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Effects of a Group of Dibenzodiazepines on Fatal Systemic Anaphylaxis in Mice, Rats, and Guinea Pigs

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A series of dibenzodiazepines was tested for protection against fatal systemic anaphylaxis in mice, rats, and guinea pigs. Structure–activity relationships are discussed. An aliphatic tertiary amine in position 5 appeared to enhance antianaphylactic action in general. Compounds active in one species were not necessarily active in another. The mechanism of action is not known. There was some correlation between inhibition of α-chymotrypsin and antianaphylactic activity, and since it has been postulated that a chymotrypsin-like enzyme is involved in release of mediators, this effect may have some bearing on the observed protection against anaphylactic shock.

It is well known that the manifestations of anaphylactic shock vary in different species. These variations in response may be explained by the involvement of different target organs and mediators in the different species.[1] Depending on the species and the routes of injection of antigen, the organs affected may be lungs, liver, or vascular tissues, and the mediators may be histamine, serotonin, slow reacting substance in anaphylaxis (SRS-A), or bradykinin as well as acetylcholine, heparin, and possibly others. In an effort to find a broad spectrum antiallergy agent with properties other than, or in addition to, histamine antagonism, compounds were tested for protection against anaphylaxis in mice, rats, and guinea pigs. It should be pointed out that active compounds do not necessarily protect by antagonizing the mediators involved. They might inhibit an earlier stage in the anaphylactic reaction such as antibody–antigen combination, release or activity of hydrolytic enzymes known to be involved, or even the release of mediators.[2]

A group of compounds showing varying degrees of antianaphylactic activity in these three species is seen among the dibenzodiazepines. The synthesis of representative compounds in this series has been described previously by Hanze, *et al.*,[3] and by Hunziker, *et al.*[4] The latter authors stated that a group of 11-oxodibenzodiazepines showed antihistamine and antianaphylactic activity in guinea pigs. The preparation of such compounds has also been described with claims for their use in the treatment of allergic conditions in a patent by Schmutz and Hunziker.[5] The effects on anaphylaxis of a number of dibenzodiazepines are described below.

Experimental Section

A. Fatal Systemic Anaphylaxis. 1.—Mice were sensitized according to a modification of the procedure of Waalkes and Coburn.[6] Each mouse was injected with 1 ml ip and 0.5 ml sc of a mixture of 3 parts horse serum and 2 parts pertussis vaccine. They were challenged 14 or 15 days later, the time of peak sensitivity, with horse serum iv. The dose used was the lowest volume producing close to 100% mortality.

2.—Rats were sensitized according to Goth's[7] modification of a procedure by Sanyal and West.[8] Each rat was injected ip and sc with a total of 1.0 ml of pertussis vaccine (Lilly) mixed with 0.5 ml of bovine serum albumin (BSA) soln (60 mg/ml). They were challenged 14 or 15 days later with the minimum vol of a soln contg 60 mg/ml of BSA injected iv which produced close to 100% mortality.

3.—Guinea pigs were sensitized with 1 ml ip and 1 ml sc/guinea pig of a soln contg 10% egg albumin and 0.5% phenol as described by Austen and Brocklehurst.[2a] They were challenged from 3 weeks to a few months later with 10% egg albumin, injected ip, in the lowest dose causing close to 100% mortality.

Compounds to be tested were dissolved or suspended in a vehicle contg 1% carboxymethylcellulose, 0.4% polysorbate 80, and 0.04% propylparaben starting with a max dose of 10% of the mouse LD_{50} for mice, and, in most cases, 7% of the mouse LD_{50} for rats and guinea pigs. If this was effective the compound was tested in doses reduced by a factor of 0.5 until a dose was reached which afforded no protection. Control animals received vehicle alone. The vol of soln or suspension was 0.1 ml/10 g for mice, 0.5 ml/100 g for rats and guinea pigs. The dose recorded in Table I is the lowest dose tested at which statistically significant differences ($p = 0.05$ or less) between the treated and control groups were obtained. It is possible, and indeed likely in some instances, that if larger numbers of animals were tested this statistically effective dose would be reduced somewhat. However, as we were mainly interested in finding the most active compounds this was not feasible.

