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BLOCKADE OF NOREPINEPHRINE UPTAKE BY *N,N'-BIS*-(1-NAPHTHYLMETHYL)-1,4-CYCLOHEXANE *BIS*-(METHYLAMINE) DIHYDROCHLORIDE IN RODENTS

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Abstract—The effects in rodents of *N,N'-bis*(1-naphthylmethyl)-1, 4-cyclohexane *bis*(methylamine)dihydrochloride (AY-9928) on the uptake and storage of the monoamines and various other properties were determined. AY-9928 caused a decline in the ^{3}H-norepinephrine content in the heart of both the mouse and rat at 2 hr when given before, but not after, the ^{3}H-norepinephrine. AY-9928 increased the ^{3}H-catechol content in the mouse heart at 24 hr when injected after the ^{3}H-norepinephrine. The endogenous levels of catecholamines or serotonin or of both in the heart, brain and adrenals of the mouse were not changed after administration of AY-9928. The compound caused no inhibition of monoamine oxidase or catechol-*O*-methyl transferase activity *in vivo*. After a pretreatment with AY-9928, the norepinephrine-releasing activity in the mouse heart of metaraminol or α-methylmetatyrosine was prevented, whereas that of reserpine was not. AY-9928 caused a decrease in the norepinephrine-induced release of free fatty acids *in vitro*. Of 45 structurally related compounds studied, AY-9928 was the most potent in causing a decrease in the ^{3}H-norepinephrine in the mouse heart when given before the ^{3}H-norepinephrine. AY-9928 blocks the uptake of monoamines and appears to act by interfering with the transport through the nerve cell membrane.

VARIOUS drugs are known which cause alterations in the uptake, storage and release of norepinephrine in tissues. Entry into the nerve ending, rather than enzymatic destruction, is the main mechanism for the termination of the biological action of norepinephrine.[1] Drugs which inhibit this inactivation process potentiate the actions of norepinephrine.[2]

Interference in the uptake of norepinephrine has been reported to be caused by various phenothiazines and related compounds, e.g. imipramine, desmethylimipramine, amitryptyline, chlorprothixene and chlorpromazine.[3–6] Other compounds possessing this activity which have received much attention are cocaine,[6–9] tripelennamine and *d*-chlorpheniramine.[10] A search was carried out in this laboratory for compounds which block the uptake of norepinephrine, and *N,N'-bis* (1-naphthylmethyl)-1,4-cyclohexane *bis* (methylamine) dihydrochloride (AY-9928) was found to exhibit such an action. This report describes studies on this activity of AY-9928 as well as on various other properties of this compound.

METHODS AND MATERIALS

Radioactive norepinephrine levels in tissues. Male albino mice (23–25 g) or rats (60–80 g) from Canadian Breeding Laboratories were injected in the tail vein with 0·25 ml containing 5 μc *dl*-7-^{3}H-norepinephrine ·HCl (1·2 to 3·5 c/m-mole; New England Nuclear Corp.) in a solution of 0·75% sodium chloride and 0·01 N HCl. Drugs

were injected intraperitoneally (i.p.) in 0·5 ml of 2% carboxymethylcellulose–0·9% saline, unless otherwise specified. Control animals received injections of the appropriate 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-phenyloxazol-2-yl)-benzene], and the total radioactivity was measured by liquid scintillation counting. The counting efficiency was 22 per cent, except for the counts in Table 5 where the efficiency was 12 per cent due to determination in a different scintillation counter.

The radioactivity in the heart of the mouse[11] and rat[12, 13] at times comparable to those of the present studies is almost entirely due to ^{3}H-norepinephrine.

In the studies in which the animals were killed 24 hr after the test drug, the tritiated catechols were determined using acetic acid as the eluting agent.[1] Aliquots dried *in vacuo* were taken up in 2 ml water and 0·2 ml methanol and counted in 10 ml Bray's solution.[14] The counting efficiency was 15 per cent.

