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THE EFFECT OF SOME INHIBITORS OF THE POSTGANGLIONIC SYMPATHETIC MECHANISM ON MONOAMINE OXIDASE

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Abstract—The effect on liver monoamine oxidase of a series of inhibitors of postganglionic sympathetic transmission was studied. Chemically, the inhibitors are derivatives of benzyl-substituted quaternary ammonium and guanidinium as well as β-phenoxyethyl-substituted guanidinium salts.

With kynuramine as substrate these compounds inhibited liver monoamine oxidase of rats, guinea pigs, and cats. Bulkiness of *ortho*-substituents appeared to enhance the inhibition of both the enzyme *in vitro* and adrenergic transmission *in vivo*. Such parallelism was not observed with compounds whose guanidine portion was methylated.

In vivo, typical representatives were found to be relatively well-absorbed, peripherally-acting inhibitors of monoamine oxidase.

It is suggested that guanidinium analogs of bretylium, in addition to their adrenergic blocking properties, may also inhibit the monoamine oxidase in peripheral sympathetic fibers. The localization and possible role of the enzyme in adrenergic fibers is discussed briefly.

THE role of monoamine oxidase in adrenergic mechanisms is not fully understood.[1] It is likely that the enzyme is involved in the limitation of adrenergic transmission[2]; in addition, since sympathetic ganglia contain monoamine oxidase,[3–5] the enzyme may influence their mechanism of transmission.[6]

As far as inhibition of monoamine oxidase is concerned, much attention has been devoted to the effect of monoamine oxidase inhibitors on the central nervous system, and a number of such agents has been introduced into clinical medicine.[7, 8] Amongst other side effects (*e.g.* Reference 9) postural hypotension has usually been observed, and it appeared that a causal relationship existed between monoamine oxidase inhibition and orthostatic lowering of blood pressure.[10, 11*]

In order to investigate the role of monoamine oxidase in adrenergic mechanisms, we have examined the influence of a series of sympathetic postganglionic blocking agents[14] on this enzyme *in vitro*, and typical representatives have been tested *in vivo*. Chemically, these compounds are derivatives of benzyl-substituted quaternary ammonium (I) and guanidinium (II), as well as β-phenoxyethyl-substituted guanidinium (III) salts. Some of these compounds were first reported by the Wellcome group and described as powerful adrenergic neuron-blocking agents.[15, 16] Costa *et al.*[17]

* In contrast to Horwitz and Sjoerdsma,[11] Bucci and Saunders[12] found no postural hypotension in clinical studies with the monoamine oxidase inhibitor N-benzyl-N-methyl-2-propynylamine (MO-911).[13]

have recently examined the influence of a few of these compounds on the norepinephrine levels in the heart of rats.

The effect on monoamine oxidase of guanethidine[18] was also investigated.

METHODS

The quaternary ammonium (I) and guanidinium (II, III) salts were synthesized in our laboratories.[14]

Monoamine oxidase activity or inhibition was measured by the direct spectrophotometric method of Weissbach *et al.*[19] using kynuramine as a substrate and measuring its disappearance at 360 mμ. A Beckman model DK-1 spectrophotometer was used.

The enzyme system used was a crude tissue extract prepared by homogenizing the liver (or brain) in five volumes of distilled water followed by centrifugation at 1,000 rpm for 15 min. The incubation medium consisted of 100 μg (0·23 μmole) of kynuramine dihydrobromide, 0·3 ml of 0·5 M phosphate buffer at pH 7·4, 0·1 or 0·2 ml of the liver homogenate, and distilled water to a total volume of 3·0 ml. Incubations were run at room temperature. Activity is expressed as the change in absorbance at 360 mμ in 5 min measured against a blank cuvet in which water replaced kynuramine. In inhibition studies a chosen concentration of the compound under investigation was added to both cuvets.

In the studies *in vivo*, at selected time intervals after administration of the compound under investigation, the animals were sacrificed and the liver (or brain) homogenized as described above. Inasmuch as the tissue sample undergoes extensive dilution before and during the assay, it is probable that the values obtained for inhibition of monoamine oxidase *in vivo* represent minimal values and that in the intact animal the enzyme is inhibited to a greater extent.

