced the norepinephrine-depleting activity of metaraminol and α-methyl-metatyrosine. Neither compound prevented the norepinephrine depletion, tremors or lacrimation caused by tremorine. C and T did not cause any, or caused only partial, antagonism, whereas imipramine reversed the sedation, decreased locomotor activity and blepharospasm caused by tetrabenazine. C (5×10^{-7} M) and T (5×10^{-5} M) inhibited the free fatty acid mobilization *in vitro* induced by norepinephrine.

RECENTLY it has been shown by Lippmann[1] that *N,N'*-bis (1-naphthylmethyl)-1,4-cyclohexane bis (methylamine) dihydrochloride (AY-9928) (Fig. 1) blocks the uptake of norepinephrine into storage sites. In studies on the related activities of AY-9928, it was found that the compound blocks the uptake, and does not cause a release, of norepinephrine in the heart of both the mouse and rat. AY-9928 has no effect on the endogenous levels of catecholamines or serotonin or of both in the heart, brain and adrenals of the mouse. Monoamine oxidase or catechol-*O*-methyl transferase activity *in vivo* is not altered by AY-9928. The norepinephrine-depleting activity of metar-



2 HCl

FIG. 1. *N,N'*-bis (1-naphthylmethyl)-1,4-cyclohexane bis (methylamine) dihydrochloride.

* Presented in part at the Meeting of the Federation of American Societies for Experimental Biology (April 13–18, 1969).

aminol or α-methyl-metatyrosine is prevented, whereas that of reserpine is not, by a prior administration of AY-9928. AY-9928 inhibits the free fatty acid mobilization *in vitro* induced by norepinephrine. Since AY-9928 is a mixture of the *cis* and *trans* isomers, studies were carried out in order to determine the related activities of the pure *cis* (C) and *trans* (T) isomers and the results are reported here.

METHODS

For the determination of the radioactive norepinephrine levels in tissues, male albino mice (Canadian Breeding Laboratories, 23–25 g) or male albino rats (60–80 g) were injected in the tail vein with 5 μc *dl*-7-^{3}H-norepinephrine ·HCl (1·2 to 3·5 c/m-mole; Radiochemical Centre) in 0·25 ml of a solution of 0·75% sodium chloride and 0·01 N HCl. Drugs were injected intraperitoneally in 0·5 ml of bidistilled water. Control animals received injections of the vehicle. The tissue samples were homogenized in ice-cold 0·4 N perchloric acid and centrifuged. A portion of the supernatant fluid was transferred to a vial containing a mixture of 1 ml methanol, 3 ml ethanol and 10 ml toluene phosphor [0·4% 2,5-diphenyloxazole and 0·005% 1,4-bis-(5-phenyl-oxazol-2-yl)-benzene], and the total radioactivity was measured by liquid scintillation counting; the counting efficiency was 12 per cent. The radioactivity in the heart of the mouse[2, 3] and rat[4] at times comparable to those of the present studies is almost entirely due to ^{3}H-norepinephrine (^{3}H-NE). The endogenous levels of heart norepinephrine in the acetic acid eluates from aluminium oxide columns[5] were determined by oxidation with ferricyanide.[6]

The amount of free fatty acids released from minced rat epididymal fat pads was determined essentially according to the method of Itaya and Ui,[7] a modification of the method of Duncombe.[8] Tissue (about 100 mg) in 2·8 ml Krebs–Ringer bicarbonate buffer, pH 7·4, containing 3% bovine serum albumin (fatty acid poor) was incubated at 37° under 7 lb oxygen for 30 min. The test compound was added followed by norepinephrine (1×10^{-4} M, final concentration) to a total volume of 3 ml. The mixture was incubated in a Dubnoff metabolic shaker for an additional 30 min, filtered, and 1·5-ml aliquots were acidified and extracted with chloroform.

The rats utilized (Charles River albinos, Canadian Breeding Laboratories, 170–190 g) for the tremorine studies were female, and male for the tetrabenazine studies. Drugs employed in these studies and their sources were imipramine hydrochloride (Geigy, Canada Ltd.), desmethylimipramine hydrochloride (Geigy, Canada Ltd.), α-methyl-metatyrosine (Mann Research Lab.), metaraminol bitartrate (Merck, Sharp & Dohme Ltd.), tremorine hydrochloride (Abbott Laboratories), atropine sulfate (May and Baker Ltd.), chlorpromazine hydrochloride (Poulenc Ltd.) and tetrabenazine (Roche Ltd.). AY-compounds studied were synthesized by Dr. L. G. Humber (Ayerst Laboratories) and were in the form of the hydrochloride salts. Student's *t*-test was used in the evaluation of the data.

