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BLOCKADE OF NOREPINEPHRINE UPTAKE AND OTHER ACTIVITIES OF 5-(3′-DIMETHYLAMINOPROPYL)-DIBENZO[a,d] [1,4]CYCLOHEPTADIENE HYDROCHLORIDE (AY-8794) AND STRUCTURALLY RELATED COMPOUNDS

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Abstract—The effects of AY-8794 [5-(3′-dimethylaminopropyl)dibenzo[a,d] [1,4] cycloheptadiene hydrochloride] and structurally related compounds on the uptake of norepinephrine into the storage sites and other activities were determined. AY-8794 blocks the uptake of ^{3}H-norepinephrine into the mouse and rat heart. AY-8794 is a potent inhibitor of gastric acid secretion in the rat. The free fatty acid mobilization *in vitro* from minced rat epididymal fat pads induced by norepinephrine is inhibited at a high level and is stimulated at a low level of AY-8794. AY-8794 exhibits anti-inflammatory activity in the rat. The structural requirements for various of these activities were determined.

THE PRINCIPAL mechanism for the inactivation of endogenously liberated (sympathetic nerve stimulation) norepinephrine[1] and injected norepinephrine[2,3] is through the uptake of norepinephrine into the storage sites of sympathetic nerve endings. Drugs which inhibit this inactivation process potentiate the actions of norepinephrine.[4] Various drugs which have been reported to cause interference in the uptake of norepinephrine are the tricyclic derivatives, such as imipramine, desmethylimipramine and amitriptyline, chlorprothixene and chlorpromazine,[5–8] and cocaine,[8–11] tripelennamine and *d*-chlorpheniramine.[12] The present report concerns the finding that the tricyclic derivative 5-(3′-dimethylaminopropyl) dibenzo[a, d] [1, 4] cycloheptadiene hydrochloride (AY-8794) and some structurally related compounds inhibit the uptake of norepinephrine *in vivo*. Other activities of this series of compounds were also determined and are reported here.

METHODS AND MATERIALS

Radioactive norepinephrine levels in tissues. Male albino mice (Canadian Breeding Laboratories), weighing 23–25 g, were injected in the tail vein with 0·25 ml containing 1·5 or 5 μc *dl*-7-^{3}H-norepinephrine ·HCl (1·8–3·5 c/m-mole; New England Nuclear Corp.) in a solution of 0·75% sodium chloride and 0·01 N HCl. Drugs were injected i.p. in 0·5 ml of 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 12 per cent. The radioactivity in the heart of the mouse[13] and rat[14,15] at times comparable to those of the present studies is almost entirely due to ^{3}H-norepinephrine.

Gastric acid secretion. Gastric acid secretion was determined by a modified method of Shay *et al.*[16] Charles River female albino rats (Canadian Breeding Laboratories; 170–190 g) were caged individually 48 hr prior to treatment. After the first 24 hr of food deprivation, the animals were given access to 8% sucrose in 0·2% sodium chloride for 8 hr. Water was permitted *ad libitum* except during the 8 hr of sucrose. Three hr after the pyloric ligation, the animals were anesthetized with ether and the amount of acid in the stomach was determined by titration against 0·1 N sodium hydroxide in a direct reading pH meter to 7·0.

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,[17] a modification of the method of Duncombe.[18] 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. Aqueous insoluble compounds were added in 0·1 ml dimethylsulfoxide (which alone did not affect the release). 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.

