Brit. J. Pharmacol. (1953), **8**, 461.

THE PROTECTIVE ACTION OF ANTIHISTAMINIC AND SYMPATHOMIMETIC AEROSOLS IN ANAPHYLACTIC MICROSHOCK OF THE GUINEA-PIG

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(RECEIVED JULY 13, 1953)

In two earlier papers we have described the protective effect of varying amounts of a number of antihistamines (Armitage, Herxheimer, and Rosa, 1952) and sympathomimetic amines (Herxheimer and Rosa, 1953) given by injection. This paper deals with experiments in which some of these substances were given by aerosol. In some other experiments the duration of the protective effect by injection was investigated in order to compare it with that following aerosol inhalation.

METHOD

Our method as previously described (Herxheimer, 1952a ; Armitage *et al.*, 1952) was modified. Instead of the protecting substance being injected, usually given one hour before exposure to the shocking agent, the animals inhaled it in an aerosol. The dosage of protecting substance was varied by altering either its concentration or the period of inhalation. The test substances were dissolved in a mixture of equal parts of water and propylene glycol. The aerosolization of the protective substances was produced by compressed air from a cylinder, the air pressure being controlled by a flowmeter and kept constant. In a few experiments a commercial nebulizer was used, but in most a " midget scrubber " as described by Dautrebande (1951). These two instruments differ in that the droplets produced by the commercial nebulizer are larger, probably of a diameter between 1 and 3 μ, while those produced by the midget scrubber are, according to Dautrebande, of more uniform size, with a diameter well below 1 μ. The smaller droplets penetrate further into the lungs, but adhere less easily to the walls of the air passages. In addition their total weight, and therefore their action, is smaller than that of the larger particles. Though an exact comparison of both methods of nebulization is difficult, we have tried to compare them clinically by measuring the effect of isoprenaline aerosols in mild induced attacks of asthma. Such a comparison showed that the aerosol with uniform droplet size below 1 μ diameter is the less effective.

The percentage retention of a drug inhaled as an aerosol will depend partly on the amount of the drug inhaled. How much is inhaled will depend on the aerosol density in the aerosol chamber, the respiratory volume, and the dead space. Other factors include the aerosol density in the air passages, the size of the particles, their electrical charge, and the nature of the inhaled substance.

Accurate data about the retention of aerosol in the lungs are lacking. Abramson, Reiter, Gettner, and Sklarofsky (1949) have found in experiments with a phenolsulphonphthalein aerosol in man that not more than one-tenth of the inhaled amount was retained. According to Dautrebande (1951) the output of the midget scrubber nebulizer at 8 lb./sq. in.—the pressure we have applied—is 3 ml./hr. This equals 30 mg./hr. or 0.5 mg./min. of the substance used, if it is in 1% solution. As the output of air by this instrument is 5 l./min. at the same pressure, the concentration of aerosol in the chamber will be approximately 1 mg./10 l.

According to Ainsworth (1953), to whom I am obliged for these data, the tidal air of a guinea-pig of 300–500 g. weight varies from 2–3 c.c., and the respiratory volume from 240–400 c.c./min. If these data are used as the basis of a very approximate calculation, the animal would inhale about 333 c.c./min. of the aerosol containing 1 mg. of the drug in 10 l. of air, or about 0.033 mg./min. It would retain one-tenth of this, namely about 3 μg. in one min., or 0.5 μg. in 10 sec. This calculated amount is probably a little too great, as the respiration of the guinea-pig is very shallow (resp. rate 110–144/min.) and the proportion of dead space ventilation in the respiratory minute volume therefore high. Our calculation shows that the amounts of drug retained during short periods must be very small indeed. They are smaller than the lowest doses of sympathomimetic or antihistaminic substances which gave definite protection by intramuscular injection (Armitage *et al.*, 1952 ; Herxheimer and Rosa, 1953).

The guinea-pigs were sensitized with egg albumin as in our previous experiments. Shock exposure was always by aerosol through the same commercial nebulizer at the same pressure.

Before a typical experiment the aerosol chamber is filled with the protecting aerosol. Some animals are then transferred into the chamber and left there, the

aerosol flowing through the chamber at a constant rate for the period intended. At the end of this period the chamber is lifted off its base and quickly ventilated, the animals removed, the chamber replaced and filled with albumin aerosol for the shock experiment. The animals are then put back into the chamber and the preconvulsion time is determined. In each animal the "percentage protection" index was calculated by the modified method described by Herxheimer and Rosa (1953).