RESULTS

Effects of AY-20,552 (C) and AY-20,553 (T) on uptake and release of ^{3}H-NE in the rat and mouse heart

Rats receiving C before ^{3}H-NE exhibited a reduction in the ^{3}H-NE in the heart at 10, but not at 5 mg/kg, i.p. (Table 1). T caused a greater decline at 10 mg/kg, i.p., and also a moderate decrease was observed at 5 mg/kg, i.p. AY-9928 decreased the ^{3}H-NE

at 5 mg/kg, i.p. There was no reduction in the ^{3}H-NE after any of the drugs (10 mg/kg, i.p.) when each was given after the ^{3}H-NE. Thus, the reductions in ^{3}H-NE caused by the drugs were due to blockade of uptake and not increased release of ^{3}H-NE.

In another species, the mouse, C reduced the ^{3}H-NE in the heart at 2·5 and 5 mg/kg, i.p., when given before ^{3}H-NE (Table 2). After T, larger depletions at these levels were observed. As in the rat, the depletions were due to a blockade of uptake, as no declines were observed when the drugs (5 mg/kg, i.p.) were given after the ^{3}H-NE. When C (5 mg/kg) and T (2·5 mg/kg) were administered orally, activities similar to those after intraperitoneal injections were observed.

Duration of blockade of ^{3}H-NE uptake in the rat heart

In order to determine the duration of the blockade of uptake of ^{3}H-NE in the rat heart by C and T, the animals were injected with ^{3}H-NE at various times after the test drugs and were killed 30 min later. Since C was found to be less potent in action than T (Table 1), it was injected at 20 mg/kg, i.p., whereas T was injected at 10 mg/kg, i.p. The known blockers of ^{3}H-NE uptake, imipramine and desmethylimipramine,[9, 10] were also examined (10 mg/kg, i.p.). After 0·25 hr, both C and T caused large blockades

TABLE 1. EFFECTS OF AY-20,552 AND AY-20,553 ON UPTAKE AND RELEASE OF ^{3}H-NOREPINEPHRINE IN THE RAT HEART*

Compound	Dose (mg/kg, i.p.)	Time drug given before or after ^{3}H-NE (min)	^{3}H-NE content (cpm/g ± S. E.)	% of Control
None			6240 ± 556	
AY-20,552	5	45, before	6216 ± 634	100
AY-20,553	5	45, before	4313 ± 204†	69
AY-9928	5	45, before	4158 ± 242‡	67
None			6172 ± 332	
AY-20,552	10	45, before	3847 ± 387‡	62
AY-20,553	10	45, before	2197 ± 169‡	36
AY-9928	10	45, before	3047 ± 297‡	49
None			5642 ± 252	
AY-20,552	10	45, after	6219 ± 416	110
AY-20,553	10	45, after	5475 ± 331	97

* The rats were killed 2 hr after injection of the test compound. There were twelve to fourteen animals in the control group and eight to ten in the treated group.
† $P < 0·01$.
‡ $P < 0·001$.

of uptake and these were increased slightly after 1 hr (Fig. 2). There were almost complete depletions after imipramine and desmethylimipramine at 0·25 and 1 hr. The ^{3}H-NE content after C had risen to about one-half at 3 hr and to two-thirds that of controls at 7 hr, while that after T was still greatly reduced. Imipramine and desmethylimipramine still showed very large declines after 3 and 7 hr. By 16 hr, the levels of ^{3}H-NE after T and imipramine were approaching those of the controls. At 24 hr, the ^{3}H-NE contents after T, imipramine and desmethylimipramine were essentially those of the controls.