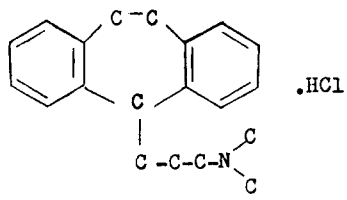
Anti-inflammatory activity. For the determination of anti-inflammatory activity utilizing carragheenin-induced edema, rats were starved for 16 hr and a method similar to that of Winter *et al.*[19] was employed. The drugs were administered by gastric gavage in a volume of 1 ml water per 100 g body weight followed by administration of water to a total of 4 ml per 100 g body weight. One hr later, 0·1 ml carragheenin (1%) was injected, subplantarly, in both hind paws and the increase in each foot volume after 3 hr was determined. The paws were immersed to the level of the lateral malleous in a mercury bath on a Mettler balance and balance readings made. There were six rats (~160 g) in each group. For the adjuvant-induced arthritis, the method of Newbould[20] was employed. The thickness of the left hind-foot was measured by mercury displacement and 0·05 ml of a fine suspension of dead tubercle bacilli in liquid paraffin B.P. (concn, 5 mg/ml) was injected intradermally into the plantar surface of the left hind-foot with a No. 26 needle. The test drug was then given by gastric gavage. The drug treatment was given daily and the increase in the thickness of the injected foot determined after 14 days. There were six rats (150–160 g) in each group.

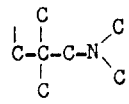
Drugs employed in these studies and their suppliers were: imipramine and desmethylimipramine hydrochlorides (Geigy, Canada Ltd.), trimipramine (Poulenc Ltd.) and amitriptyline hydrochloride (Merck, Sharp & Dohme Ltd.). AY-compounds were synthesized by Dr. S. Winthrop (Ayerst Laboratories). The structures of the compounds studied are shown in Fig. 1. Student's *t*-test was used in the evaluation of the data.

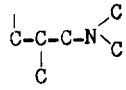
RESULTS

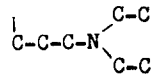
Uptake and release of ^{3}H-norepinephrine (^{3}H-NE)

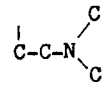
In Table 1 are shown the effects of AY-8794 and related tricyclic compounds on

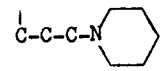
AY-8794  I

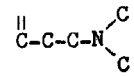
AY-8644  II

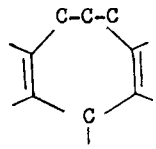
AY-8609  III

AY-8802  IV

AY-8633  V

AY-8606  VI

Amitriptyline  VII

AY-8828  VIII

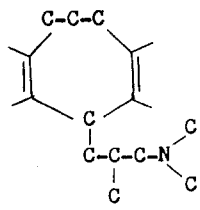
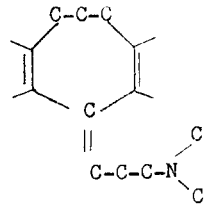
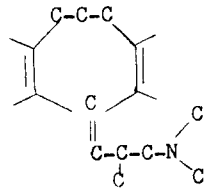
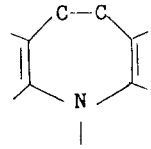
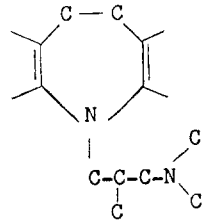
AY-8829  IX

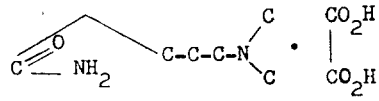
Figure 1 continued on page 2520

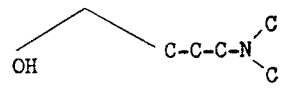
AY-8820  X

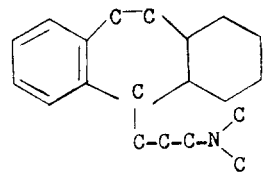
AY-8827  XI

Imipramine  XII

Trimipramine  XIII

AY-8693  XIV

AY-8793  XV

AY-8799  XVI

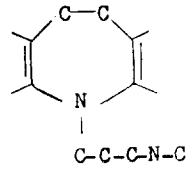
Desmethylimipramine  XVII

FIG. 1. Structures of compounds studied.