The LD_{50} values reported in Tables 3, 4, 5, and 6 were obtained by administering intravenously several doses of the compound to mice (3 to 5 animals per dose). No statistical evaluation of data was made. The LD_{50} values were approximated from 10 to 15 mice per compound.

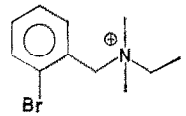
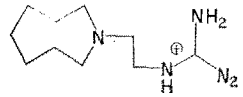
Contraction of the nictitating membrane was measured in cats. Animals were anaesthetized with an intravenous dose of 60 mg of chloralose/kg, and the cervical sympathetic chain and the superior cervical ganglion were isolated. Postganglionic fibers were stimulated electrically by 4 to 6 v of 2 msec duration at a frequency of 20/sec for 3 sec. The contractions of the nictitating membrane were recorded on a revolving drum.

RESULTS

Bretylium (Table 1) and bretylium-like quaternary ammonium salts (Table 2), as well as guanidinium analogs of bretylium (Tables 3 and 6) and TM-10[20] (Table 4) inhibit monoamine oxidase *in vitro*. The extent of inhibition varied according to the animal species (cf. Reference 21), and variation seemed to be greater with quaternary ammonium salts.

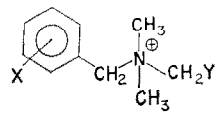
In both the quaternary ammonium and guanidinium series *ortho*-substituents appear to enhance the inhibition of monoamine oxidase (Tables 2, 3, and 4). The effects of the same *ortho*-substituents in both series on the LD_{50}, on the response of a nictitating

TABLE 1. *IN VITRO* INHIBITION OF LIVER MONOAMINE OXIDASE IN VARIOUS MAMMALIAN SPECIES BY BRETYLIUM* AND GUANETHIDINE†

	% Inhibition at concentration										
	1×10^{-2} M				1×10^{-3} M				1×10^{-4} M		
	G. pig	Cat	Dog	Rat	G. pig	Cat	Dog	Rat	G. pig	Cat	Dog
 Bretylium				28	94	41	49	8	61	13	9
 Guenethidine	59	67	53	0	19	11	11				

* Mol. wt: 414·36.
† Mol. wt. 247·35.

TABLE 2. EFFECT OF AROMATIC AND SIDE CHAIN SUBSTITUTION ON INHIBITION OF GUINEA PIG LIVER MONOAMINE OXIDASE *IN VITRO* BY QUATERNARY AMMONIUM SALTS OF TYPE (I)



(I)

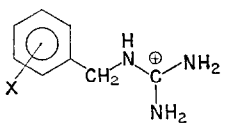
X	Y									
	—CH_2OH			—CH_2Cl		—CH—CH_2			—CH_2—C≡N	
	1×10^{-4}*	1×10^{-5}	1×10^{-6}	1×10^{-4}	1×10^{-5}	1×10^{-3}	1×10^{-4}	1×10^{-5}	1×10^{-4}	1×10^{-5}
H	56†	12		30	0					
2-CH_3	68	27		32	0	71	32		50	13
2-Cl	84	47		43	6	84	46	4		
2-Br	83	36	6	50	7	85	44	3	72	22
2-OCH_3	59	17		37	9					
2-CN	63	17	0	11	0	57	15			
2,6-Cl_2	95	78	46	76	30	91	68	23	88	48

* Molar concentration of compound.
† Per cent inhibition.

membrane, and on the degree of monoamine oxidase inhibition *in vitro* are presented in Table 5. The effects of methylation of the guanidine portion are snown in Table 6.

With kynuramine as substrate, two typical representatives of these types of compounds, namely bretylium and compound 12,695 (α-naphthyl-methylguanidinium chloride) act competitively, as shown by a pair of parallel lines obtained with modified (s/v *vs.* s)[22] Lineweaver-Burk plots.