The substances used were tripelennamine, promethazine, diphenhydramine, chlorcyclizine, isoprenaline, adrenaline, noradrenaline, ephedrine, and aminophylline. Detailed dose-response relationships were investigated only for one antihistamine (tripelennamine), one sympathomimetic amine (isoprenaline), and for aminophylline. The duration of the protective effect after injection was investigated with promethazine, diphenhydramine, chlorcyclizine, and aminophylline.

Results

The experiments with tripelennamine (Table I) show that aerosol inhalation for only one minute confers a definite, though not very strong, protection. Inhalation of a 1% solution for 3 min. gives about as strong a protection as an intramuscular injection of 1.0 or 3.0 mg./kg. No greater protection is given if the 1% solution is replaced by the 2% solution, or if the inhalation period is extended to 15 min.

The protection thus achieved is of short duration. One hour later it had decreased, often considerably, whatever the period of inhalation. Two hours later there was no longer evidence of protection in two out of three experiments. In the third, protection was still present but very weak. By contrast, after injection of 1 mg. or 3 mg./kg. a significant degree of protection was still present 4 and 6 hours later (Table I).

Diphenhydramine, chlorcyclizine, and promethazine (Table I) were examined in less detail. A 2% diphenhydramine aerosol gave no significant protection when inhaled for 5 or 10 min. If this period was extended to 15 or 30 min., the protection given was pronounced, about the same as that estimated for intramuscular injection of 2.0 mg./kg. and that obtained for 6.0 mg./kg. Two hours after inhalation no significant protection was present. When 6 mg. was injected intramuscularly, protection was still at its maximum after 6 hr. but had decreased sharply after 8 and 12 hr. After the injection of 2 mg./kg. no protection could be found after 8 hr.

Inhalation of 0.5% promethazine gave no protection after 2 min., but good protection after 5 min. The 3% solution gave a very high protection after inhalation for 10 min. When the animals were exposed for 30 min., they became dyspnoeic whilst in the promethazine aerosol. The same happened to two out of five animals breathing a 10% aerosol for 15 min. The duration of this protection was not followed up, but that following intramuscular injection was found to remain at or near its maximum for at least 12 hr. After 17 and 22½ hr. it was weak but still present.

TABLE I

EFFECT OF ANTIHISTAMINIC DRUGS ON ANAPHYLACTIC MICROSHOCK

Substance and Route of Administration	Conc. Inhaled (%)	Duration of Inhalation (min.)	Dose Injected (mg./kg.)	No. of Animals	% Protection ± Standard Error	Interval between Drug and Exposure to Shock (min.)
Tripelennamine						
(*a*) Aerosol	1	1		5	40·4± 3·7*	0
	1	1		5	24·8±10·8	0
	1	3		4	59·0± 9·2*	0
				3	60·0± 1·2*	0
	1	5		3	64·0± 3·5*	0
				3	68·7± 6·3*	0
	2	5		3	78·0± 4·4*	0
	2	15		5	63·0±10·9*	0
	1	5		6	52·7± 3·9*	60
	1	5		6	10·7± 8·9	120
	2	5		6	34·0± 8·0*	60
	2	5		6	25·3± 8·9*	120
	2	15		7	48·9±10·9*	60
	2	15		6	8·3±10·1	120
	2	15		5	23·0±10·5	300
(*b*) Injection			1·0	5	62·2± 8·2*	60
			1·0	6	25·0± 8·7*	360
			3·0	5	54·2± 8·4*	60
			3·0	5	59·8± 2·9*	240
Diphenhydramine						
(*a*) Aerosol	2	5		6	31·5±13·4	0
	2	10		5	20·2±17·4	0
	2	15		6	59·3± 6·9*	0
	2	30		7	46·4±10·1*	0
	2	15		5	− 6·6±14·7	120
(*b*) Injection			0·9	7	33·3± 3·2*	60
			2·0[1]	6	17·3±11·5[3]	60
			6·0[1]	6	44·7± 9·7*	60
			2·0	6	−11·3± 8·4	480
			6·0	4	67·0± 7·5*	360
			6·0	5	37·2± 9·8*	480
			6·0	6	32·3± 7·0*	540
Promethazine						
(*a*) Aerosol	0·5	2		7	17·9±14·5	0
	0·5	5		6	51·3± 4·3*	0
	3	10		5	66·2± 4·9*	0
	10	15		2	69·0± 7·0	0
(*b*) Injection			0·75[1]	6	68·8± 7·4*	60
			0·75[1]	7	75·6± 5·1*	60
			1·5[1]	7	66·0± 6·7*	60
			1·0	5	75·2± 6·6*	390
			1·0	5	70·0± 4·6*	510
			1·0	6	56·7± 7·0*	540
			1·0	6	21·2±10·1	1,020
			1·0	6	20·8± 6·1*	1,350
Chlorcyclizine						
(*a*) Aerosol	2	5		5	1·6±11·9	0
	2	15		3	3·3±12·7	0
	5	15		5	69·0± 7·5[2]*	0
(*b*) Injection			6·0[1]	5	58·2± 2·4*	60
			6·0	6	30·7±11·1*	480
			6·0	4	50·0± 8·2*	720