TABLE 2. EFFECTS OF AY-20,552 AND AY-20,553 ON UPTAKE AND RELEASE OF ^{3}H-NOREPINEPHRINE IN THE MOUSE HEART*

Compound	Dose (mg/kg)	Time drug given before or after ^{3}H-NE (min)	^{3}H-NE content (cpm/g ± S. E.)	% of Control
None			9054 ± 491	
AY-20,552	2·5, i.p.	45, before	6394 ± 302†	71
	5·0, i.p.	45, before	4758 ± 517†	53
AY-20,553	2·5, i.p.	45, before	4004 ± 419†	44
	5·0, i.p.	45, before	2648 ± 305†	29
None			10,871 ± 620	
AY-20,552	5·0, i.p.	45, before	6152 ± 437†	57
	5·0, oral	45, before	6338 ± 1150†	58
AY-20,553	2·5, i.p.	45, before	4506 ± 350†	41
	2·5, oral	45, before	5945 ± 730†	55
None			10,745 ± 1008	
AY-20,552	5·0, i.p.	45, after	9441 ± 677	88
AY-20,553	5·0, i.p.	45, after	8539 ± 389	79

* The mice were killed 2 hr after injection of the test compound. There were fourteen animals in the control group and ten in the treated group.
† $P < 0{\cdot}001$.

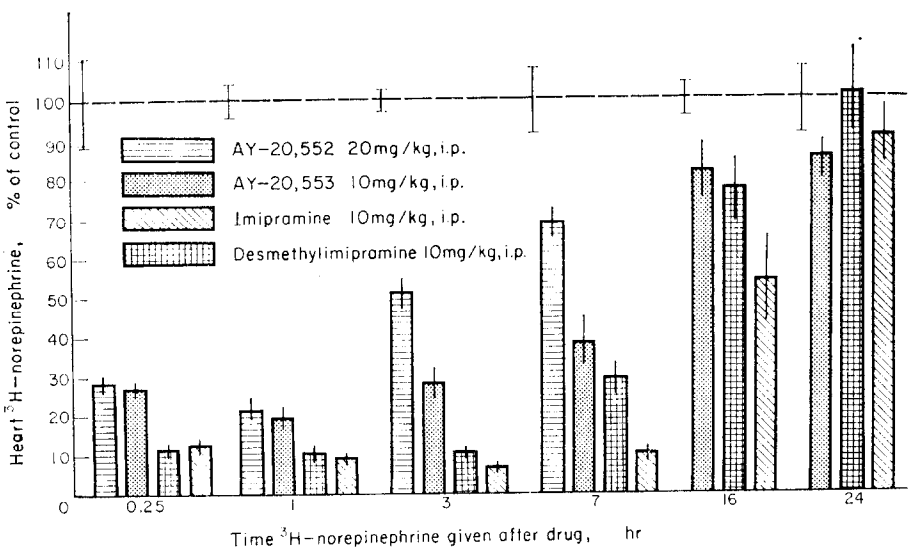


FIG. 2. Duration of blockade of ^{3}H-norepinephrine uptake in the rat heart. Rats were injected with 5 μc ^{3}H-norepinephrine at the various times indicated after the test drug and were killed 30 min after the latter injection. There were eleven animals in the control group and seven to nine in the treated.

Effects of C and T on the activity of norepinephrine-releasing agents in the mouse heart

In Fig. 3 are shown the effects of C and T on the activity of the norepinephrine-releasing agents, metaraminol and α-methyl-metatyrosine. Mice received ^{3}H-NE 15 min before C, T or imipramine, and 5 min after the latter treatment the animals were injected with the norepinephrine-releasing agent; the animals were killed 1 hr after the initial treatment. Metaraminol (0·3 mg/kg, i.v.) alone caused a decrease in the heart