The number of animals at the dose recorded was small even though significant. Whenever a dose was effective, lower doses were tested so there are a large number of experiments not reported to support the data provided, and when doses lower than 10% of the mouse LD_{50} are tabulated their preceding higher doses also gave significant protection. The dose recorded protected between 60 and 100% of the animals and so is higher than the ED_{50}. Since we do not have results with doses lower than ED_{50} in all cases with a sufficient number of animals, we have not calculated or estimated ED_{50} and have chosen the procedure described above for recording effectiveness.

B. Enzyme Activity. 1. Chymotrypsin Activity. α-Chymotrypsin was purchased from Worthington Biochemical Co. The procedure for measuring activity was that of Schwert and Takenaka[9] using acetyltyrosine ethyl ester as substrate and measuring the change in absorbancy at 237 mμ. Compds were tested for inhibitory activity at 20, 10, and 5 μg/ml. Since these compds occasionally interfered with the absorption at 20 and frequently at 30 μg/ml, concns giving 50% inhibition were usually not attained. If inhibitions less than 20% were found at 20 μg the compds were considered inactive. In Table I, compds considered active inhibited at least 25% in concns between 6×10^{-5} and 10^{-5} *M*.

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(1) (a) K. F. Austen and J. H. Humphrey, *Advan. Immunol.*, **3**, 1 (1963). (b) G. B. West, *Clin. Pharmacol. Ther.*, **4**, 749 (1963).

(2) (a) K. F. Austen and W. E. Brocklehurst, *J. Exp. Med.*, **113**, 521 (1961). (b) E. L. Becker and K. F. Austen, *ibid.*, **120**, 491 (1964). (c) K. F. Austen and E. L. Becker, *ibid.*, **124**, 397 (1966).

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(4) F. Hunziker, F. Künzle, and J. Schmutz, *Helv. Chim. Acta*, **46**, 2337 (1963).

(5) J. Schmutz and F. Hunziker, U. S. Patent 3,150,125 (1962). Assignors to Dr. A. Wander.

(6) T. P. Waalkes and H. Coburn, *J. Allergy*, **31**, 151 (1960).

(7) A. Goth, personal communication (1964).

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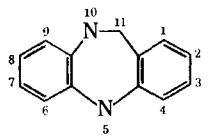
Table I

Effect of Substituted Dibenzodiazepines on Fatal Systemic Anaphylaxis and on Chymotrypsin Activity



