Anaphylactic Microshock in the Screening of Some Theophyllines and Benzylphenoxyaminoalkanes for Antiasthmatic Activity

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The prevention of Herxheimer's anaphylactic microshock has been used as a screen for antiasthmatic activity in a series of 7-substituted theophylline derivatives. A number of soluble, neutral derivatives were found which were equal in activity to aminophylline. One of these, 7-(3-hydroxypropyl)-theophylline, showed true synergism with two isomeric benzylphenoxyaminoalkanes which were in themselves antiasthmatic by the same test.

Over the last ten years a large number of theophylline derivatives and aralkylamines of diverse structure have been prepared in our laboratories. They have been subjected to pharmacological characterization using generally accepted techniques involving both isolated organ and whole animal preparations. In order to characterize the compounds with respect to their antiasthmatic potency, it was considered desirable to supplement the values obtained from the use of the isolated guinea pig ileum stimulated with histamine, and from the histamine-aërosol provoked syndrome of bronchial constriction in the guinea pig, with results obtained from an experimental technique more closely resembling the pathophysiological conditions in clinical asthma.

The early observation of Busson and Ogata[1] that a condition resembling an asthmatic attack in man is caused in the sensitized guinea pig by prolonged inhalation of antigen, has been extended by Alexander, Becke and Holmes.[2] Manteufel and Preuner,[3] and most recently by Herxheimer,[4,5] who developed a technique for production of repeated microshocks of predetermined severity useful for assessing prophylactic antiasthmatic effects. Friebel and Basold,[6] on the other hand, developed a sustained microshock method for the measurement of curative antiasthmatic effect. The similarity of these types of microshock to the human asthmatic attack has been pointed out by Kallos and Pagel.[7]

The present communication reports on the antiasthmatic activities of nineteen theophyllines substituted at position 7 with hydroxyalkyl-, haloalkyl-, or aminoalkyl groups. The results are expressed in terms of ability to *prevent* or alleviate Herxheimer's microshock. It was found that by combining one of the active members, 7-(3-hydroxypropyl)-theophylline, with antiasthmatic compounds representing a different chemical class a prophylactic effect can be obtained, which is much greater than that obtained with either of the two components separately. The two active agents alone and in combination were further tested for ability to prevent or relieve an already existing asthmatic attack according to the method of Friebel using aminophylline as a standard.[8]

Chemistry.—The structures, melting points and analytical data of the theophylline derivatives not reported in the literature are shown in Table I. Directions for the preparation of these compounds are given in the Experimental section.

These theophylline derivatives were prepared essentially according to the literature: 7-(3-hydroxypropyl)-theophylline (IV),[9] 7-(4-hydroxybutyl)-theophylline (V),[10] 7-(2-diethylaminoethyl)-theophylline (VII),[11] 7-(2-dimethylaminopropyl)-theophylline hydrochloride (IX),[12] 7-(2-dimethylaminoethyl)-theophylline hydrochloride (XV),[13] 7-(2-aminoethyl)-theophylline hydrochloride (XVIII),[14] 7-(2-hydroxypropyl)-theophylline (XX),[9] 7-(2,3-dihydroxypropyl)-theophylline (XXI)[15] and 7-theophyllineacetic acid monohydrate (XXII).[16] Two antihistaminic drugs, N,N-dimethyl-2-(α-phenyl-*o*-toloxy)-propylamine hydrochloride (I) and 2-(*o*-benzylphenoxy)-N,N-dimethylisopropylamine hydrochloride (II) also were prepared.[17] III is a mixture of about equal parts of I and II as obtained by following example 24 of the reference.[17]

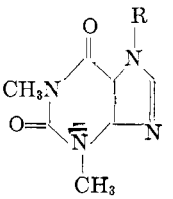
Experimental[18]

β-Dimethylaminoethyl 7-theophyllinylethyl Ether Hydrochloride (VI). Method A.—7-(2-Hydroxyethyl)-theophylline[19] (60 g.) was added to a solution of sodium ethoxide (from 6.1 g. of sodium and 400 ml. of ethanol). The solution was evaporated to dryness, and a solution of dimethylaminoethyl chloride (34.5 g.) in toluene (290 ml.) was added. The mixture was heated under reflux for 6 hr. with stirring and the sodium chloride was removed by filtration. The toluene was distilled off and the residue dissolved in 3 *N* hydrochloric acid. After evaporation the residue was crystallized twice from ethanol.