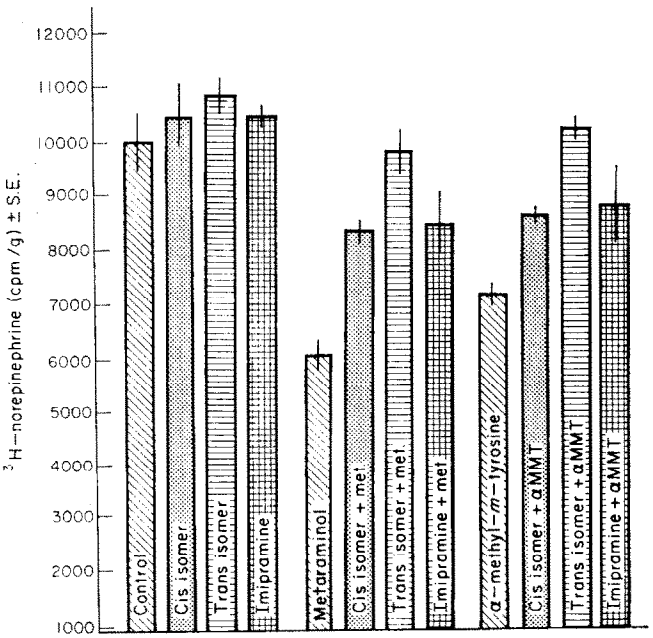


FIG. 3. Effects of AY-20,552 and AY-20,553 on the activity of norepinephrine-releasing agents in the mouse heart. Mice were injected with ^{3}H-norepinephrine and after 15 min AY-20,552 (15 mg/kg, i.p.), AY-20,553 (15 mg/kg, i.p.) or imipramine (15 mg/kg, i.p.) was given. Five min later, metaraminol (0·3 mg/kg, i.v.) or α-methyl-metatyrosine (50 mg/kg, i.v.) was administered. The animals were killed 1 hr after the ^{3}H-norepinephrine injection. There were twelve control and eight to ten treated animals in each group.

TABLE 3. EFFECT OF AY-20,552 AND AY-20,553 ON TREMORINE-INDUCED DEPLETION OF NOREPINEPHRINE IN THE RAT HEART*

Drug (mg/kg, i.p.)		Heart norepinephrine	% of	Symptoms		
A	B	(μg/g ± S.E.)	Control	Lac	Tr	Sed
None	None	0·54 ± 0·03		—	—	—
None	Tremorine (20)	0·37 ± 0·03	69†	+++	++	—
AY-20,552 (10)	None	0·56 ± 0·04	104	—	—	—
AY-20,552 (10)	Tremorine (20)	0·34 ± 0·02	63†	+++	+++	—
AY-20,553 (10)	None	0·50 ± 0·04	93	—	—	—
AY-20,553 (10)	Tremorine (20)	0·35 ± 0·02	65†	+++	++	—
Atropine (10)	None	0·56 ± 0·05	104	—	—	—
Atropine (10)	Tremorine (20)	0·60 ± 0·03	111	—	—	—
Chlorpromazine (10)	None	0·62 ± 0·02	115‡	—	—	+++
Chlorpromazine (10)	Tremorine (20)	0·15 ± 0·01	28†	+++	—	+++

* Rats were injected with drug A followed in 30 min by drug B and were killed 30 min after the latter injection. There were eleven animals in the control group and seven to eight in the drug-treated groups. Symptoms are rated as no effect (—) to maximum (+++); Lac = lacrimation; Tr = tremors; Sed = sedation.

† $P < 0.001$.

‡ $P < 0.05$.

^{3}H-NE to about one-half that of controls, whereas there was no decrease or only a slight decrease when the animals were pretreated with C, T or imipramine (15 mg/kg, i.p.). The reduction in ^{3}H-NE in the animals injected with only α-methyl-metatyrosine (50 mg/kg, i.v.) was not as great in the animals receiving a prior injection of C, T or imipramine (15 mg/kg, i.p.). Thus, C, T and imipramine block the norepinephrine-releasing activities of metaraminol and α-methyl-metatyrosine.

Effects of C and T on tremorine-induced depletion of norepinephrine in the rat heart

Tremorine had been shown to cause a decrease in norepinephrine in the rat heart; atropine, an anticholinergic drug, prevented this action, whereas chlorpromazine, which causes a blockade of ^{3}H-NE uptake, increased the depletion.[11] Similar effects were observed in the present studies (Table 3). C (10 mg/kg, i.p.) and T (10 mg/kg, i.p.) did not cause any alterations in the norepinephrine depletion caused by tremorine. Atropine (10 mg/kg, i.p.) and chlorpromazine (10 mg/kg, i.p.) prevented the tremors, atropine prevented the lacrimation caused by tremorine (20 mg/kg, i.p.), and chlorpromazine alone caused sedation; C (10 mg/kg, i.p.) and T (10 mg/kg, i.p.) did not exhibit any of these activities.