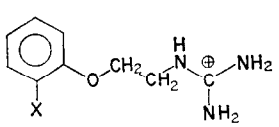
TABLE 3. EFFECT OF AROMATIC SUBSTITUTION ON INHIBITION OF RAT LIVER MONOAMINE OXIDASE *IN VITRO* BY GUANIDINIUM SALTS OF TYPE (II)



(II)

X	Compound no.	LD_{50} (μmole/kg)	% Inhibition at concentration 1×10^{-3} M	1×10^{-4} M	1×10^{-5} M
H	12,681	436	67	18	0
2-CH_3	12,694	250	77	33	4
3-CH_3	12,741	234	39	41	2
4-CH_3	12,740	248	87	54	4
2-F	12,703	120	83	40	7
2-Cl	12,682	195	92	56	17
3-Cl	12,706	322	92	59	8
4-Cl	12,705	280	98	44	2
2-Br	12,684	131	96	78	26
3-Br	12,738	183	87	51	10
4-Br	12,723	166	85	55	7
2-I	12,721	135	83	51	9
2,4-Cl_2	12,696	144	89	79	30
3,4-Cl_2	12,708	180	81	69	18
α-naphthylmethyl	12,695	72		90	56

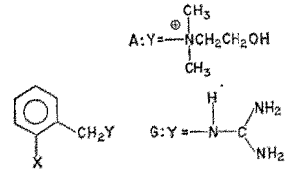
TABLE 4. EFFECT OF *ORTHO*-SUBSTITUENTS ON INHIBITION OF GUINEA PIG LIVER MONOAMINE OXIDASE *IN VITRO* BY GUANIDINIUM SALTS OF TYPE (III)



(III)

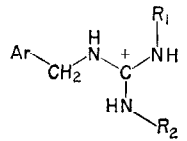
X	Compound no.	LD_{50} (μmole/kg)	% Inhibition at concentration 1×10^{-4} M	1×10^{-5} M
H	12,717	246	42	6
—$CH(CH_3)_2$	12,730	48	45	11
2,6-$(CH_3)_2$	12,719	73	64	26

TABLE 5. COMPARISON OF *ORTHO*-SUBSTITUTED BENZYL-QUATERNARY AMMONIUM (Y = A) AND GUANIDINIUM (Y = G) SALTS

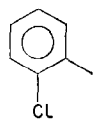
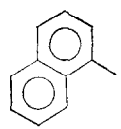


X	Y	Compound no.	LD_{50} (μmole/kg)	% Inhibition of contraction of nictitating membrane 60 min after 10 μmoles/kg i.v.	% MAO inhibition *in vitro*, at concentration 1×10^{-3} M		1×10^{-4} M		1×10^{-5} M			1×10^{-6} M	
					Rat	Cat	Rat	G. pig	Cat	Rat	G. pig	Cat	G. pig	Cat
H	A	12,606	252	2		31		56	16		12			
	G	12,681	436	13	67	68	18	29	24	0	10	5		
CH_3	A	12,609	181	14		31		68	4		27			
	G	12,694	250	19	77	78	33	64	21	4	1			
Cl	A	12,619	158	56		55		84	14		47		16	
	G	12,682	195	35	92		56	75	58	17	30	20	16	9
Br	A	12,634	130	59		45		83	3		36		6	
	G	12,684	131	21	96		78	76	75	26	18	24		0
2,6-Cl_2	A	12,685	114	70		79		95	35		78	4	46	
	G	12,701	62	44				90	74		55	42	18	5
Naphthyl	G	12,695	72	63		100	90	87	78	56	56	38	17	12

TABLE 6. EFFECT OF N-METHYLATION ON INHIBITION OF GUINEA PIG AND CAT LIVER MONOAMINE OXIDASE *IN VITRO* BY GUANIDINIUM SALTS OF FORMULA (IV)