Catecholamine and serotonin levels in tissues. Brain catecholamine levels were determined as described by Lippmann and Wishnick[15] essentially according to the procedure of Maynert and Klingman,[16] a modification of the Shore and Olin method[17] in which ferricyanide was substituted for iodine[18] and norepinephrine was used as the standard. Brain serotonin levels were determined by the fluorometric procedure of Bogdanski *et al.*[19] on aliquots of the final acid extract.[20] The levels of heart norepinephrine in acetic acid eluates from aluminum oxide columns[2] were determined by oxidation with ferricyanide. Adrenal catecholamines were isolated and determined as previously described by Lippmann and Wishnick.[15]

Monoamine oxidase and catechol-O-methyl transferase activities in tissues. Monoamine oxidase activity in brain and liver tissues was measured by the method of Kraml[21] and catechol-*O*-methyl transferase activity in liver tissue according to the method of Anderson and D'Iorio.[22]

Free fatty acid release in vitro. The amount of free fatty acids released from minced rat epididymal fat pads was determined essentially according to the method of Itaya and Ui,[23] a modification of the method of Duncombe.[24] Tissue (about 100 mg) in 2·8 ml of 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.

Drugs employed in these studies were α-methyl-metatyrosine (Mann Research Laboratory), metaraminol bitartrate (Aramine; Merck, Sharpe & Dohme, Ltd.), imipramine hydrochloride (Tofranil; Geigy Ltd.) and desmethylimipramine hydrochloride (Pertofrane, Geigy Ltd.). AY-9928 and the structurally related compounds were synthesized by Dr. L. G. Humber (Ayerst Laboratories).

Student's *t*-test was used in the evaluation of the data.

RESULTS

Effects of AY-9928 on the uptake and release of ^{3}H-NE in the mouse and rat heart

Mice injected with AY-9928 (15 mg/kg, i.p.) showed a decrease in the level of ^{3}H-NE

in the heart at 2 hr (Table 1). The decline (72%) was observed when AY-9928 was given before ^{3}H-NE, but there was no appreciable change when AY-9928 was given after ^{3}H-NE. Thus, AY-9928 caused a block of uptake and did not cause an increased rate of release of ^{3}H-NE in the heart.

When mice received ^{3}H-NE before AY-9928 (20 mg/kg, i.p.) and the hearts were examined 24 hr after the latter treatment, there was an increase (42%) in the radioactivity content (Table 1). No increase in the endogenous level of norepinephrine was observed.

AY-9928 (10 mg/kg, i.p.) also caused a decline (57%) in ^{3}H-NE content of the heart

TABLE 1. EFFECTS OF AY-9928 ON UPTAKE AND RELEASE OF ^{3}H-NOREPINEPHRINE IN THE MOUSE HEART

Drug*	Time drug given before or after ^{3}H-NE (min)	Time animals killed after drug (hr)	^{3}H-NE content (cpm/g ± S.E.)	(% of control)	P
None	15 before	2	21,608 ± 968		
AY-9928	15 before	2	6307 ± 631	28	<0·001
None	15 after	2	19,188 ± 419		
AY-9928	15 after	2	20,875 ± 732	108	>0·05

Drug†	Endogenous norepinephrine content (μg/g ± S.E.)	P	^{3}H-NE content (cpm/g ± S.E.)	(% of control)	P
None	0·39 ± 0·03		4696 ± 288		
AY-9928	0·46 ± 0·02	>0·1	6657 ± 411	142	<0·01

*AY-9928 was administered at 15 mg/kg, i.p. There were fifteen animals in the control and 10 in the treated group.

†The animals were pretreated with 15 μc ^{3}H-norepinephrine 18 hr before AY-9928 (20 mg/kg, i.p.) was administered. The animals were killed 24 hr later and the radioactivity of three combined hearts was determined. There were twenty-four animals in the control and eighteen in the treated group.

