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THE PROTECTIVE EFFECT OF SYMPATHOMIMETIC AMINES AND OF AMINOPHYLLINE IN THE ANAPHYLACTIC MICROSHOCK OF THE GUINEA-PIG

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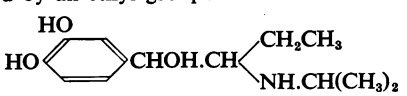
Recently we have attempted to assay the protective effect of antihistamines in the anaphylactic shock of the guinea-pig (Armitage, Herxheimer, and Rosa, 1952). In this paper we shall deal with the effect of a number of sympathomimetic amines and of aminophylline.

METHOD

The method has been described earlier (Herxheimer, 1952), and the procedure in the present experiments is similar to that described in our study of the antihistamine effect.

The substances used were adrenaline, noradrenaline, isoprenaline (N-*iso*propylnoradrenaline), ephedrine, methylephedrine, cobefrin (corbasil), neosuprel, and aminophylline. Neosuprel (WIN 3046) is a derivative of isoprenaline in which an α-H-atom has been replaced by an ethyl group:



Methylephedrine is the N-methyl derivative of ephedrine, so that it contains a dimethylamino group.

All substances except the aminophylline were given intramuscularly; aminophylline was given intraperitoneally, as its intramuscular injection tends to cause necrosis. All except ephedrine and methylephedrine were given 15 min. before exposure to shock; ephedrine and methylephedrine were given one hour beforehand. The interval of 15 min. was chosen because the maximum effect of a subcutaneous or intramuscular injection of these substances in man can be expected at about that time. With ephedrine a longer interval was preferred, as oral ephedrine usually takes almost an hour until it acts and as its action may be delayed after injection (Starr *et al.*, 1937). All dosages of 10 mg./kg. or more of ephedrine and methylephedrine caused ruffling of the hair and, in the higher doses, tremor. These symptoms started in some animals 15 min. after injection and lasted for several hours. Isoprenaline 0.2 mg./kg. caused death in two of the eight animals used; with 0.1 mg./kg. one death occurred in 16 animals. Adrenaline 0.2 mg./kg. caused tremor. No other toxic effects were observed.

The statistical methods applied in the present study differ in two respects from those described by Armitage *et al.* (1952). First, the method previously used for measuring the protective effect of each substance occasionally resulted in very large negative responses, when an individual animal happened to show a shorter pre-convulsion time under treatment than in its control observations. To avoid this difficulty we now define the response as follows:

$$y = 100(1 - C/T), \text{ if T is greater than C;}$$
$$y = -100(1 - T/C), \text{ if T is less than C;}$$

where C is the control pre-convulsion time (mean of 2 controls), T the drug pre-convulsion time in seconds. This method differs from that used previously only for negative responses, and the effect on the dose-response relationships will be negligible except possibly at the doses giving very low protection.

Secondly, in order to make the procedure of fitting logistic curves to the dose-response relationships as objective as possible, we have estimated the upper limits of the curves from the data by statistical methods rather than by a preliminary guess.

Some of the substances were tested at too small a number of doses to permit curves to be fitted with any useful degree of accuracy. Logistic curves were fitted for ephedrine, methylephedrine, isoprenaline, and neosuprel. The mean response at 15 mg./kg. ephedrine, and that in one of the experiments with 0.1 mg./kg. isoprenaline, differed widely from the responses at adjacent doses, and have been omitted in the construction of the curves because of the possibility of a technical error. As the responses of ephedrine lay close to those of methylephedrine, the curves of these 2 substances were so fitted that they differed by a constant log-dose interval. This corresponds on the graph to a constant horizontal distance. The results for aminophylline, the highest dose of which (100 mg./kg.) produced complete protection in all four animals, cannot be fitted satisfactorily by a logistic curve. The shape of the dose-response relationship

at high doses of aminophylline is uncertain, and a freehand curve (shown by a dotted line in Fig. 1) has been drawn.

Results

The detailed results are shown in Table I and Fig. 1. It can be seen that all the substances investigated had a protective effect, with the exception of cobefrin. The dose-response curve appears, for those compounds of which a sufficient number of doses have been tried, to have a sigmoid shape. Only aminophylline achieved full protection. Isoprenaline and neosuprel were, weight for weight, the strongest of the amines, followed by adrenaline and noradrenaline. Ephedrine reached approximately the same maximum protective effect as noradrenaline, although with a much higher dosage. Methylephedrine was more effective in very high doses which were not tried with ephedrine. Because of the toxic side-effects of the latter, we have refrained from giving doses higher than 60 mg./kg. It does not seem advisable to use dosages which cause obvious toxic side-effects. We have had some fatalities with 0.1 and 0.2 mg./kg. of isoprenaline and a considerable amount of tremor with 60 mg./kg. of ephedrine. The possibility cannot be excluded that such toxic side-effects influence the usual action of a drug in a decisive way.