TABLE 1. EFFECTS OF AY-8794 AND RELATED TRICYCLIC COMPOUNDS ON UPTAKE AND RELEASE OF ^{3}H-NOREPINEPHRINE IN THE MOUSE HEART*

Compound	^{3}H-norepinephrine content					
	Dose (mg/kg, i.p.) given before ^{3}H-NE				Dose (mg/kg, i.p.) given after ^{3}H-NE	
	5		10		5	
	(cpm/g ± S.E.)	(% of control)	(cpm/g ± S.E.)	(% of control)	(cpm/g ± S.E.)	(% of control)
AY-8794 (I)	†1369 ± 86‡ ††1795 ± 202‡	32 43			†††3122 ± 224	103
III			†4502 ± 224	104		
VIII	†2261 ± 81‡	52			†††3308 ± 105	110
IX			†3651 ± 172	84		
XII	†1906 ± 301‡	44			†††3136 ± 114	104
XIII			†4563 ± 225	105		

* Mice were injected with the test compound (in 0·025% acetic acid) 45 min before or after the administration of 1·5 μc ^{3}H-norepinephrine and were killed 75 min after the injection of the test compound. There were seven to ten amimals in each group.
† Controls = 4339 ± 162; †† 4212 ± 210; ††† 3021 ± 134.
‡ $P<0·001$.

the uptake and release of ^{3}H-NE in the mouse heart. AY-8794, VIII and XII caused a decrease in the ^{3}H-NE when given before, but not after, the ^{3}H-NE. Thus, AY-8794 blocked the uptake of the ^{3}H-NE. The blockade of uptake at 5 mg/kg, i.p., was observed whether the central ring was an eight-membered carbocyclic (VIII) or a seven-membered heterocyclic with nitrogen (XII) instead of a seven-membered carbocyclic (AY-8794). The respective compounds containing a branched aliphatic side chain (IX, XIII, III) did not cause a blockade of uptake.

In another species, the rat, AY-8794 also caused a prevention of uptake of ^{3}H-NE in the heart (Table 2). Neither AY-8794 nor any of the other compounds at 10 mg/kg, i.p., caused a decrease in ^{3}H-NE when the compound was given 15 min after the ^{3}H-NE and the animals were killed 1 hr after the administration of the compound (Table 2). The blocking activity at 5 mg/kg was lost when in the dimethylaminopropyl side chain there was a di-(II) or mono-substitution (III) of methyl groups for the β-hydrogens, substitution of ethyl groups for the methyl groups on the nitrogen (IV), shortening of the chain length from three to two carbons (V), or replacement of the dimethylamine group by a piperidino group (VI). Loss of activity was also observed when the side chain was attached to the ring by a double bond (VII) instead of a single bond (AY-8794).

When the central ring was eight-membered (VIII, IX) rather than seven-membered (AY-8794), there was a reduction in activity. A loss in activity occurred when the attachment of the side chain was through a double bond (X) instead of a single bond (VIII); furthermore, branching of the aliphatic side chain with a β-methyl group (XI) also caused a fall in activity, as observed at 10 mg/kg, in comparison with the straight-chain-containing compound (X).

Replacement of the seven-membered carbocyclic central ring (AY-8794) by a seven-membered nitrogen heterocyclic ring (XII) yielded a highly potent compound. Here also, when the latter compound contained the branched β-methyl side chain

TABLE 2. EFFECTS OF AY-8794 AND RELATED TRICYCLIC COMPOUNDS ON UPTAKE AND RELEASE OF ^{3}H-NOREPINEPHRINE IN THE RAT HEART*

Compound	^{3}H-norepinephrine content					
	Dose (mg/kg, i.p.) given before ^{3}H-NE				Dose (mg/kg, i.p.) given after ^{3}H-NE	
	5		10		10	
	(cpm/g ± S.E.)	(% of control)	(cpm/g ± S.E.)	(% of control)	(cpm/g ± S.E.)	(% of control)
AY-8794 (I)	†4672 ± 223††	64	‖3496 ± 146††	36	**3226 ± 145	99
	‡5696 ± 270††	52				
II	†8748 ± 532	121	‖10,079 ± 367	104	3167 ± 249	97
III	†7569 ± 691	104	‖10,384 ± 358	107	3272 ± 238	100
IV	†7278 ± 400	100	‖7687 ± 355††	80	3281 ± 209	101
V	††6506 ± 314	89	‖6512 ± 321††	67	3531 ± 190	108
VI			‡9590 ± 738	88	3254 ± 170	100
VII‡‡	‡6272 ± 523	92	‖5409 ± 261††	56	3227 ± 204	99
VIII	†5418 ± 457§§	74	‖3372 ± 203††	35	3088 ± 171	95
IX††	§6654 ± 496§§	80	§4441 ± 305††	54	3295 ± 208	101
IX	†6236 ± 350‖‖	85	‖4751 ± 423††	49		
X	†6698 ± 350	92	‖7323 ± 520††	76	2949 ± 174	90
XI	†7430 ± 437	102	‖9748 ± 359	101	3128 ± 145	96
XII‡‡	†2358 ± 108††	32			3193 ± 209	98
XIII‡‡			§9444 ± 459‖‖	114	3090 ± 150	95
XIV			‡10,522 ± 490	97	2983 ± 220	91
XV	¶4095 ± 105	96	¶3167 ± 245††	74	2984 ± 146	92
XVI	†5848 ± 272§§	80	‖6013 ± 231††	62	3054 ± 177	94