TABLE 2. EFFECTS OF AY-9928 ON UPTAKE AND RELEASE OF ^{3}H-NOREPINEPHRINE IN THE RAT HEART*

Drug	No. of animals	Time drug given before or after ^{3}H-NE (min)	Time animals killed after drug (hr)	^{3}H-NE content (cpm/g ± S.E.)	(% of control)	P
None	14	15 before	2	5648 ± 282		
AY-9928	9	15 before	2	2419 ± 178	43	<0·001
Imipramine	9	15 before	2	857 ± 93	15	<0·001
None	11	15 after	2	4741 ± 264		
AY-9928	6	15 after	2	5292 ± 378	112	>0·1
Imipramine	10	15 after	2	5449 ± 303	115	>0·1

*Rats (60–80 g) received AY-9928 or imipramine at 10 mg/kg, i.p., and 5 μc ^{3}H-NE, i.v.

of rats when given before, but not after, ^{3}H-NE (Table 2). Imipramine, a known blocker of uptake, also decreased the level of ^{3}H-NE in the tissues under these conditions.

Effects of compounds structurally related to AY-9928 on the ^{3}H-NE content in the mouse heart

Table 3 shows the effects of structurally related *N,N'*-di(aralkyl and nonaromatic) derivatives of 1,4-*bis*(aminomethyl)-cyclohexane (Fig. 1) on the ^{3}H-NE contents in

TABLE 3. EFFECTS OF *N,N'*-DI(ARALKYL AND NONAROMATIC) DERIVATIVES OF CYCLOHEXANE-1,4-*BIS*(AMINOMETHYL) CYCLOHEXANE* ON THE ^{3}H-NE CONTENT IN HEARTS OF MICE RECEIVING ^{3}H-NE

Compound No.	^{3}H-NE content Dose (mg/kg, i.p.) 15 (cpm/g ± S.E.)	15 (% of control)	5 (cpm/g ± S.E.)	5 (% of control)
1	† 4422 ± 101	21	†††10,878 ± 2442	48
2	†20,138 ± 1028	97		
3	†21,570 ± 1618	104		
4	†20,548 ± 724	99		
5	†21,924 ± 1036	106		
6	†15,844 ± 1042	76		
7‡	†21,570 ± 2532	104		
8	†20,672 ± 1140	100		
9	†26,464 ± 2288	127		
10	†22,284 ± 1274	107		
11	†19,688 ± 568	95		
12	††23,865 ± 1487	94		
13§	††25,032 ± 750	99		
14§	††25,904 ± 1657	102		
15	††26,267 ± 1582	104		
16	††25,214 ± 1649	100		
17	†23,398 ± 580	113		
18	†19,808 ± 1476	95		
19§	†18,600 ± 1008	90		
20	†21,242 ± 1030	102		
21	†21,886 ± 1372	105		
22	††25,462 ± 1561	101		
23	††29,033 ± 1673	115		
24‖	†21,804 ± 1968	104		
25	†††† 5932 ± 462	29	††††14,287 ± 959	68
25a	† 8136 ± 686	39	†††16,302 ± 1364	72
26	†15,786 ± 1236	76		
27	††23,528 ± 1177	93		
28	††23,784 ± 843	94		
29	††23,670 ± 1786	93		
30	††21,363 ± 1153	84		
31¶	††19,153 ± 2285	76		
32	††23,050 ± 1534	91		

*All basic compounds were administered as dihydrochloride salts except where indicated to the contrary. The drugs were administered 45 min before the ^{3}H-NE and the animals were killed 75 min later. There were five to six animals in each group.

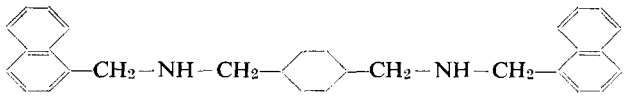
†Controls = 20,768 ± 726; ††25,324 ± 1330; †††22,609 ± 1092; ††††20,881 ± 1232.

‡Administered as the free base.

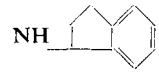
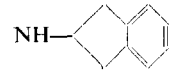
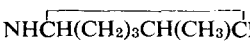
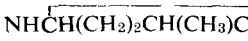
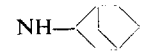
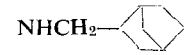
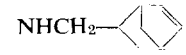
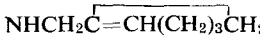
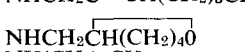
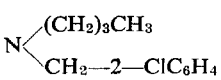
§Administered as the diacetate salt.

‖This compound is a mixture of the 6- and 7-octenyl derivatives.

¶Administered as the dihydrobromide salt.