Effects of C and T on the tetrabenazine syndrome

Rats were injected with C, T or imipramine (30 or 10 mg/kg, i.p.) followed 1 hr later by tetrabenazine (20 mg/kg, i.p.). Tetrabenazine alone caused sedation, decreased motor activity and caused blepharospasm. Two hr after the tetrabenazine treatment, the animals receiving C (30 mg/kg, i.p.) exhibited no differences, while the T-treated animals were not sedated and did not show blepharospasm, but still exhibited decreased motor activity; impramine-treated animals exhibited alertness, increased locomotor activity and exophthalmus. After T at 10 mg/kg, i.p., the changes observed at 30 mg/kg were not present; after imipramine at 10 mg/kg, i.p., some of the animals exhibited the antagonism of tetrabenazine. At 1 hr after tetrabenazine, part of the group receiving T at 30 mg/kg resembled those at 30 mg/kg for 2 hr and some of the imipramine-treated animals at 30 mg/kg exhibited antagonism of the tetrabenazine.

TABLE 4. EFFECTS OF AY-20,552 AND AY-20,553 ON NOREPINEPHRINE-INDUCED LIPOLYSIS *IN VITRO**

Compound	Free fatty acids released (μmoles/g tissue ± S. E.)			
	Norepinephrine + compound (M)			
	5×10^{-4}	5×10^{-5}	5×10^{-6}	5×10^{-7}
AY-20,552	6·12 ± 0·59†,‡(23)	8·23 ± 1·00†,‡(42)	8·65 ± 0·90†,‡(46)	10·08 ± 1·18§,‖(54)
AY-20,553	8·34 ± 0·65‡,§(35)	8·22 ± 0·81‡,§(34)	11·42 ± 1·57§(68)	

* Numbers in parentheses represent % of norepinephrine-induced $= 100 \times \frac{[NE + Compound] - Control}{NE - Control}$. There were four to five samples in each group.

† Control = 3·65 ± 0·15; norepinephrine = 14·48 ± 0·76.
‡ $P < 0.01$.
§ Control = 5·04 ± 0·45; norepinephrine = 14·46 ± 0·63.
‖ $P < 0.05$.

Effects of C and T on norepinephrine-induced lipolysis in vitro

In Table 4 are shown the effects of C and T on norepinephrine-induced lipolysis *in vitro*. At 5×10^{-4} M and 5×10^{-5} M, C inhibited the release of free fatty acids 77 and 58 per cent, while T exhibited inhibitions of 65 and 66 per cent, respectively. C inhibited at 5×10^{-6} M (54%) and 5×10^{-7} M (46%), whereas T did not cause any significant change at 5×10^{-6} M. Under similar conditions, desmethylimipramine caused an inhibition at 5×10^{-4} M of 57 per cent and a stimulation of 76 per cent in free fatty acid release at 1×10^{-5} M.[1]

DISCUSSION

As was found with the *cis/trans* mixture, AY-9928,[1] both AY-20,552, the *cis* isomer (C), and AY-20,553, the *trans* isomer (T), cause a blockade of uptake and not an increased release of norepinephrine in the heart of rodents. This was indicated by the findings that there is a lowering in the ^{3}H-NE in the heart when the compounds are given before, but not after, the ^{3}H-NE. In comparison between the two isomers, T is more potent than C in blocking the uptake of norepinephrine in the rat heart, since after T, but not C, a significant fall in the ^{3}H-NE is observed at 5 mg/kg, i.p; also there is a larger decline at 10 mg/kg after T than after C. With respect to mode of administration, both compounds are active when administered orally and exhibit activities similar to those observed after intraperitoneal injection. The blocking action is not species specific, since this activity is observed in the mouse in addition to the rat; as in the rat, T is more potent than C. In comparison between the two species, both compounds are more potent in the mouse, since significant declines are observed at 2·5 mg/kg, i.p., in contrast to 5 or 10 mg/kg in the rat.

The blockade of ^{3}H-NE uptake has a rapid onset, as both C and T cause a large decline at 15 min with the decrease being only slightly greater at 1 hr. T exhibits a more prolonged duration of action than C, since after 7 hr there is still a 62 per cent reduction of ^{3}H-NE with T, whereas the level of ^{3}H-NE after C is recovered to 69 per cent of the control; by 16 hr, the level after T has risen to 82 per cent of the control. In comparison, other blockers of uptake, imipramine and desmethylimipramine,[9, 10] also have a rapid onset of action and the latter drugs are more potent in their action, as they exhibit greater depletions of ^{3}H-NE up to 3 hr. In the subsequent recovery of the uptake activity, T and imipramine are similar, whereas desmethylimipramine exhibits a more prolonged duration of action, since after 16 hr there is still a 54 per cent decrease in uptake. After 24 hr, T, imipramine and desmethylimipramine are similar in their actions as the recovery after each is essentially complete.