7-(3-Dimethylaminopropyl)-theophylline Hydrochloride (XII). Method B.—A mixture of sodium theophyllinate (20 g.) and γ-dimethylaminopropyl chloride (14.5 g.) in toluene (100 ml.) was heated under reflux for 6 hr. The mixture was worked up as de-

(1) B. Busson and N. Ogata, *Wien. klin. Wochschr.*, 820 (1924).
(2) H. L. Alexander, W. G. Becke and J. A. Holmes, *J. Immunol.*, **11**, 175 (1926).
(3) P. Manteufel and R. Preuner, *Z. Immunitätsforschg.*, **80**, 65 (1933).
(4) H. Herxheimer, *J. Physiol.*, **117**, 251 (1952).
(5) H. Herxheimer, *Arch. intern. pharmacodynamie*, **106**, 371 (1956).
(6) H. Friebel and A. Basold, *Arch. exptl. Pathol. Pharmakol.*, **217**, 13 (1953).
(7) P. Kallos and W. Pagel, *Acta Med. Scand.*, **91**, 292 (1937).
(8) These experiments (reported in Table VI) were kindly carried out by Professor H. Friebel, Pharmacological Institute, Bonn University, West Germany. The authors gratefully acknowledge the permission to quote the results.
(9) Gane's Chemical Works Inc., British Patent 736,594 (1956).
(10) J. R. Parikh and A. Burger, *J. Am. Chem. Soc.*, **77**, 2386 (1955).
(11) A. Quevauviller, P. Chabrier and H. Morin, *Bull. soc. chim. biol.*, **31**, 532 (1949).
(12) R. Zelnik, M. Pesson and A. Polonovski, *Bull. soc. chim. France*, 1773 (1956).
(13) R. C. Batterman, A. J. Grossman, P. Leifer and G. J. Mouratoff, *Am. J. Med. Sci.*, **236**, 162 (1958).
(14) E. Stieglitz and H. Stamm (to Arzneimittelfabrik Krewel-Leiffen G.m.b.H.), German Patent 1,122,534 (1962).
(15) J. W. Jones and P. V. Maney (to the State University of Iowa), U. S. Patent 2,575,344 (1951).
(16) J. Baisse, *Bull. soc. chim. France*, 769 (1949).
(17) S. B. Binkley and L. C. Cheney (to Bristol Laboratories, Inc.) U. S. Patent 2,703,324 (1955).
(18) The analyses were carried out by Mrs. G. Speggers, Pharmacia A.B.
(19) German Patent 193,799 (to Bayer & Co.) (1908).

TABLE I
THEOPHYLLINE DERIVATIVES



Compound	R	Method[a]	M.p., °C.	Recryst. solvent	Yield, %	Formula	Analysis Calcd.	Analysis Found
VI	$(CH_2)_2O(CH_2)_2N(CH_3)_2 \cdot HCl$	A	223–225	EtOH	29	$C_{13}H_{22}ClN_5O_3$	Cl, 11.8	11.1
VIII	$CH_3CH_2N^+(C_2H_5)_3 \cdot Br^-$	D	195–198	EtOH–ether	56	$C_{15}H_{26}BrN_5O_2$	Br, 20.6	20.4
X	$CH_2CH(CH_3)N^+(CH_3)_3 \cdot I^-$	D	223–226	MeOH	43	$C_{13}H_{22}IN_5O_2$	I, 31.2	30.5
XI	$CH_2CH(CH_3)N^+(CH_3)_2(C_2H_5) \cdot Br^-$	D	192–195	EtOH	31	$C_{14}H_{24}BrN_5O_2$	Br, 21.4	21.1
XII	$(CH_2)_3N(CH_3)_2 \cdot HCl$	B	271–272	MeOH–ether	46	$C_{12}H_{20}ClN_5O_2$	Cl, 11.8	11.9
XIII	$(CH_2)_3N^+(CH_3)_3 \cdot I^-$	D	238–239	MeOH	60	$C_{13}H_{22}IN_5O_2$	I, 31.2	30.7
XIV	$(CH_2)_3N^+(CH_3)_2(C_2H_5) \cdot Br^-$	D	195–197	EtOH–ether	72	$C_{14}H_{24}BrN_5O_2$	Br, 21.4	21.3
XVI	$CH_2CH_2N^+(CH_3)_3 \cdot I^-$	D	264–265	EtOH–H_2O	88	$C_{12}H_{20}IN_5O_2$	N, 32.3	32.3
XVII	$CH_2CH_2N^+(CH_3)_2(C_2H_5) \cdot Br^-$	D	220–221	MeOH–ether	59	$C_{13}H_{22}BrN_5O_2$	Br, 22.2	21.9
XIX	$CH_2CHClCH_2Cl \cdot 0.25C_2H_5OH$	C	112–114	EtOH	37	$C_{10}H_{12}Cl_2N_4O_2 \cdot 0.25C_2H_5OH$	N, 23.4	23.5

[a] See Experimental section. [b] All melting points are uncorrected.