* Rats were injected with the test compound 45 min before or after the administration of ^{3}H-norepinephrine and were killed 60 m in afterthe injection of the test compound. In the last two experiments, 2·5 μc ^{3}H-norepinephrine was injected instead of the usual 5 μc. There were seven to ten animals in each group.

† Controls = 7311 ± 273; ‡10,872 ± 284; §8300 ± 317; ‖9666 ± 313; ¶4274 ± 100; **3261 ± 125.

†† $P<0·001$.

‡‡ Compound was administered in 0·025% acetic acid.

§§ $P<0·01$.

‖‖ $P<0·05$.

(XIII), the activity was lost. The compound containing one saturated ring (XVI) in place of one of the aromatic rings exhibited a reduction in activity. The activity observed with AY-8794 was lost when a carboxamido group (XIV) was substituted for the hydrogen on the carbon atom bearing the aliphatic side chain; the activity was reduced when a hydroxyl group (XV) was similarly substituted.

Gastric secretion

The effects of AY-8794 and the related tricyclic compounds on gastric secretion in the rat are shown in Table 3. AY-8794 caused inhibition of gastric secretion at 5 mg/kg. This activity was lost when the aliphatic side chain contained two (II) or one (III) methyl group in the β-position, ethyl groups in place of the methyl groups on the nitrogen (IV), or two carbons instead of three (V). The compound containing the aliphatic side chain attached to the ring by a double bond (VII) instead of a single bond also exhibited high activity.

Compounds which contained an eight-membered (VIII, IX, XI) instead of a seven-membered (AY-8794) central ring showed either no activity or greatly reduced activity. The compound containing a seven-membered heterocyclic nitrogen ring

(XII) in place of the carbocyclic ring (AY-8794) exhibited a high activity similar to that of AY-8794; however, the compound in which the side chain was branched by a β-methyl group (XIII) showed no activity. High potency was observed in the compound in which one of the benzene rings was reduced (XVI). Compounds containing a carboxamido (XIV) or a hydroxyl (XV) group substituted for the hydrogen on the carbon atom bearing the aliphatic side chain did not exhibit any activity.