Compd	Substituents at position 5	10	11	Substituents at other positions	Salt	LD_{50}[a] mice, mg/kg ip	Fatal systemic anaphylaxis[b] — Mice: Dose, mg/kg ip	Mice: Survivors, total no. Treated	Mice: Survivors, total no. Control	Rats: Dose, mg/kg ip	Rats: Survivors, total no. Treated	Rats: Survivors, total no. Control	Guinea pigs: Dose, mg/kg ip	Guinea pigs: Survivors, total no. Treated	Guinea pigs: Survivors, total no. Control	Chymotrypsin[c]
I	$CCCN(CH_3)_2$	H	O			167	2.0	7/9	5/21	4.2	6/6	2/10	16.7	8/10	1/13	I
II	$CCCN(CH_3)_2$	H	H_2			198	1.3	5/6	2/12	2.0	5/6	2/9	10.0	9/10	2/12	I
III	$CCN(C_2H_5)_2$	H	O			300	8.0	11/12	13/30	30.0	4/6	0/9	30.0	7/7	1/7	I
IV	$CCCN(CH_3)_2$	H	H_2	3-Cl		178	5.0	5/6	4/14	5.0	7/8	4/16	17.8	3/3	0/3	I
V	$CCN(C_2H_5)_2$	$CCCN(CH_3)_2$	O		2HCl	533	11.0	10/15	10/36	11.0	5/5	2/9	13.4	4/5	1/8	I
VI	$CCCN(C_2H_5)_2$	H	H_2			167	5.0	3/3	0/6	8.4	7/11	2/14	16.7	NS		I
VII	H	$CCN(CH_3)_2$	O	7-Cl	HCl	75	10.0	6/6	5/15	2.0	10/12	3/18	10.0	NS		I
VIII	$CCN(C_2H_5)_2$	H	H_2			65	6.5	16/23	10/51	5.0	11/18	3/31	10.0	NS		I
IX	$CCCNCH_3$	H	H_2		2HCl	178	17.8	3/3	1/6	8.9	8/12	3/16	17.8	NS		I
X	$CCCN(CH_3)_2$	H	O	3-Cl	HCl	178	17.8	3/3	1/6	5.0	4/5	0/5				—
XI	$CCN(\rightarrow O)(C_2H_5)_2$	H	O			1000	100.0	4/5	2/12	70.0	3/3	1/6				O
XII	$CCN(CH_3)_2$	$\Delta^{10,11}$	C_6H_5	2-Cl		1000	5.0	3/3	1/6	35.0	NS					I
XIII	$CCCN(\rightarrow O)(CH_3)_2$	H	O			300	7.5	3/3	1/6	21.0	NS		40.0	3/3	0/3	I
XIV	$CCCN(C_2H_5)_2$	H	O			200	5.0	3/3	1/6	14.0	NS		20.0	NS		O
XV	H	$CCCNCH_3$	O		HCl	178	17.8	6/6	4/18	17.8	NS		17.8	NS		O
XVI	H	$CCCNCH_3$	H_2		2HCl	56	5.6	NS		4.5	NS		5.6	NS		I
XVII	H	$CCCN(C_2H_5)_2$	O			200	20.0	3/3	1/6	20.0	NS		10.0	NS		I
XVIII	H	$CCN(C_2H_5)_2$	O			200	20.0	NS		14.0	NS					—
XIX	H	$CCCN(CH_3)_2$	H_2		2HCl	65	6.5	5/6	3/18	4.6	NS					I
XX	$CCCN(\rightarrow O)(C_2H_5)_2$	H	O			533	53.3	5/6	0/6	53.3	NS					O
XXI	H	$CCCN(CH_3)_2$	H_2	3-Cl	2HCl	75	7.5	3/3	1/6	5.3	NS					Color interference
XXII	H	CH_3	O			1000	100.0	NS		70.0	3/3	1/6				—
XXIII	H	$CCN(C_2H_5)_2$	H_2		HCl	65	6.5	NS		4.6	NS					I
XXIV	H	CCCN⟨⟩NC	O		$2(CHCOOH)_2$		50.0	NS		14.0	NS					O
XXV	H	H	O			>1000	100.0	NS		100.0	NS					—
XXVI	H	$CCCN(CH_3)_2$	O			100	10.0	NS		7.0	NS					I
XXVII	H	H	H_2		HCl	533	53.3	NS		37.3	NS					—
XXVIII	H	$\Delta^{10,11}$	H	1,2,3,4-H_4	H_2O	562	56.2	NS		39.3	NS					—
XXIX	CH_3	CH_3	H_2			178	17.8	NS		12.5	NS					O
XXX	H	H	S			1000	100.0	NS		70.0	NS					—
XXXI	CCCNC	H	O		HCl and H_2O	100	10.0	NS		7.0	NS					I
XXXII	$CCNC(CC)_2I^-$	CH_3	O			65	6.5	NS		4.6	NS					I
XXXIII	H	CCCN⟨⟩NC	H_2		$2.5(CHCOOH)_2$		20.0	NS		14.0	NS					O
XXXIV	H	$\Delta^{10,11}$	C_6H_5	2-Cl		>1000	10.0	NS		70.0	NS					—

XXXV	CO—CC_4H_9—CONa—O	H	O	1000	100.0	NS	—

[a] Mice LD_{50} values were supplied by W. Veldkamp, Pharmacology Department, The Upjohn Co., Kalamazoo, Mich. [b] The lowest dose tested which protected against anaphylaxis is given; otherwise the dose is 10% of the mouse LD_{50} for mice or either 10 or 7% of the mouse LD_{50} for rats and guinea pigs. Where figures are given under "survivors" the differences between control and treated are statistically significant when determined from a 2×2 contingency table evaluated either by χ^2 or by exact probability. NS indicates the differences were not significant. Assistance with statistics was kindly given by Dr. C. M. Metzler of Research Computer Services, The Upjohn Co., Kalamazoo, Mich. [c] I indicates that the compound inhibited at least 25% in a concentration between 6×10^{-5} and 10^{-5} M; —, compound not tested; O, no inhibition at 10^{-4} M.