TABLE II
PROTECTIVE ANTI-ASTHMATIC EFFECTS OF A SERIES OF THEOPHYLLINE DERIVATIVES

Test compound	Dose, mg./kg.	Animals	Effect, min.	Effect, % of standard
IV	20	6	3.2	89
V	30	10	3.7	97
VI	20	7	0	0
VII	20	8	0.8	21
VIII	20	7	1.1	44
IX	20	6	1.7	106
X	30	6	0.1	2
XI	30	7	0	0
XII	30	7	1.5	65
XIII	30	7	1.7	33
XIV	30	6	1.4	32
XV	40	11	1.6	30
XVI	40	13	0.3	11
XVII	40	10	1.3	33
XVIII[a]	30	22	1.3	25
XIX[a]	30	23	1.7	44
XX	40	11	1.0	15
XXI	40	8	1.0	15
XXII	40	8	1.4	21

[a] Combined results from two tests.

TABLE III
PROTECTIVE EFFECT OF SUBSTITUTED THEOPHYLLINES

Test compound	Expts.	Dose in mg./kg.	Animals	Effect in min.	Effect, % of standard
IV	3	40	31	2.9	78
Aminophylline	3	40	30	3.7	100
V	3	30	30	3.3	82
Aminophylline	3	30	30	4.0	100
IX	2	30	28	3.3	103
Aminophylline	2	30	23	3.2	100
XII	3	30	27	2.4	44
Aminophylline	3	30	22	5.5	100

scribed above for the preparation of VI. The crude product was crystallized twice from methanol-ether.

7-(2,3-Dichloropropyl)-theophylline (XIX). Method C.—A mixture of 7-(2,3-dihydroxypropyl)-theophylline (XXI)[15] (200 g.) and thionyl chloride (224 ml.) was heated under reflux for 4 hr. and then left standing for 16 hr. at room temperature. Ethanol (1 l.) was added slowly and the mixture refluxed for 4 hr. The ethanol solution was concentrated under vacuum and the precipitate (141 g.) was removed by filtration and crystallized twice from ethanol.

Method D.—The quaternary compounds 7-(2-diethylaminoethyl)-theophylline ethobromide (VIII), 7-(2-dimethylaminopropyl)-theophylline methiodide (X), 7-(2-dimethylaminopropyl)-theophylline ethobromide (XI), 7-(3-dimethylaminopropyl)-theophylline methiodide (XIII), 7-(3-dimethylaminopropyl)-theophylline ethobromide (XIV), 7-(2-dimethylaminoethyl)-theophylline methiodide (XVI) and 7-(2-dimethylaminoethyl)-theophylline ethobromide (XVII) were prepared by conventional procedures involving reaction in ethanol of the appropriate terti-

TABLE IV
PROTECTIVE EFFECT OF THE ANTIHISTAMINIC COMPOUNDS I, II AND III[a]

Expt.	Test compound	Dose, mg./kg.	Animals	Effect in min.	Effect in % of standard
1	III	10	10	3.1	37
	Aminophylline	30	8	8.4	100
2	III	20	7	10.1	125
	Aminophylline	30	6	8.1	100
3	I	15	10	3.1	100
	II	15	10	6.4	206
4	I	15	10	2.6	100
	II	15	10	6.7	258
5	I	15	10	3.3	100
	II	15	10	5.2	158

[a] In experiments 3–5 the relative activities of I and II were compared, the activity of compound I being set at 100.

TABLE V
SYNERGISTIC PROTECTIVE EFFECT OF COMPOUNDS II AND IV[a]

Expt.	Test compound	Dose in mg./kg.	Animals	Effect in min.	Effect in % of the standard
1	IV	15	20	0.7	23
2	II	2.5	20	0.3	14
3	IV	15	20	4.9	140
	+II	2.5			

[a] In each experiment, a group of 20 guinea pigs was injected with 30 mg./kg. of the standard aminophylline, and served as positive control group.