Norepinephrine-induced lipolysis in vitro

In Table 4 are shown the effects of the compounds on norepinephrine-induced

TABLE 3. EFFECTS OF AY-8794 AND RELATED TRICYCLIC COMPOUNDS ON GASTRIC ACID SECRETION IN THE RAT

Compound	Gastric acid secretion Dose (mg/kg, i.p.)							
		2·5		5·0		10·0	
	None	(m-equiv./3 hr ± S.E.)	(% of control)	(m-equiv./3 hr ± S.E.)	(% of control)	(m-equiv./3 hr ± S.E.)	(% of control)
AY-8794 (I)	0·55 ± 0·03	0·42 ± 0·07	76	0·27 ± 0·03*	49		
	0·56 ± 0·04	0·45 ± 0·04	80	0·27 ± 0·07†	48		
	0·45 ± 0·03	0·33 ± 0·05	73	0·24 ± 0·05†	53		
	0·55 ± 0·07	0·40 ± 0·03	73	0·27 ± 0·03‡	49		
	0·61 ± 0·07	0·51 ± 0·04	77	0·35 ± 0·03‡	56		
	0·61 ± 0·05	0·44 ± 0·06‡	72	0·35 ± 0·07†	57		
II	0·49 ± 0·03			0·46 ± 0·04	94	0·23 ± 0·03*	47
III	0·55 ± 0·03			0·54 ± 0·03	98	0·36 ± 0·03*	65
IV	0·45 ± 0·03			0·44 ± 0·04	98	0·15 ± 0·03*	33
V	0·45 ± 0·03			0·51 ± 0·02	114	0·16 ± 0·04*	36
VII	0·55 ± 0·03	0·43 ± 0·04‡	78	0·18 ± 0·04*	33		
VIII	0·56 ± 0·04			0·45 ± 0·04	80	0·23 ± 0·03*	41
IX	0·56 ± 0·04			0·51 ± 0·04	91	0·38 ± 0·05‡	68
XI	0·63 ± 0·05			0·45 ± 0·05‡	72	0·38 ± 0·05†	59
XII	0·50 ± 0·04			0·33 ± 0·04‡	66	0·15 ± 0·02*	30
XIII§	0·50 ± 0·04			0·37 ± 0·04	74	0·37 ± 0·04	74
XIV	0·55 ± 0·07			0·59 ± 0·05	108	0·58 ± 0·03	107
XV	0·61 ± 0·07			0·54 ± 0·03	89	0·58 ± 0·08	95
XVI	0·55 ± 0·07	0·44 ± 0·06	81	0·21 ± 0·04†	38		

* $P<0·001$.
† $P<0·01$.
‡ $P<0·05$.
§ Compound was administered as a suspension.

lipolysis *in vitro*. Inhibition of the release of free fatty acids was caused by AY-8794, by the compound containing an eight-membered central ring and with a branched side chain which was attached through a double bond (XI), by the compound containing a hydroxyl group substituted for the hydrogen on the carbon atom bearing the aliphatic side chain (XV), and by the compound containing a seven-membered heterocyclic nitrogen ring and a side chain with one methyl group on the nitrogen (XVII), at 5×10^{-4}M; at 1×10^{-4}M, only AY-8794 and XV exhibited inhibitions. XI, at the lower concentrations of 1×10^{-4}M and 1×10^{-5}M, caused increases in free fatty acid release; at 1×10^{-5}M, AY-8794 also caused an increase and this was greater at 1×10^{-6}M. An increase was observed with XVII at 1×10^{-5}M. Increased release at 5×10^{-4}M was caused by II, V, VII and X. As the levels were lowered, i.e.

TABLE 4. EFFECTS OF AY-8794 AND RELATED TRICYCLIC COMPOUNDS ON NOREPINEPHRINE-INDUCED LIPOLYSIS *IN VITRO*

Compound	Free fatty acids released (μmoles/g tissue ± S.E.)*					
			Norepinephrine + compound (M)			
	Control	Norepinephrine	5×10^{-4}M	1×10^{-4}M	1×10^{-5}M	1×10^{-6}M
AY-8794 (I)	1·70 ± 0·14	7·57 ± 0·61	4·91 ± 0·23† (55)	5·06 ± 0·67‡ (57)		
	1·73 ± 0·13	6·89 ± 1·02			9·93 ± 1·31§ (159)	11·44 ± 0·57‡ (188)
II	2·22 ± 0·43	9·40 ± 0·53	11·32 ± 0·46§ (127)			
III	1·93 ± 0·14	10·86 ± 0·97	8·83 ± 0·60 (77)			
IV	1·98 ± 0·23	6·74 ± 0·38	7·74 ± 0·83 (121)			
V	2·22 ± 0·43	9·40 ± 0·53	11·58 ± 0·69§ (130)			
VII	1·73 ± 0·13	6·89 ± 1·02	8·43 ± 0·96§ (130)	11·36 ± 0·86‡ (187)	12·75 ± 0·49† (213)	
VIII	1·35 ± 0·15	5·42 ± 0·40	4·48 ± 0·37 (77)	6·58 ± 0·28 (128)		
IX	2·22 ± 0·43	9·40 ± 0·53	8·76 ± 0·48 (91)			
X	1·98 ± 0·23	6·74 ± 0·38	9·11 ± 0·90§ (150)			
XI	2·01 ± 0·18	8·38 ± 0·76	6·01 ± 0·83§ (63)	11·33 ± 0·87§ (146)	12·05 ± 0·38‡ (158)	
XII	1·52 ± 0·05	4·90 ± 0·36	4·16 ± 0·29 (88)			
XIII	1·52 ± 0·05	4·90 ± 0·36	5·20 ± 0·58 (109)			
XIV	2·31 ± 0·21	6·30 ± 0·67	6·95 ± 0·16 (116)			
XV	1·92 ± 0·06	12·27 ± 1·05	6·05 ± 1·07‡ (40)	9·17 ± 1·06§ (70)	9·66 ± 0·73 (75)	
XVI	1·98 ± 0·23	6·74 ± 0·38	7·96 ± 0·80 (126)			
XVII	2·31 ± 0·21	6·30 ± 0·67	4·01 ± 0·23‡ (43)	7·54 ± 0·70 (131)	9·32 ± 1·33§ (176)	