2. C'1 esterase was prepared from human serum according to the procedure of Yuce and Stefanini.[10] Its activity was determined by measuring the rate of hydrolysis of acetyltyrosine ethyl ester.

Results and Discussion

In this series of dibenzodiazepines the compounds (I–V) which showed protection against fatal systemic anaphylaxis in the 3 species used, namely mice, rats, and guinea pigs, all had an aliphatic tertiary amine in position 5. Those protecting mice and rats (VI–XI) also were substituted in position 5 with one exception—VII. Demethylation of the amine on position 5 may reduce activity (compare I with XXXI and IV with IX).

Compounds active in mice and rats are not necessarily active in guinea pigs (VI–IX), and compounds active in mice are not necessarily active in rats (XII–XXI).

It should be made clear that in the experiments reported, guinea pigs were challenged by ip administration of antigen and, under these conditions, mediators and target organs are different from those when antigen is administered iv or by aerosol.[11] Under the latter conditions, where antihistamines have some effect, Tarpan (VII) protected guinea pigs from anaphylactic shock[12] whereas it was inactive in the guinea pigs challenged by ip administration of antigen.

The mechanism of action of these compounds is not known. One of the most active ones, namely I, has good antihistamine activity *in vivo* but little antiserotonin activity. It is thus difficult to explain its marked activity in mice where histamine is not the only mediator and serotonin as well as bradykinin and SRS-A may be implicated,[13] as well as in rats where histamine, serotonin, and possibly SRS-A and bradykinin may be involved.

There are other possible sites of action which have not been investigated for all compounds. One is on the release of histamine and/or other mediators. Compound I inhibited release of histamine from sensitized guinea pig lung by antigen over a very narrow range. Paradoxically it stimulated release over a much wider range. This latter property is characteristic of certain antihistamines.[14]

Since there was some indication of inhibition of histamine release from guinea pig lung by I, it was decided to determine whether this series of diazepines showed any inhibitory action on the type of enzyme believed to be involved in release of histamine, namely chymotrypsin.

The idea of an enzyme theory of anaphylaxis was developed by Mongar and Schild.[15] They showed that the antigen–antibody reaction caused a transient activation of an intracellular heat-labile, calcium-requiring enzyme system which was responsible for the release of histamine and perhaps other pharmacologically active substances. Austen and Brochlehurst[2a] elaborated this

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(14) (a) J. Pellerat and M. Murat, *C. R. Soc. Biol.*, **140**, 297 (1946). (b) I. Mota and W. D. DaSilva, *Brit. J. Pharmacol. Chemother.*, **15**, 396 (1960).

(15) J. L. Mongar and H. O. Schild, *Physiol. Rev.*, **42**, 226 (1962).

idea and showed that the release of histamine from guinea pig lung was prevented by chymotrypsin substrates and inhibitors but not by trypsin, carboxypeptidase, or leucine amino peptidase substrates or by soy bean trypsin inhibitor. They concluded that the activation of a chymotrypsin-like enzyme was a necessary condition for anaphylactic release of histamine. Becker and Austen[2c,16] found that a similar enzyme was required for release of histamine from rat mast cells.

The dibenzodiazepine compounds were found to show varying degrees of inhibition of chymotrypsin activity, and there was a definite trend in the correlation between chymotrypsin inhibition and antianaphylactic activity. Of the compounds protecting at lease 2 species against anaphylaxis in doses of 30 mg/kg or less (I–X), all those tested inhibited chymotrypsin activity, *i.e.*, 9/9 (X was not available). Of the remaining compounds which were tested 7/16 inhibited. It is unfortunate that so many of the compounds in the second group could not be tested either because they were not available or they formed precipitates in the buffer.

The dibenzodiazepines caused little change in the behavior of mice[17] in the doses tested for antianaphylactic activity. A few showed some CNS effects at higher doses, *e.g.*, I at 30, II at 30, and VII at 10 mg/kg caused CNS stimulation; IV at 100, VIII at 30, XVI at 100, and XXIV at 100 mg/kg caused clonic convulsions; V at 100, XIX at 10, XXI at 30, and XXII at 100 mg/kg caused depression.