AY-9928

No.	$-R_1$	1,4 Config.
1	$NHCH_2$-1-naphthyl	*cis/trans*
2	$NHCH_2$-2-naphthyl	*cis/trans*
3	$NHCH_2$-2-furyl	*cis/trans*
4	$NHCH_2$-2-pyridyl	*cis/trans*
5	$NHCH_2$-4-pyridyl	*cis/trans*
6		*trans*
7		*trans*
8	$NHCH_2$-cyclohexyl	*trans*
9	$NHCH_2$-cyclohexyl	*cis*
10	$NHCH_2$-cylobutyl	*trans*
11	$NHCH_2$-cyclopentyl	*cis/trans*
12	NH-cyclohexyl	*trans*
13	NH-cyclooctyl	*trans*
14		*trans*
15		*trans*
16		*trans*
17		*trans*
18		*trans*
19		*trans*
20		*trans*
21	$NH(CH_2)_6CH_3$	*trans*
22	$NHC(CH_3)_3$	*trans*
23	$NHC(CH_3)_2CH_2C(CH_3)_3$	*trans*
24	$NH(CH_2)_3CH(CH_3)CH=C(CH_3)_2$	*cis/trans*
25	$NH(CH_2)_2C_6H_5$	*trans*
25a	$NH(CH_2)_2C_6H_5$	*cis/trans*
26	$NHCH_2CH(CH_3)C_6H_5$	*cis/trans*
27	$N(CH_3)CH_2C_6H_5$	*trans*
28	Pyrrolidino	*trans*
29	Hexamethyleneimino	*trans*
30		*trans*

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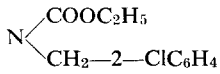
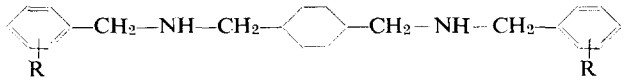
32  *trans*



No.	R
33	H
34	2-Chloro
35	4-Chloro
36	2-Bromo
37	3-Bromo
38	4-Bromo
39	2-Fluoro
40	3-Fluoro
41	4-Fluoro
42	2-Methyl
43	4-Methyl
44	4-Isopropyl
45	2,3-Dimethoxy
46	3,4-Dimethoxy

FIG. 1. Structures of compounds studied.

the hearts of mice receiving ^{3}H-NE. The drugs were given 45 min before ^{3}H-NE and the animals were sacrificed 75 min later. At 15 mg/kg, i.p., large decreases of greater than 40 per cent in the ^{3}H-NE content were observed only after the 1-naphthylmethyl (1)* [79%] and the β-phenethyl (25, 25a) [71%, 61%] derivatives. None of the other compounds examined caused a decrease of more than 25 per cent. At 5 mg/kg, i.p., there was a decline of 52 per cent after the 1-naphthylmethyl (1) and of 32 and 28 per cent after the β-phenethyl (25, 25a) derivatives.

The structurally related *N,N'*-dibenzyl derivatives of 1,4-*bis* (aminomethyl)-cyclohexane were studied and the results are shown in Table 4. At 15 mg/kg, i.p., declines greater than 40 per cent were observed after the benzyl (33) [55 per cent] and the 2,3-dimethoxybenzyl (45) [45 per cent] derivatives; at 5 mg/kg, the benzyl (33) and the 2,3-dimethoxybenzyl (45) derivatives caused declines of 36 and 35 per cent, respectively.

The compounds which caused at least a 40 per cent decline in the ^{3}H-NE at 15 mg/kg when given before the ^{3}H-NE (i.e. 1, 25, 33 and 45) did not cause a decrease when given after the ^{3}H-NE (Table 5). Thus, these various compounds block the uptake and do not cause an increased release of ^{3}H-NE.

Effect of A Y-9928 on catecholamine and serotonin content of various tissues of the mouse

Mice injected with AY-9928 (15 mg/kg, i.p.) and killed 6 hr later exhibited no changes in the catecholamine levels in the heart and brain and in the serotonin level in the brain. Monoamine oxidase and catechol-*O*-methyl transferase activities were not altered 1 hr after administration of AY-9928 (15 mg/kg, i.p.).