* Numbers in parentheses represent % of norepinephrine-induced =

$$100 \times \frac{[\text{NE} + \text{compound}] - \text{control}}{\text{NE} - \text{control}}.$$

There were 4–5 samples in each group.
† $P < 0{\cdot}001$.
‡ $P < 0{\cdot}01$.
§ $P < 0{\cdot}05$.

1×10^{-4}M and 1×10^{-5}M, VII caused greater increases. No changes in free fatty acid release were observed at 5×10^{-4}M with III, IV, VIII, IX, XII, XIII, XIV or XVI.

Anti-inflammatory activity

AY-8794 exhibited significant anti-inflammatory activity at 100 and 25 mg/kg, orally (Table 5). Similar results were obtained with the compound containing one methyl group in the β-position of the aliphatic side chain (III) and with the compound in which the side chain was attached to the ring by a double bond (VII). The compound containing an eight-membered carbocyclic central ring (VIII) also caused significant inhibition at both levels.

TABLE 5. EFFECTS OF AY-8794 AND RELATED TRICYCLIC COMPOUNDS ON CARRAGHEENIN-INDUCED EDEMA*

Compound	Edema formation			
	Dose (mg/kg, i.p.)			
	100		25	
	(g ± S.E.)	(% inhibition)	(g ± S.E.)	(% inhibition)
None	34·0 ± 2·4			
AY-8794 (I)	14·8 ± 1·4†	56	26·0 ± 2·3‡	23
None	34·0 ± 1·0			
III	12·2 ± 1·4†	64	26·7 ± 2·7‡	22
None	42·7 ± 2·3			
VII	25·0 ± 1·8†	41	32·7 ± 1·7§	23
VIII	21·7 ± 1·5†	49	25·8 ± 1·4†	39

*There were six animals in each group.
†$P<0.001$.
‡$P<0.05$.
§$P<0.01$.

DISCUSSION

Evidence indicates that the major mechanism of inactivation of norepinephrine is through the uptake of the norepinephrine into the storage sites of sympathetic nerve endings, whether the norepinephrine is injected[2,3] or endogenously liberated (sympathetic nerve stimulation).[1] Blockade of the uptake mechanism could lead to a higher level of norepinephrine at the receptor sites and thus could result in a potentiation of the effects of the norepinephrine on the effector organs. Various tricyclic compounds such as imipramine, chlorpromazine and desmethylimipramine[4,21] have been found to block the uptake of norepinephrine *in vivo*. In the present studies the tricyclic compound, AY-8794, has been found to cause blockade of uptake of ^{3}H-norepinephrine into the storage sites. In addition, the compound inhibits gastric acid secretion, norepinephrine-induced free fatty acid release (*in vitro*) and induced edema formation.