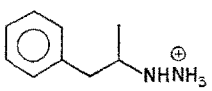
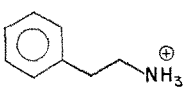
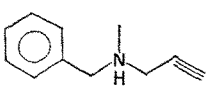
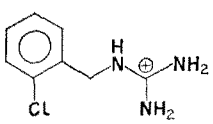
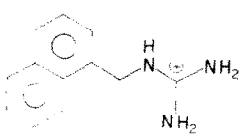


(IV)

Ar	R_1	R_2	Compound no.	LD_{50} (μmole/kg)	% Inhibition of contraction of nictitating membrane, 60 min after 10 μmoles/kg i.v.	% MAO inhibition *in vitro* at concentration 1×10^{-3} M		1×10^{-4} M		1×10^{-5} M		1×10^{-6} M	
						G. pig	Cat	G. pig	Cat	G. pig	Cat	G. pig	Cat
	H	H	12,681	436	13		68	29	24	10	5		
	H	CH_3	12,764	212	79	73	72	43	30	11	4		
	CH_3	CH_3	12,761	92	100	71	54	23	23	7	9		
	H	H	12,682	195	35			75	58	30	20	16	9
	H	CH_3	12,763	135	83		100	80	93	27	28	18	11
	CH_3	CH_3	12,755	52	98	71	67	43	39	24	19		
	H	H	12,695	72	63		100	87	78	56	38	17	12
	H	CH_3	12,752	35	57	81		56		24			
	CH_3	CH_3	12,758	68	34	74		42		10			

For the purpose of comparison, the known monoamine oxidase inhibitors catron (α-phenyl-isopropylhydrazine),[23] parnate (2-phenylcyclopropylamine),[24] and MO-911 (N-methyl-N-benzyl-propynylamine)[13] were tested *in vitro* under the same conditions and their activity compared with compounds 12,682 and 12,695 (Table 7).

TABLE 7. COMPARISON OF DIFFERENT MONOAMINE OXIDASE INHIBITORS

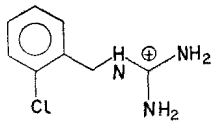
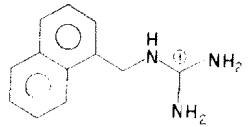
Formula	Name	% Inhibition of liver MAO *in vitro*, at concentration 1×10^{-4} M		1×10^{-5} M		1×10^{-6} M	
		G. pig	Cat	G. pig	Cat	G. pig	Cat
	Catron		100	70	61	19	13
	Parnate			100	93	20	30
	MO-911	100		60	95	12	38
	12,682	75	58	30	20	16	9
	12,695	87	78	56	38	17	12

Compounds 12,682 (2-chlorobenzyl-) and 12,695 (α-naphthylmethyl-guanidinium chloride) were tested *in vivo* in guinea pigs (Table 8).

Comparison of inhibitory effects on monoamine oxidase *in vitro* and *in vivo* is complicated, (1) because crude enzyme preparations were used whose homogeneity has not been established,[25] and (2) *inter-* as well as *intra*-species differences in the pattern of monoamine oxidase substrate and inhibitor specificity[26] are more complex with reversible inhibitors.[27]

Since very high concentrations of guanethidine were required for partial inhibition of liver monoamine oxidase *in vitro* (Table 1), it is most unlikely that this can contribute to the mechanism of action of guanethidine.

TABLE 8. INHIBITION OF LIVER MONOAMINE OXIDASE IN THE GUINEA PIG *IN VIVO* AFTER 50 μMOLE OF COMPOUNDS 12,682* AND 12,695/KG†

Formula	Compound no.	Route	Change in absorbance at 360 mμ in 5 min			
			Hours after dose			
			0	1	2	4
	12,682	i.p.	0·118 ± 0·025‡	0·069 ± 0·004	0·063 ± 0·012 (47%)§	0·083 ± 0·012
		p.o.	0·114 ± 0·018	0·089 ± 0·014 (22%)	0·105 ± 0·017	0·100 ± 0·018
	12,695	i.p.	0·120 ± 0·010		0·072 ± 0·004 (40%)	
		p.o.	0·093 ± 0·011	0·068 ± 0·008	0·066 ± 0·006 (29%)	0·082 ± 0·007

* Mol. wt: 220·11.
† Mol. wt: 235·71.
‡ Standard error; at least five animals were used for each value.
§ Maximal inhibition of monoamine oxidase.