The most active compounds (I and II) were superior to tripelennamine in protecting mice against fatal anaphylaxis and were comparable to cyproheptadine in this species. Compound I was comparable to cyproheptadine in protecting rats against anaphylaxis and was superior to this compound in guinea pigs. In guinea pigs exposed to a histamine aerosol, I was active in doses as low as 1 mg/kg ip; tripelennamine was active in slightly lower doses.

(16) E. L. Becker and K. F. Austen, *J. Exp. Med.*, **124**, 379 (1966).

(17) W. Veldkamp, personal communication (1967).

Analogs of Amphetamine. 4. Synthesis of Metabolites of 1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane (DOM)[1,2]

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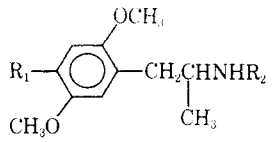
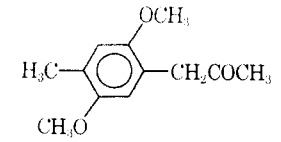
Three amphetamine derivatives and one substituted 1-phenyl-2-propanone, which are possible metabolites of 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM or STP, **1**), were synthesized. A formyl group was introduced to the ring C_4 of 1-(2,5-dimethoxyphenyl)-2-aminopropane (**6**) while the amino function was protected by a phthaloyl group. The resulting compound **8** was oxidized by Ag_2O–NaOH to the acid **3**. Reduction of **8** by $Al(O\text{-}i\text{-}Pr)_3$ followed by removal of the phthaloyl group gave the hydroxymethyl compound **2**. 1-(2,5-Dimethoxy-4-methylphenyl)-2-propanone (**5**) was prepared by the Fe–HCl reduction of 1-(2,5-dimethoxy-4-methylphenyl)-2-nitropropene, and the *N*-acetyl derivative **4** was obtained by acetylation of **1**.

During the course of investigation on structures and actions of some hallucinogens the metabolic study of 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM or STP, **1**) was undertaken. This compound has multifunctional groups and is structurally related to amphetamine and to a lesser extent to mescaline, whose metabolic fates in a number of species have been well documented. In particular, the 4-CH_3 group of **1** is vulnerable to biotransformation. Gillette[3] reported the oxidation of the CH_3 group of *p*-nitrotoluene by liver microsomal system to a CH_2OH group which was oxidized further by dehydrogenases in the soluble fraction to a COOH group. In a similar fashion 4-hydroxymethylacetanilide was formed from 4-methylacetanilide.[4] By analogy, the conversion of the 4-CH_3 group of **1** in animals or human to CH_2OH was expected to give 1-(2,5-dimethoxy-4-hydroxymethylphenyl)-2-aminopropane (**2**). Further oxidation of **2** to 2,5-dimethoxy-4-(2-aminopropyl)benzoic acid (**3**) occurred as predicted. Other metabolites of **1** might be the *N*-acetyl DOM (**4**) and/or 1-(2,5-dimethoxy-4-methylphenyl)-2-propanone (**5**) resulting from the oxidative deamination of **1**.

Chemistry.—Compounds **2** and **3** were prepared from 1-(2,5-dimethoxyphenyl)-2-aminopropane (**6**).[2a] A previous attempt to prepare **3** from the $KMnO_4$ oxidation of *N*-acetyl DOM (**4**) was unsuccessful; the isolated acidic product showed no NH absorption in its ir spectrum and was, therefore, not characterized further. The amine function of **6** was protected by a phthaloyl





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1, $R_1 = CH_3$; $R_2 = H$
2, $R_1 = CH_2OH$; $R_2 = H$
3, $R_1 = COOH$; $R_2 = H$
4, $R_1 = CH_3$; $R_2 = Ac$
6, $R_1 = H$; $R_2 = H$
7, $R_1 = H$; R_2H = phthaloyl
8, $R_1 = CHO$; R_2H = phthaloyl
9, $R_1 = CH_2OH$; R_2H = phthaloyl

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