The blockade of uptake by AY-8794 is not species specific, since the activity is observed both in the mouse and rat. It appears that in the blockade of uptake the following structural elements in this tricyclic series of 5-(dialkylaminoalkyl)-dibenzocycloalkanes are of importance: two benzene rings, a seven-membered central ring, which can be carbocyclic or heterocyclic (nitrogen in the 5-position), or an eight-

membered central carbocyclic ring, the aliphatic side chain linear and of three carbon atoms in length and attached to the ring through a single bond, and the dialkyl groups being methyl groups.

AY-8794 is a potent inhibitor of gastric acid secretion in the rat. This antisecretory activity is retained in the compound in which the aliphatic side chain is attached to the ring through a double bond instead of a single bond (VII) and in the compound in which one of the benzenes is reduced (XVI). The compound containing a central ring which is heterocyclic with nitrogen (XII) instead of carbocyclic also exhibits antisecretory activity under these conditions. As was observed with respect to blockade of uptake activity, the linearity of the side chain is also of importance for the antisecretory activity, since branching of the side chain leads to reduction in activity (III vs. AY-8794; XIII vs. XII). In this relation it is of interest that the compound G-31.002 [*N*-(γ-dimethylaminoisopropyl)-iminodibenzyl hydrochloride], which is structurally related to XII and contains the branched side chain dimethylaminoisopropyl instead of the straight chain dimethylaminopropyl, does not reduce gastric acid secretion whereas XII does.[22] Furthermore, XII, in contrast to G-31.002, reduces induced ulcer incidence;[22] the activity of AY-8794 in this respect is of interest.

Further similarities between the blockade of uptake and the antisecretory activities are found with the compounds which exhibit reduced, or no, activity of both, i.e. in the compounds in which the dimethylaminopropyl side chain is β-substituted with methyl groups in place of the hydrogens (II), substituted with ethyl groups for the methyl groups on the nitrogen (IV), or shortened to two carbons in length (V). Also, when a group, such as a carboxamido (XIV) or a hydroxyl (XV), is substituted for the hydrogen on the carbon atom bearing the aliphatic side chain, both activities are lost. With respect to the relationship of the catecholamines and gastric secretion, it has been shown that catecholamines as well as serotonin inhibit gastric secretion.[23,25] Prevention of the inactivation of norepinephrine through the blockade of uptake by the compounds could lead to a higher level of endogenous norepinephrine and result in inhibition of gastric acid secretion. Another tricyclic compound, XII, which causes blockade of uptake,[21] also exhibits gastric antisecretory action.[22,26,27] It is possible that other activities of the compound are of importance in their observed antisecretory actions. In this connection anticholinergic activities have been demonstrated for AY-8794,[28] VII[29] and XII.[30]

It has been widely shown that addition of norepinephrine or epinephrine to the epididymal fat pad of the rat *in vitro* causes stimulation of the release of free fatty acids.[31,32] Some of the compounds studied [i.e. the compound in which there is a disubstitution of methyl groups for the β-hydrogens on the dimethylaminopropyl side chain (II) of AY-8794, the compound having a shortened linear side chain of two carbons in length (V) or the dimethylaminopropyl side chain attached to the ring by a double bond (VII) instead of a single bond, and the compound in which the central ring is eight-membered instead of seven-membered and the attachment of the side chain is through a double bond (X)] cause an increase in the free fatty acid release induced by norepinephrine *in vitro*. As pointed out, compounds which prevent the uptake of norepinephrine, and thus its inactivation, could cause an increase in the activity of the norepinephrine. It is of interest that in consideration of the attachment of the linear side chain to the central ring, those compounds in which the attachment is through a double bond (VII and X) instead of a single bond cause an increase in the

induced free fatty acid release. It should be noted that the free fatty acid release studies were *in vitro* while the norepinephrine uptake studies were *in vivo* and this could be of importance in differences observed with respect to various of the other compounds studied.