C and T cause a blockade of the norepinephrine-releasing activity of metaraminol and α-methyl-metatyrosine. Two different amine-concentrating mechanisms appear to be present in the adrenergic cells, one in the cell membrane and the other in the storage granules.[12] Imipramine and desmethylimipramine block the uptake of norepinephrine by interfering with the active transport through the nerve cell membrane.[9] Desmethylimipramine and imipramine block the release of norepinephrine caused by metaraminol or α-methyl-metatyrosine.[13, 14] C and T, like AY-9928,[1] appear to act by interfering with the active transport through the nerve cell membrane.

Tremorine causes a decline in the norepinephrine content of the rat heart and this action is accentuated by chlorpromazine; these findings are in accord with those of Anton *et al.*[11] Chlorpromazine blocks the uptake of norepinephrine,[9] blocks the

norepinephrine-releasing action of agents[15] and potentiates the actions of catecholamines,[16, 17] apparently by acting on cellular membranes The enhancement of the tremorine effect by chlorpromazine is rapid in onset and it has been suggested that the action is one on the membrane.[11] C and T exhibit similar activities to chlorpromazine, as they also block the uptake of norepinephrine in the rat heart, interfere with the norepinephrine-releasing action of various agents and probably act on the membrane. However, they differ from chlorpromazine as no enhancement of the tremorine-induced norepinephrine-depleting activity is observed under similar conditions. It would appear that under the conditions examined this enhancing property is not a general one with respect to blockers of uptake. In this regard imipramine, which blocks the tuptake of norepinephrine both in the heart[9] and in the brain,[10] also does not accentuate the depletion under similar conditions;* imipramine also does not cause sedation. It is possible that the action of chlorpromazine is associated with the type of central activities of the drug.

In contrast to the enhancement of the tremorine-induced depletion of norepinephrine by chlorpromazine, atropine, which blocks the peripheral muscarinic effects of acetylcholine, has been shown in the present studies and by Anton *et al.*[11] to prevent the depletion. Here also, C and T appear to be different from this type of agent, since these compounds exhibit no such action. Furthermore, C and T are not atropine-like, since they do not prevent the tremors and lacrimation caused by tremorine, whereas atropine does. This is indicative of a lack of central action in this respect by the AY-compounds as the anti-Parkinson-like syndrome appears to be centrally mediated; the peripherally acting atropine methyl bromide does not block the syndrome, but does block the tremorine-induced heart norepinephrine depletion.[11] That AY-compounds lack some central activities of other compounds which block the uptake of norepinephrine is shown by the results which are obtained in tetrabenazine reversal studies. C does not antagonize the sedation and decreased motor activity caused by tetrabenazine. T-treated animals are not sedated but still show decreased motor activity whereas imipramine reverses the tetrabenazine effects and causes increased motor activity. This reversal of the tetrabenazine by imipramine or desmethylimipramine appears to be due to potentiation of the released brain catecholamines at central adrenergic receptors.[18] The sedation observed after administration of drugs like chlorpromazine is also not seen after C and T.

C and T both inhibit the free fatty acid mobilization *in vitro* induced by norepinephrine. However, C is more potent in its action as activity is observed at 5×10^{-7} M, whereas T does not exhibit inhibition activity at 5×10^{-6} M, but does at 5×10^{-5} M. This activity of C relative to T is the reverse of that found in the blockade of norepinephrine uptake. It may be of importance that the free fatty acid release studies were *in vitro* while the norepinephrine uptake studies were *in vivo*. Desmethylimipramine at 5×10^{-4} M also inhibits;[1] other workers[19] have reported that desmethylimipramine causes an inhibition at 1×10^{-3} M. Thus both C and T are more potent inhibitors than desmethylimipramine. Furthermore, they also differ from desmethylimipramine since the latter compound causes a stimulation instead of inhibition at 1×10^{-5} M.[1] The lipolytic enzymes are activated by 3',5'-cyclic adenosine monophosphate, which is produced from the catecholamine-stimulated conversion of adenosine triphosphate.[20, 21] The effects resulting from the action of the catecholamines on rat epididy-