DISCUSSION

The pharmacodynamic properties of bretylium are believed to be caused by suppression of release of norepinephrine from sympathetic nerve ends.[28] It has been recently suggested that bretylium prevents the physiological, acetylcholine-mediated release of norepinephrine by blocking cholinergic receptor sites at the catecholamine store.[29] A similar mechanism has been postulated for *ortho*-substituted guanidinium analogs of bretylium.[16, 17]

According to the present results, a group of guanidinium analogs of bretylium, known to inhibit the postganglionic sympathetic mechanism,[14, 16] also inhibits liver monoamine oxidase. Furthermore, bulkiness of the *ortho*-substituent increases the toxicity of these compounds and appears to enhance their inhibitory effects both on the response of a nictitating membrane and on liver monoamine oxidase activity *in vitro*.* Methylation of the guanidine portion, on the other hand, while enhancing the blocking effect on the nictitating membrane and increasing the toxicity, does not seem to affect the ability of the compounds to inhibit monoamine oxidase.

Two members of the series, compounds 12,682 and 12,695, were tested and found to inhibit liver monoamine oxidase *in vivo*. The difference in degree of monoamine oxidase inhibition resulting from intraperitoneal and oral administration (cf. Reference 32) seems to indicate a relatively good gastrointestinal absorption. An intraperitoneal dose of 100 μmole of compound 12,695/kg caused in guinea pigs after 2 hr a 54% inhibition of liver monoamine oxidase. The finding that at the same time brain monoamine oxidase activity remained unchanged, suggests inability of the compound to pass the blood-brain barrier. Hence, these inhibitors of peripheral adrenergic transmission are also peripherally-acting inhibitors of liver monoamine oxidase.

Belleau *et al.*[2] have established recently, that liver monoamine oxidase and the monoamine oxidase involved in adrenergic mechanisms display identical absolute stereospecificity. From this they have concluded that these two enzymes may be very similar in properties and mechanism of action. Since the compounds we have investigated inhibited liver monoamine oxidase *in vivo*, and in view of their affinity for adrenergic fibers it is, in accordance with Belleau's conclusion, tempting to postulate that these compounds may also inhibit the monoamine oxidase involved in adrenergic mechanisms.

The fact that the sympathomimetic response (the rate of degradation by monoamine oxidase of deuterated tyramine) is determined by the configuration-dependent isotope effect prompted Belleau *et al.*[2, 33] to conclude that the enzyme involved is intimately associated with adrenergic neuroeffector cells. However, since tyramine acts indirectly—*i.e.* by displacing from the catecholamine store norepinephrine[34–37] which in turn triggers an excitatory response at the effector cells[38]—Belleau's results reveal close proximity of monoamine oxidase to the catecholamine store rather than to neuroeffector cells. It is feasible that the physiological function of this enzyme in adrenergic mechanism is that of an oxidative barrier which controls the displacement of norepinephrine by potential sympathomimetic amines. If monoamine oxidase is indeed associated with the catecholamine store, inhibition of the enzyme may influence the accessibility of norepinephrine-binding sites to norepinephrine-displacing amines.

* In the structurally somewhat related[30] series of *ortho*-substituted S-benzyl-isothiouronium salts, Fastier and Hawkins[31] have observed *in vitro* a similar enhancement of inhibition of rabbit liver monoamine oxidase.

Evidence is accumulating that, in contrast to their apparently similar adrenergic blocking actions,[39, 40] bretylium and guanethidine differ in their interactions with the receptor sites involved in the physiological release of norepinephrine. It appears that bretylium and related quaternary ammonium as well as guanidinium derivatives paralyze, whereas guanethidine stimulates[41, 42] the release mechanism. In accordance with our results, guanidinium analogs of bretylium may, in contrast to guanethidine, also inhibit monoamine oxidase in peripheral sympathetic fibres.

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