Table I

PERCENTAGE PROTECTION UNDER TREATMENT, MEASURED BY INCREASE OF PRECONVULSION TIME (See Method)

Substance	Dose (mg./kg.)	Log Dose	No. of Animals	Mean Protection ± Standard Error	Estimated Protection from Fitted Curve
Adrenaline	0·005	−2·30	7	5·3±14·7	
	0·02	−1·70	6	28·7±12·2	
	0·2	−0·70	7	57·1± 5·8	
Aminophylline	5·0	0·70	6	8·3± 2·7	
	10·0	1·00	6	11·8±10·4	
	20·0	1·30	7	39·4± 9·6	
	40·0	1·60	5	56·0±13·3	
	100·0	2·00	4	100·0	
Cobefrin ..	0·005	−2·30	5	36·2±18·7	
			4	2·0±12·1	
			4	4·8±28·1	
	0·02	−1·70	5	9·2±12·7	
	0·2	−0·70	5	−1·6± 5·1	
Ephedrine	4·0	0·60	7	2·0± 7·8	8
	10·0	1·00	6	20·8±10·2	20
	15·0	1·18	7	*−24·0± 7·7	27
	20·0	1·30	7	42·0±11·0	35
	30·0	1·48	4	48·8± 4·5	44
			4	18·8±11·4	
			6	47·2± 8·5	
	40·0	1·60	6	54·8± 7·1	49
	60·0	1·78	7	31·3±16·3	56
			5	52·2± 3·8	
Isoprenaline	0·001	−3·00	7	14·4±13·0	8
	0·005	−2·30	4	52·0± 7·9	53
			3	39·0±21·6	
	0·02	−1·70	3	46·0±15·2	69
			7	78·3± 5·4	
	0·1	−1·00	5	*38·6± 3·5	70
			3	85·3±14·7	
			7	68·4± 5·0	
	0·2	−0·70	3	61·0±11·8	70
			3	76·3±16·3	
Methylephedrine	10·0	1·00	6	−5·2±10·5	7
	30·0	1·48	6	43·2± 3·6	40
	60·0	1·78	5	47·0± 6·1	53
	100·0	2·00	4	48·5±14·5	60
	160·0	2·20	6	62·2± 2·9	64
	200·0	2·30	6	72·3± 3·8	66
Neosuprel	0·005	−2·30	7	16·4± 8·9	0
			7	18·1±10·6	
	0·02	−1·70	6	4·7±14·9	20
			5	28·6±11·5	
	0·06	−1·22	4	73·8± 7·7	77
	0·1	−1·00	7	80·1± 1·9	78
	0·2	−0·70	7	77·0± 2·4	79
Noradrenaline	0·02	−1·70	4	20·2±12·1	
			5	9·2± 9·2	
	0·2	−0·70	6	18·8±10·0	
	0·4	−0·40	6	31·3±12·1	
			5	36·6± 8·8	
	1·0	0	6	55·7± 3·5	

* These experiments have been omitted in the construction of the graph.

Discussion

If the actions of the various substances are compared, the protective effect of aminophylline appears clearly as the strongest. Full protection is not achieved by any of the sympathomimetic amines, nor by any antihistamine (Armitage *et al.*, 1952). The amount of aminophylline necessary for full protection (100 mg./kg.) is greater than that used with the other substances except methylephedrine, and, theoretically, it seems possible that a similar amount of an adrenaline-like substance would also have given full protection had its action not been too toxic to be tolerated. It is interesting to note that in human asthma aminophylline, given intravenously, is probably the most reliable drug for cutting off acute attacks or relieving the asthmatic state. It is more reliable than the sympathomimetics, as tolerance to it is not so easily acquired and its action is less fleeting. The amount used for intravenous injection in man is 4–8 mg./kg.

The differences in the protective action of the adrenaline-like substances are of a similar order to that found in human asthma. In our experiments isoprenaline and neosuprel are strongest, followed by adrenaline and noradrenaline. In man isoprenaline has been found stronger than adrenaline by Gay and Long (1949) and Bresnick, Beakey, Levinson, and Segal (1949). This is in accordance with our own (unpublished) clinical observations. Neosuprel has been found by Hershfus *et al.* (1951) to be somewhat weaker than isoprenaline. Data about the action of noradrenaline in man are lacking, but our own spirometric observations on the