AY-8794 inhibits the free fatty acid mobilization *in vitro* induced by norepinephrine. This inhibitory activity is also observed with the compound containing an eight-membered central ring and with a branched side chain which is attached through a double bond (XI), the compound containing a hydroxyl group substituted for the hydrogen on the carbon atom bearing the aliphatic side chain (XV), and desmethylimipramine (XVII). With respect to the inhibitory activity, it is of interest that the substituents on the nitrogen of the aliphatic side chain are of importance, since the compound which contains only one methyl group (XVII) inhibits, whereas the disubstituted compound (XII) does not. The type of group substituted on the carbon to which the aliphatic side chain is attached plays a role in the inhibitory activity, since the compound containing a hydroxyl group (XV) is inhibitory, whereas the compound containing a carboxamido group (XIV) is not. When the central ring is an eight-membered carbocyclic (VIII) or a seven-membered heterocyclic (XII) instead of a seven-membered carbocyclic (AY-8794), the inhibitory activity is lost, thus showing the importance of the nature of the central ring. The influence of the two aromatic rings in AY-8794 is shown by the finding that when one ring is a saturated one (XVI) the inhibitory activity is lost.

The importance of the level of the compound is indicated by the finding that the compound may exhibit dual activities. At lower concentrations AY-8794, XI and XVII cause stimulation, whereas at a higher level they cause inhibition of the norepinephrine-induced release of free fatty acids.

The activation of lipolytic activity by the catecholamines involves the catecholamine-stimulated conversion of adenosine triphosphate to 3′5′-cyclic adenosine monophosphate, which in turn activates the lipolytic enzymes.[33,34] *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 noncompetitively.[35,36] The β-receptor blocking agents appear to be inhibiting at the receptor level and the α-adrenergic blocking agents act by nonspecifically impairing the activation of lipase by cyclic adenosine monophosphate.[37] As observed in the present studies, XVII, which causes blockade of norepinephrine uptake,[38] has also been shown by others[39] to inhibit at a high concentration the lipolytic activity in adipose tissue. With XVII, the antagonism of free fatty acid mobilization occurs whether the mobilization is induced by catecholamines or by other means, and the addition of XVII to an already activated lipase preparation causes a prompt cessation of lipolytic activity. It has been suggested that the XVII directly antagonizes lipolytic enzymes and that the effects on the adrenergic receptor are secondary to the primary inhibition phase.[39] It is possible that at the high concentrations AY-8794, XI and XV also act by a similar mechanism. At the lower concentrations of AY-8794, XI and XVII, it is possible that the effects on the adrenergic receptor are of importance with respect to the stimulation of the norepinephrine-induced release. It is of interest that, in addition to AY-8794 and XVII, the compound AY-9928 [*N,N′-bis*(1-naphthylmethyl)-1,4-cyclohexane *bis* (methylamine) dihydrochloride], which causes a blockade

of uptake of norepinephrine but is not a tricyclic derivative, also inhibits free fatty acid release induced by norepinephrine.*

Various of the compounds [i.e. AY-8794, the compound in which in the dimethylaminopropyl side chain there is a monosubstitution of a methyl group for a β-hydrogen (III), and the compound containing a central eight-membered (VIII) instead of a seven-membered ring] were examined for their ability to interfere with the carrageenin-induced edema and each was found to inhibit the edema formation. It has been demonstrated that catecholamines, released by stimuli, inhibit partially or completely the acute inflammatory response, probably by acting as local anti-inflammatory hormones.[40] Potentiation of the endogenous catecholamines through their blockade of uptake of norepinephrine could thus be of importance with respect to the anti-inflammatory activities of the above compounds. In relation to this, other tricyclic compounds which block the uptake of norepinephrine, such as VII, XII, XVII and nortriptyline, also exhibit anti-inflammatory activities.[41] Furthermore, that the catecholamines are of importance in the anti-inflammatory activity of these tricyclic compounds has been demonstrated by the finding that this effect is antagonized by adrenalectomy, demedullation, norepinephrine-depleting drugs and drugs which inhibit norepinephrine biosynthesis.[41] In the present studies, III exhibited anti-inflammatory activity although, in contrast to AY-8794, VIII and XVI, the compound does not cause a blockade of uptake. However, in this respect it is of importance that the uptake studies were carried out under different conditions from those of the anti-inflammatory studies, i.e. at a lower dose level, for a shorter time period, and with an intraperitoneal injection instead of oral administration.

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