* Statistically significant protection.
[1] Experiment taken from earlier work (Armitage *et al.*, 1952).
[2] All animals dyspnoeic in chlorcyclizine aerosol.
[3] Estimated protection 44·9.

Inhalation of a 2% chlorcyclizine solution gave no protection. If a 5% solution was used, all animals became slightly dyspnoeic but were nevertheless well protected. After intramuscular injection of 6 mg. chlorcyclizine a moderate protection was observed after 8 and 12 hours.

The protection given by 90 to 105 sec. of 1% isoprenaline (Table II) was strong, but it disappeared almost completely after 30 or 60 min. Even inhalations as short as 10 and 15 sec. gave a mild but definite protection. The experiments with 1% adrenaline (Table II) gave similar results. The effect of noradrenaline was weaker (Table II).

Ephedrine hydrochloride 1% given for 15 min. caused dyspnoea. The ½% aerosol had no such effect and gave a limited protection.

The experiments with aminophylline (Table III) had no clear result. By contrast with the antihistamines no dyspnoea occurred with the higher concentration used (12½%), but the protection given even by long exposures was slight or absent. The lower concentrations (1%–5%) seemed to give the same or even a better protection than the higher ones regardless of the period of exposure. In no instance did the percentage protection reach the maximum values seen with tripelennamine, adrenaline and isoprenaline.

The duration of the effect of the isoprenaline and adrenaline (Table II) aerosols was extremely short; the isoprenaline effect had disappeared after 30 min. and that of adrenaline after 1 hr. The injection of both substances (Table II) was effective for several hours. Aminophylline injections (Table III), which gave complete immediate protection, lost their effect 6 hr. later.

TABLE II

EFFECT OF SYMPATHOMIMETIC DRUGS ON ANAPHYLACTIC MICROSHOCK

Substance and Route of Administration	Conc. Inhaled (%)	Duration of Inhalation (sec.)	Dose Injected (mg./kg.)	No. of Animals	% Protection ± Standard Error	Interval between Drug and Exposure to Shock (min.)
Isoprenaline						
(*a*) Aerosol	1	10		5	28·0±12·2	0
	1	10		4	33·3± 6·8*	0
	1	15		5	36·4±10·9*	0
	1	30		5	45·2± 7·0*	0
	1	30+15[1]		2	68·0	0
	1	90+15[1]		3	85·7± 7·3*	0
	1	90+15[1]		9	1·9± 4·7	30
	1	90+15[1]		6	17·5±12·8	60
(*b*) Injection			0·1	3[2]	85·3±14·7*	15
			0·1	7[2]	68·4± 5·0*	15
			0·1	3	83·7± 1·8*	30
			0·1	5	72·2± 9·2*	60
			0·1	3	44·7± 4·1*	180
			0·1	3	30·3±15·9	360
Adrenaline						
(*a*) Aerosol	1	10		5	53·0± 9·6*	0
	1	15		5	69·7± 6·0*	0
	1	20		4	65·0±13·7*	0
	1	60		5	79·2± 1·4*	0
	1	90+15[1]		6	47·3± 2·1*	30
	1	90+15[1]		4	40·5± 6·6*	30
	1	90+15[1]		4	13·8± 7·6	60
(*b*) Injection			0·2	7[2]	57·1± 5·8*	15
			0·2	4	35·0± 9·0*	60
			0·2	6	66·8± 4·4*	60
			0·2	4	52·5± 6·4*	120
			0·2	3	11·7± 7·5	180
			0·2	3	71·6±23·5	180
			0·2	5	54·4±21·6	240
Noradrenaline						
Aerosol	1	20		5	52·2± 5·2*	0
	1	20		4	50·5± 7·1*	0
	1	60		5	41·4± 8·4*	0
	1	60		6	52·2± 8·1*	0
	1	120		3	75·0± 5·8*	0

* Statistically significant protection.
[1] After 90 sec. exposure the inflow of aerosol was stopped and the animals allowed to remain 15 sec. longer in the aerosol chamber. In the other experiments they were removed immediately.
[2] Experiment taken from earlier work (Herxheimer and Rosa, 1953).