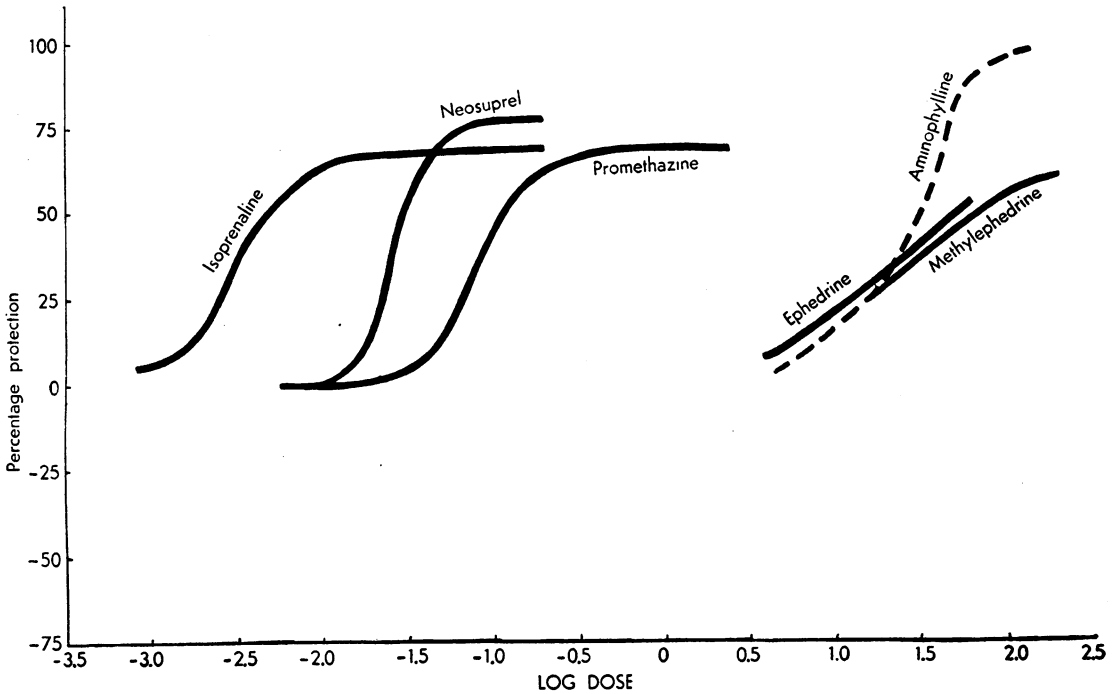


FIG. 1.—Log dose-response curves for isoprenaline, neosuprel, promethazine, ephedrine, methylephedrine, and aminophylline. The log dose has been plotted against the percentage protection given by the dose concerned. Promethazine, one of the stronger antihistamines, has been included for comparison.

increase in vital capacity in the induced asthmatic attack indicate that noradrenaline is considerably weaker than adrenaline (Herxheimer, unpublished). It is remarkable that the order of efficiency is similar in the guinea-pig and in man, although the relative dosage used in the former is very much greater and would be toxic in man.

The protective effect of maximum doses of isoprenaline and neosuprel appears similar to that of the stronger group of antihistamines, whilst maximum doses of adrenaline and noradrenaline appear to reach only the effect of the weaker group of antihistamines. The lowest doses of adrenaline and noradrenaline giving a protective effect do not differ much from the corresponding doses of the weaker antihistamines. Isoprenaline and neosuprel show a protective effect with 5 μg./kg. which is equalled by mepyramine, tripelennamine, and BW 295/C/51. The comparison between sympathomimetic amines and antihistamines thus does not show the fundamental differences which are seen in human asthma. One of the causes for this may be the considerable difference in dosage in man and guinea-pig. The latter requires 30–100 times more of the sympathomimetic amines than man, whilst with some of the antihistamines the dosage is approximately the same, and with others the differences are small.

Ephedrine and methylephedrine, which do not differ in this respect from the other amines, cause toxic side-effects with a comparatively moderate dosage. As can be seen from the figure, ephedrine appears to have, weight for weight, the same protective action as methylephedrine, but the animals tolerate a greater amount of the latter. In human asthma we have found that a double to fourfold dose of methylephedrine is required if ephedrine is to be replaced. In this connection it should be kept in mind that the close similarity of the fitted curves for ephedrine and methylephedrine does not necessarily represent the true relation of the dose-responses of these drugs. As can be seen from Table I, there is a considerable scatter of points, and it appears possible to fit curves representing a greater difference and showing ephedrine, weight for weight, 2–3 times stronger than methylephedrine. Many more experiments would be required to state this relation in more accurate terms.

Cobefrin has not shown any protective action. As far as we know, it has not been tried in human asthma.

SUMMARY

1. The protective effect of aminophylline, adrenaline, noradrenaline, isoprenaline, neosuprel, cobefrin, ephedrine, and methylephedrine in mild anaphylactic shock of the guinea-pig has been investigated.

2. Aminophylline is the only substance with which full protection can be achieved. The other substances achieve, in non-toxic doses, partial protection only. Cobefrin does not protect.

3. The order of efficiency of these substances is approximately the same as that observed in human asthma.

4. The dose-response curve in some of these substances has, as in most antihistamines, a sigmoid shape.

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