TABLE VI
SYNERGISTIC CURATIVE EFFECT OF COMPOUNDS II AND IV[a]

Test compound	Animals	Animals without symptoms: Number	Animals without symptoms: %	Animals greatly relieved: Number	Animals greatly relieved: %
Saline (controls)	17	0	0	3	18
Aminophylline	22	17	77	5	23
IV	22	2	9	13	59
II	20	6	30	12	60
IV + II	20	18	90	2	10

[a] s.c. dosage: aminophylline = 48 mg./kg., IV = 48 mg./kg., II = 5 mg./kg.

ary amines with ethyl bromide or methyl iodide, respectivly, at 100° in an autoclave (VIII, XIV and XVII), at reflux temperature (X, XI, and XIII) or at room temperature (XVI) for several hr.

Pharmacology. Method and Materials.—Herxheimer's[4] technique was used with these modifications: Dilutions of native eggwhite, 1:40–1:200, were used as aerosol fluid instead of solutions of crystalline egg albunin. The volume of the exposure chamber was 15 instead of 55 l. The calculation of results was done as follows: the protective effect was measured as prolongation of preconvulsion time and expressed as effect in min., *i.e.*, as the difference between preconvulsion times of the treated and the control animals. The effect in min. of the test compound then was compared to that of the standard and expressed as per cent. of the standard. Comparisons always were made relative to the standard on each day of testing.

Test Compounds.—Aminophylline Leo, ampoules, containing 24 mg./ml. (corresponding to 18 mg./ml. of theophylline) was used as standard drug. Nineteen theophylline derivatives were tested. Further, the three antihistaminics I, II and III were investigated alone and one of these (II) in combination with theophylline derivative IV. Test compounds were dissolved in 0.9% saline. All injections were made i.p. 20 min. prior to the exposures.

Results and Discussion

In a first run, a single test was applied to a series of theophylline derivatives. The results obtained are presented in Table II. Those substances which showed promising protective effect were submitted to further tests (Table III). Two to three tests each were performed with compounds IV, V, IX, and XII. Excepting XII these substances had a protective effect roughly comparable to that of aminophylline. Substance IV was chosen for preliminary clinical trials because of a favorable ratio of effect to toxicity and proved promising in these experiments. It was studied for a possible synergistic effect with compounds representing structures related to antihistaminic drugs.

The antihistaminic III, representing a mixture of the two isomers I and II, had roughly twice the activity of aminophylline (Table IV). When the protective activities of isomers I and II were compared with each other in three experiments, II had about twice the activity of I and was therefore chosen for further trials.

In a final series, near-threshold doses of II and IV were tested alone and in combination for the detection of possible synergistic action. The results in Table V demonstrate that a synergism is obtained, when both substances are given simultaneously at near-threshold doses. The protective effect increased from 14 and 23%, respectively, for the single substances to 140% for the combination. The synergistic effect of compounds II and IV also has been verified with the sustained microshock method of Friebel and Basold[6] (Table VI). With this method, where curative effect is measured, the percentage of animals cured, *i.e.*, without symptoms, was 9 with 48 mg./kg. of IV, and 30 with 5 mg./kg. of II. When both compounds were given simultaneously at the same dosage, 90% of the animals were cured.

The Herxheimer method is designed to measure a protective or preventive antiasthmatic effect. Herxheimer has pointed out that the precision of his method is limited by the fact that some animals may react differently on successive occasions and that the end point is difficult to assess at long preconvulsion times. The suitability of the method however for screening purposes is demonstrated by the fairly constant protective effect obtained with the same compounds in successive trials, as can be seen by comparing results in Tables II and III. The unusually high protective effect of compound IV at a dose of 20 mg./kg. (Table II) as compared to later tests with higher doses may be attributed to the relatively small number of animals used in the first test.

No systematic influence of the chemical structure or the size of the substituent in position 7 on antiasthmatic activity could be found among 19 theophylline derivatives. Development of maximum activity is thus independent of the length of the substituent chain as well as of the appearance of a tertiary amine or a quaternary ammonium function.

Water solubility did not influence potency. Among two soluble compounds, 7-(2-hydroxypropyl)-theophylline (XX) attained satisfactory blood concentrations but was quite ineffective both in experimental asthma and in clinical trials, whereas 7-(3-hydroxypropyl)-theophylline (IV), which is less soluble than XX, was effective both in experimental asthma and in clinical trials.[20]

(20) H. Colldahl, St. Görans Sjukhus, Stockholm, Sweden, personal communication.