TABLE III

EFFECT OF AMINOPHYLLINE ON ANAPHYLACTIC MICROSHOCK

Route of Administration	Conc. Inhaled (%)	Duration of Inhalation (min.)	Dose Injected (mg./kg.)	No. of Animals	% Protection ± Standard Error	Interval between Drug and Exposure to Shock (min.)
Aerosol ..	1	10		5	35·0± 8·3*	0
	1	60		5	50·6± 8·2*	0
	2	10		4	43·0± 9·0*	0
	2	30		4	41·0± 5·7*	0
	5	3		3	11·7±10·9	0
	5	5		3	10·3±11·7	0
	5	10		5	41·2± 8·0*	0
	5	15		3	41·3± 2·8*	0
	5	20		4	59·8± 2·3*	0
	5	20		4	51·3±12·8*	0
	5	60		3	22·3± 1·8*	
	12·5	10		2	16·5±18·5	0
	12·5	20		5	18·5±13·1	0
	12·5	20		2	10·5± 0·5	0
	12·5	30		5	48·6± 9·6*	0
	12.5	60		4	25·0±10·4	0
Injection			100	4[1]	100·0*	20
			100	6	83·0± 3·0*	60
			100	4	60±22·0	300
			100	5	37·4±17·6	360

* Statistically significant protection.
[1] Experiment taken from earlier work (Herxheimer and Rosa, 1953).

DISCUSSION

The high degree of protection achieved by inhalation may seem surprising. It is certain that the amounts of substance inhaled and absorbed during an exposure of 10 sec. must be very small indeed. Moreover, the effects of the absorption of these small amounts appear within a few seconds. A similar observation has been made in man. In an attack of induced asthma the effect of 10 sec. isoprenaline inhalation becomes apparent about 20 sec. after the end of the inhalation, when the respiration becomes deeper and the rate increases. This appears to be due to a central stimulating effect on respiration. The

decrease of bronchial obstruction, measured by the speed of the expiratory flow or by the vital capacity, shows the effect of the drug at the same time and reaches its maximum after 1 min. or a little later. If the substance is taken perlingually, its effect does not become noticeable for 3 or 4 min., and the amount needed is many times greater. The longer period of time is needed for the absorption of the substance and its transport by the blood stream to its site of action. By inhalation the substances are effective almost immediately, because only a negligible interval is required for absorption and transport. We are thus driven to the conclusion that the substances which act with such speed act in the cells in which they are absorbed. The few seconds which pass before their action occurs are probably required for the penetration of the film of mucus covering the cells of the mucous membrane. This would explain how an inhalation of 1% adrenaline for about 15 sec., which could not lead to an absorption of more than a few micrograms, could achieve as great a degree of protection as the injection of 200 μg. The latter amount is distributed throughout the body in order to reach the bronchi, and the amount acting on the mucous membrane can be only a fraction of the amount injected. The same is seen with tripelennamine. Here the inhalation of a 1% solution, with a probable retention of about 6 μg., gives a percentage protection of about 40, whilst by injection 50 μg. was the smallest dose with which any noticeable protection was achieved.

On the other hand, our assumption would also explain the short duration of the effect of inhalation. The small amount deposited in the mucous membrane would at once be destroyed or absorbed into the blood stream. In either event the effective concentration would be reduced considerably within a short time, and in fact the high protection given by isoprenaline and adrenaline disappears within about half an hour, whilst that given by the injection lasts for a few hours. This difference points to the fact that it is not so much destruction of the substance as its changing distribution which causes the difference in the duration of its effect. A few micrograms spread over the whole body constitute a subthreshold dose, whereas the same amount concentrated in the bronchial membrane has a demonstrable effect.

This becomes still more evident with the antihistamines. If injected, their effect lasts for many hours; in the case of promethazine it can be traced even after 17 and 22 hours; in others it is less, and, on the whole, the effective periods observed are very similar to those Bain (1949) has found for the action of these drugs against histamine. The effect of aerosols, however, disappears after 1–2 hours. Thus the effect of the antihistamines lasts longer than that of the sympathomimetic amines whether they are inhaled or injected, but for both groups the inhalation effect is much the shorter. It is interesting to note that similar differences in the effect of the aerosols of the various antihistamines are observed whether these are injected or inhaled. In agreement with clinical experience tripelennamine and promethazine have been found stronger than diphenhydramine and chlorcyclizine.