*Numbers in parentheses refer to compounds listed in Table 3.

TABLE 4. EFFECTS OF *N,N'*-DIBENZYL DERIVATIVES OF 1,4-*BIS* (AMINOMETHYL) CYCLOHEXANE* ON THE ^{3}H-NE CONTENT IN HEARTS OF MICE RECEIVING ^{3}H-NE

Compound No.	^{3}H-NE content Dose (mg/kg, i.p.)			
	15		5	
	(cpm/g ± S.E.)	(% of control)	(cpm/g ± S.E.)	(% of control)
33‡	††10,247 ± 647	45	††14,505 ± 1265	64
34‡	††14,841 ± 1431	66	†19,342 ± 1078	78
35	†††14,427 ± 846	80	†21,180 ± 2028	86
36‡	†††14,929 ± 991	82	†18,594 ± 1270	75
37	†††11,956 ± 1363	66	†21,692 ± 1850	88
38	†††14,516 ± 1070	80	†22,590 ± 1590	92
39	†††15,926 ± 646	88	†22,176 ± 1982	90
40	†††14,499 ± 330	80	†26,782 ± 2598	109
41	†††13,600 ± 1375	75	†25,296 ± 1706	103
42	†††16,115 ± 1242	75	†22,634 ± 3016	92
43	†††11,612 ± 619	64	†21,546 ± 838	87
44	†††17,646 ± 862	97	†22,452 ± 1520	91
45	††† 9949 ± 702	55	†15,956 ± 940	65
46	†††15,775 ± 723	87	†21,548 ± 2062	87

*All compounds were administered as dihydrochloride salts, except where indicated otherwise; all compounds are mixtures of *cis*- and *trans*-1,4 isomers, except where indicated. The drugs were administered 45 min before the ^{3}H-NE and the animals were killed 75 min later. There were five to six animals in each group.

†Controls = 24,658 ± 1380; ††22,609 ± 1092; †††18,104 ± 1239.

‡A *trans*-1,4 isomer.

TABLE 5. EFFECTS OF VARIOUS OF THE DERIVATIVES ON RELEASE OF ^{3}H-NE FROM THE HEARTS OF MICE*

Compound No.	^{3}H-NE content		P
	(cpm/g ± S.E.)	(% of control)	
None	10,546 ± 409		
1	9852 ± 500	93	>0·3
25	10,183 ± 663	97	>0·5
33	10,643 ± 519	101	>0·7
45	11,296 ± 854	107	>0·3

*Mice were injected with the test compound (15 mg/kg, i.p.) 15 min after the ^{3}H-NE and were killed 2 hr after administration of the test compound. There were fifteen animals in the control and eight in each treated group.

Effect of AY-9928 on the norepinephrine-induced lipolysis in vitro

AY-9928 caused inhibitions of 47 and 45 per cent of the norepinephrine-induced release of free fatty acids at 5×10^{-4}M and 1×10^{-4}M, respectively, but did not cause a significant inhibition at 1×10^{-5}M (Table 6). Desmethylimipramine exhibited an inhibition of 57% at 5×10^{-4}M, but did not cause a significant change at 1×10^{-4}M.

Effects of AY-9928 on the activity of various norepinephrine releasers in the mouse heart

The effects of AY-9928 on the activity of various norepinephrine-releasing agents are shown in Fig. 2. Metaraminol, α-methyl-meta-tyrosine and reserpine caused

TABLE 6. EFFECT OF AY-9928 ON NOREPINEPHRINE-INDUCED LIPOLYSIS *IN VITRO**

Compound	Free fatty acids released (μmoles/g tissue ± S.E.)				
	Control	Norepinephrine	Norepinephrine + Compound (M)		
			5×10^{-4}	1×10^{-4}	1×10^{-5}
AY-9928 (1)	1·80 ± 0·26	8·37 ± 0·43	5·32 ± 0·71† (53)	5·42 ± 1·26‡ (55)	8·33 ± 0·18 (99)
Desmethyl-imipramine	2·31 ± 0·21	6·30 ± 0·67	4·01 ± 0·23† (43)	7·54 ± 0·70 (131)	

* Numbers in parentheses represent % of norepinephrine-induced = 100 × ([NE + Compound]--control/NE − control). There were four to five samples in each group.
† $P<0.01$.
‡ $P<0.05$.