* W. Lippmann, unpublished observations.

mal fat pads are competitively antagonized by β-receptor blocking agents whereas α-receptor blocking drugs inhibit noncompetitively; [22, 23] the former inhibit at the receptor site, whereas the latter nonspecifically impair the activation of lipase by cyclic adenosine monophosphate.[24] Lipolytic activity in adipose tissue is also inhibited by a high level of desmethylimipramine,[19] a blocker of norepinephrine uptake.[25] Desmethylimipramine antagonizes the mobilization of free fatty acids whether induced by catecholamines or by other means. The addition of desmethylimipramine to a previously activated lipase preparation immediately terminates the lipolytic activity. Direct antagonism of the lipolytic enzymes by desmethylimipramine has been suggested, with the effects on the adrenergic receptor being secondary to the enzyme inhibition.[19] It is possible that C and T at the higher levels act by a similar mechanism, although their effects on the adrenergic receptor may play a role in their inhibition activities.

Acknowledgements—The author wishes to acknowledge the technical assistance of Miss Dorothy Mulrooney and Mrs. Ilse Marotta in carrying out these studies. The measurements of free fatty acid release were carried out by Dr. M. Cayen.

REFERENCES

1. W. Lippmann, *Biochem. Pharmac.* **18,** 2495 (1969).
2. J. W. Daly, C. R. Creveling and B. Witkop, *J. med. Chem.* **9,** 273 (1966).
3. W. R. Burack and P. R. Draskoczy, *J. Pharmac. exp. Ther.* **144,** 66 (1964).
4. R. J. Wurtman, I. J. Kopin and J. Axelrod, *Endocrinology* **73,** 63 (1963).
5. L. G. Whitby, J. Axelrod and H. Weil-Malherbe, *J. Pharmac. exp. Ther.* **132,** 193 (1961).
6. U. S. von Euler and I. Floding, *Acta physiol. scand.* **33,** suppl. 118, 57 (1955).
7. K. Itaya and M. Ui, *J. Lipid Res.* **6,** 16 (1965).
8. W. G. Duncombe, *Biochem. J.* **88,** 7 (1963).
9. J. Axelrod, G. Hertting and L. Potter, *Nature, Lond.* **194,** 297 (1962).
10. J. Glowinski and J. Axelrod, *Nature, Lond.* **204,** 1318 (1964).
11. A. H. Anton, R. E. Rodriguez and A. H. Friedman, *Life Sci.* **6,** 507 (1967).
12. A. Carlsson, N. A. Hillarp and B. Waldeck, *Acta physiol. scand.* **59,** suppl. 215 (1963).
13. C. A. Stone, C. C. Porter, J. M. Stavorski, C. T. Ludden and J. A. Totaro, *J. Pharmac. exp. Ther.* **144,** 196 (1964).
14. A. Carlsson and B. Waldeck, *Acta pharmac. tox.* **22,** 293 (1965).
15. D. E. Schwartz, W. P. Burkhard, M. Roth, K. F. Gey and A. Pletscher, *Archs. int. Pharmacodyn. Thér.* **141,** 135 (1963).
16. W. R. Martin, J. L. Riehl and K. R. Unna, *J. Pharmac. exp. Ther.* **130,** 37 (1960).
17. G. Hertting, J. Axelrod and L. G. Whitby, *J. Pharmac. exp. Ther.* **134,** 146 (1961).
18. F. Sulser, M. H. Bickel and B. B. Brodie, *J. Pharmac. exp. Ther.* **144,** 321 (1964).
19. K. F. Finger and J. G. Page, *J. pharm. Sci.* **55,** 1025 (1966).
20. M. A. Rizack, *J. biol. Chem.* **239,** 392 (1964).
21. R. W. Butcher, R. J. Ho, H. C. Meng and E. W. Sutherland, *J. biol. Chem.* **240,** 4515 (1965).
22. M. Wenke, *Adv. Lipid Res.* **4,** 69 (1966).
23. K. Stock and E. Westermann, *Life Sci.* **5,** 1667 (1966).
24. K. Stock and E. Westermann, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **254,** 334 (1966).
25. L. L. Iverson, *J. Pharm. Pharmac.* **17,** 62 (1965).