Aminophylline differs from antihistaminic and sympathomimetic drugs in various respects. If injected, its protective effect is greater than with either of these groups; the duration of its effect is less than that of the antihistamines, but longer than that of the sympathomimetic amines. The effect of aminophylline aerosol is, by contrast, disappointing. Its protective action is weak, and it cannot be increased by longer inhalation even at higher concentration. This suggests that its mechanism of action differs from that of the other substances.

Little is known of the mechanism of aminophylline action in general, and any explanation is therefore bound to be purely speculative. The low level of protection by inhalation may suggest that the aerosol does not act upon the same cells on the surface of the mucosa as do the other substances. If aminophylline were to act in a deeper layer of the tissue—if it were to act, for example, on blood vessels other than the most superficial capillaries—it would require transport to this site of action. Its accumulation at the site of absorption would not increase its effect. Once its transport had begun it would pass its particular site of action in the deeper layers of the tissue, since it is only in transit. This would prevent its accumulation at this site, and the action would thus be produced by only a part of the inhaled total when that happened to pass the reactive tissue or organs.

A curious feature of these experiments is the effect of higher concentrations of some antihistamines which cause an asthma-like dyspnoea instead of protecting against it, as lower concentrations do. Similar reactions have been found in human asthma with promethazine, tripelennamine, and ephedrine (Herxheimer, 1952b). The reasons for this are not clear; it may be recalled that Arunlakshana has found (1953) that antihistamines under some conditions cause the release of histamine. Another possible reason is the local irritant

action of some antihistamines which Bain, Hellier, and Warin (1948) found when the drugs were injected intradermally.

The practical consequences of these results for the pharmacology of human asthma are mainly confirmatory. It has been known for a long time that adrenaline and isoprenaline aerosols have an almost immediate relieving effect in bronchial asthma, and that they are less likely to cause unpleasant systemic side effects than oral or subcutaneous application. What has been less well known is the short duration of this relieving effect by inhalation. There are many chronic asthmatics whose bronchial obstruction is continuous and does not fluctuate much in intensity. These patients often insist that they must inhale every 1½–3 hr., and some clinicians are inclined to attribute this insistence to habit rather than a necessity. Our experiments show that there may often be a real necessity for repeated inhalation.

The protective action of an antihistaminic aerosol has been noticed before (Herxheimer, 1949; Friebel, 1953). In practice, the relieving effect of sympathomimetic aerosols needs less time and is stronger than that of antihistaminic aerosols; the latter therefore are not recommended for the treatment of asthma.

The long duration of the injection effect of the antihistamines is also borne out by practical experience. Not only has Bain (1949), as mentioned before, found this long duration in their action against histamine itself, but in asthmatic patients this long lasting effect can be easily observed. If a patient is given, for instance, diphenhydramine at bedtime, its antiasthmatic (and its sedative) effect decreases sharply after 6–8 hr. Promethazine and chlorcyclizine, however, still show a definite effect in the morning, often 12–13 hr. or more after administration.

Summary

1. Antihistamines and sympathomimetic amines administered by aerosol protect guinea-pigs against anaphylactic shock. Relatively small amounts inhaled give a high degree of protection, often equalling that achieved by intramuscular injection. Whilst the effect of the latter lasts for many hours and then slowly decreases, that of the former decreases almost immediately. Protection by an aerosol is hardly detectable after 2 hr. with antihistamines and after 30 min. with sympathomimetic amines.

2. For approximately equal degrees of protection, much smaller total amounts are necessary by inhalation than by injection. Given by inhalation, the drugs are initially concentrated at their site of action, but are presumably quickly removed by the circulation. Given by injection, the drugs are widely distributed and must accordingly be given in much greater amount. Their action persists for a much longer period, until they are excreted or destroyed.

3. Aminophylline aerosol has a weak protective action against anaphylactic shock, and the protection cannot be increased by longer inhalation or by an increase in the concentration of the inhalant. By contrast, the protection given by intraperitoneal aminophylline is higher than that of any antihistaminic or sympathomimetic drug. It is suggested that the relative inefficiency of the aminophylline aerosol may be accounted for by aminophylline having a different site of action from that of the antihistaminic and sympathomimetic drugs.

4. The differences between the protective strength of the various antihistamines and their duration of action correspond to the differences observed clinically.

5. The effect of antihistaminic substances lasts longer than that of sympathomimetic amines whatever the route of administration.

This work was assisted by a personal grant from the Medical Research Council and by a grant for technical assistance from the Asthma Research Council. I am also indebted to Dr. P. Armitage, of the Statistical Research Unit, M.R.C., for the statistical analysis of the results.

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