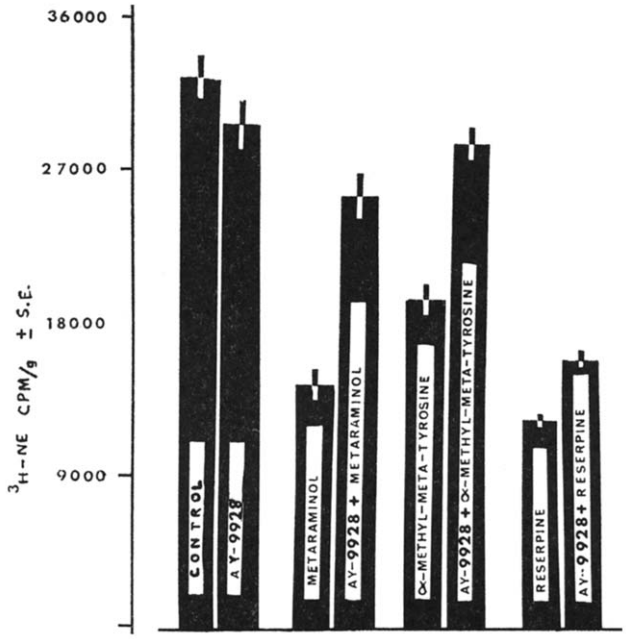


FIG. 2. Effects of AY-9928 on the activity of various norepinephrine-releasing agents in the mouse heart. Mice were injected with ^{3}H-NE and after 15 min AY-9928 (15 mg/kg, i.p.) was given. Ten min later, metaraminol (0·3 mg/kg, i.v.), α-methyl-metatyrosine (50 mg/kg, i.v.) or reserpine (0·5 mg/kg, i.v.) was administered. The animals were killed 1 hr after the ^{3}H-NE injection. There were sixteen control and seven to eight treated animals in each group.

decreases in the ^{3}H-NE content of the mouse heart of 59, 41 and 63 per cent, respectively. Administration of AY-9928 (15 mg/kg, i.p.) before these drugs prevented the releasing activities of metaraminol and α-methyl-*m*-tyrosine, whereas there was only a slight interference with the releasing action of reserpine.

DISCUSSION

In the present studies AY-9928 has been shown to inhibit the uptake of norepinephrine into the tissue storage sites in the rat and mouse, and the activities related to

this action were determined. At 2 hr, AY-9928 lowered the radioactivity in the heart of the rat and mouse when given before, but not after, ^{3}H-NE. Thus, AY-9928 does not cause an increased release of norepinephrine, but it does block the uptake. When AY-9928 is given after ^{3}H-NE and the radioactivity in the heart of the mouse is examined after 24 hr, an increase is observed in the ^{3}H-catechol content; no increase in endogenous norepinephrine is observed. Imipramine and chlorpromazine are other compounds which are known to exhibit a blockade of uptake.[3, 25, 26] The latter two drugs have also been shown to cause an increase in the ^{3}H-catechols at 24 hr.[26] As with these drugs, AY-9928 might be acting by causing changes at the cell membrane, which could lead to an inhibition of the release of the catechol(s) and thus account for the increase observed.

AY-9928 causes a blockade of uptake of ^{3}H-NE in the heart and causes no changes in the endogenous norepinephrine level in the heart and also causes no alterations in the levels of catecholamines in the brain and adrenals and of serotonin in the brain. Other drugs which exhibit the blocking activity also do not alter the endogenous catecholamine and serotonin levels.[4, 27–29]

With respect to the lowering of the ^{3}H-NE content in the heart of the animals receiving ^{3}H-NE, the importance of the attachment of the naphthylmethyl group at the 1-position, as in AY-9928 (1), is demonstrated by the finding that the 2-naphthylmethyl compound (2) does not exhibit any depleting activity. Also, the 2-furylmethyl (3), 2-pyridylmethyl (4), and 4-pyridylmethyl (5) compounds are inactive. Substitution of a 2-indanyl (6) or even a 1-indanyl (7) for the 1-naphthyl group (1) results in a loss of activity. Replacing the 1-naphthyl (1) with alicyclic groups, whether attached through one intervening methylene group (8–11) or to the nitrogens directly (12–15), causes loss of the activity whether the acyclic group is bridged (16, 17) or contains a double bond (18, 19). Replacement of the 1-naphthylmethyl (1) by alkyl groups such as the *n*-heptyl (21) or the branched *t*-butyl (22) and *t*-octyl (23) causes a loss in activity. When the aromatic 1-naphthyl (1) is replaced by a β-phenethyl group (25, 25a), the resulting compound exhibits a high activity although it is not as potent. This activity is lost when the side chain is branched with an α-methyl group (26). Also rendering of the nitrogens tertiary with a benzyl and a methyl group (27) or with various other groups (28–32) yields inactive compounds.

Elimination of the methylene group between the nitrogen and the benzyl group yields a compound with similar activity. Thus, the benzyl amino derivative (33) is similar to the β-phenethyl derivatives (25, 25a); the 1-naphthyl compound (1) is still more potent, however. Mono substitution of different halogens or alkyl groups in various positions of the benzyl ring leads to diminished activity or loss of activity (34–44). The 2,3-dimethoxy derivative (45) still shows high activity, however, whereas the 3,4-dimethoxy derivative (46) is inactive. A slight activity is observed with the 2-chloro derivative (34).

AY-9928 inhibits the free fatty acid mobilization induced by norepinephrine. AY-9928 is similar to desmethylimipramine with respect to this activity, since both compounds exhibit an inhibition at 5×10^{-4}M of comparable value. AY-9928 is, however, a more potent inhibitor since AY-9928, but not desmethylimipramine, inhibits at the lower level of 1×10^{-4}M.

The activation of lipolytic activity by the catecholamines involves the catecholamine-stimulated conversion of adenosine triphosphate to 3′5′-cyclic adenosine mono-

phosphate, which in turn activates the lipolytic enzymes.[30, 31] *In vitro*, it has been demonstrated that the β-receptor blocking agents competitively antagonize the effect of catecholamines on rat epididymal fat pads, whereas α-receptor blocking drugs inhibit non-competitively.[32, 33] The β-receptor blocking agents appear to be inhibiting at the receptor level and the α-adrenergic blocking agents act by non-specifically impairing the activation of lipase by cyclic adenosine monophosphate.[34] AY-9928 inhibits the free fatty acid mobilization *in vitro* induced by norepinephrine. As observed in the present studies, desmethylimipramine, which causes blockade of norepinephrine uptake,[6] has also been shown by others[35] to inhibit lipolytic activity in adipose tissue. With desmethylimipramine, the antagonism of free fatty acid mobilization occurs, whether the mobilization is induced by catecholamines or by other means, and the addition of desmethylimipramine to an already activated lipase preparation causes a prompt cessation of lipolytic activity. It has been suggested that the desmethylimipramine under these conditions directly antagonizes lipolytic enzymes and that the effects on the adrenergic receptor are secondary to the primary inhibition phase.[35] It is possible that AY-9928 acts in a similar manner, although the effects on the adrenergic receptor may play a role.

The decrease in ^{3}H-NE caused by the releasers metaraminol or α-methyl-*m*-tyrosine is blocked by a pretreatment with AY-9928, but that observed after reserpine is not. There appear to be two different amine-concentrating mechanisms in the adrenergic cells, one in the cell membrane and the other in the storage granules.[36] Various drugs such as imipramine, desmethylimipramine and chlorpromazine block the uptake of norepinephrine and act by interfering with the active transport through the nerve cell membrane.[25] Reserpine acts to block the incorporation of norepinephrine into the storage granule.[37, 38] Desmethylimipramine blocks the release of ^{3}H-NE induced by metaraminol or α-methyl-*m*-tyrosine, but not that of reserpine.[39, 40] Thus AY-9928 is similar to desmethylimipramine in its actions and appears to act by interfering with the active transport through the nerve cell membrane.

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