J. Pharmacol., 1965, **17**, 721–727 Received May 7, 1965

A comparison of the anti-anaphylactic properties of ethanolamine and hydrocortisone

P. GOADBY AND W. G. SMITH

Both ethanolamine and hydrocortisone potentiate the anti-anaphylactic activity of mepyramine in actively sensitised guinea-pigs subjected to anaphylactic shock by exposure to aerosols of antigen solution. The effects of these two substances have now been compared. After intramuscular injection, the peak effect with ethanolamine occurred 1 hr later whereas with hydrocortisone it occurred 18 hr later. Both substances were also effective, after 45 min and 12 hr respectively, as aerosols. The optimum intramuscular dose of ethanolamine was 10 mg/kg and that of hydrocortisone 100 mg/kg. After aerosol administration, optimum effects were observed when 5% solutions of either drug were used.

CORTISONE and synthetic analogues have been used successfully in the treatment of asthma and more particularly in status asthmaticus (Bordley, Carey, Harvey, Howard, Kattus, Newman & Winkwerder, 1949; Carryer, Koelsche, Prickman, Maytum, Lake & Williams, 1950; M.R.C., 1956).

Though several investigators have been unable to prevent the death of guinea-pigs in anaphylactic shock by pretreatment with cortisone (Leger, Leith & Rose, 1948; Dworetsky, Code & Higgins, 1950; Friedlander & Friedlander, 1950). Feinberg, Malkiel & McIntyre (1953) reported that pretreatment with cortisone 18 hr before exposure to aerosolised antigen increased the time passively sensitised guinea-pigs could withstand exposure to antigen.

We found that soluble hydrocortisone potentiated the anti-anaphylactic effects of mepyramine in actively sensitised guinea-pigs and diminished the release of SRS-A (slow reacting substance of anaphylaxis) from a sensitised guinea-pig lung shocked *in vitro* (Goadby & Smith, 1963). The results with hydrocortisone were similar to those of Smith (1961) for ethanolamine. However, although the *in vitro* conditions for the ethanolamine experiments were similar to those for the hydrocortisone study, the *in vivo* conditions were different, so that direct comparisons were not possible.

Aerosolised hydrocortisone in low dosage has produced encouraging results in the treatment of human bronchial asthma (Foulds, Greaves, Herxheimer & Kingdom, 1955; Helm & Heyworth, 1958), the most satisfactory results being in patients with allergic asthma without hypersecretion. A comparison of the anti-anaphylactic effects of ethanolamine and hydrocortisone administered by aerosol to guinea-pigs was therefore made.

Experimental

Guinea-pigs, 250–350 g, were sensitised to egg albumin by the intraperitoneal injection of 2 ml of a 5% solution. After three weeks they were exposed to an aerosol produced by applying air at 15 lb/in^2 to a

From the Research Laboratory in Biochemical Pharmacology, School of Pharmacy, The Technical College, Sunderland.

Wright nebuliser (Wright, 1958) containing a 1% solution of egg albumin. The time to onset of dyspnoea and cough was noted for each animal and termed the "collapse time". Exposure to albumin was repeated at weekly intervals for three weeks and the mean "collapse time" for the last two weeks was termed the "normal collapse time" (Goadby & Smith, 1962). The animals were then divided into groups of ten such that the mean "collapse time" for the groups was between 60 and 100 sec.

One hr before the fourth weekly exposure to antigen each animal was injected with 1 mg/kg of mepyramine (as maleate) intramuscularly and the ratio of the "treated collapse time" to the "normal collapse time" was termed the "protection ratio" (Smith, 1961). The next week the animals were exposed to antigen without pretreatment to ascertain that mepyramine had produced no lasting effect on their sensitivity.

One week later, the potentiating effect of ethanolamine or hydrocortisone on the protection afforded by mepyramine 1 mg/kg given 1 hr before exposure to antigen, was determined. Ethanolamine was used as a solution of the hydrochloride, the doses being expressed as ethanolamine base. Hydrocortisone was used as a solution of the sodium hemisuccinate, the doses being expressed as the alcohol. The times between intramuscular injection for potentiating drug and exposure to aerosolised antigen and the doses by the intramuscular route giving optimum results were investigated.

The effect of a prior inhalation of an aerosol of ethanolamine or hydrocortisone on the anti-anaphylactic effects of mepyramine was also examined. The rate of aerosolisation of the solution was 8·0 ml/hr. Animals were exposed for 15 min. The pretreatment time was taken as the time from the removal of the animals from the drug aerosol to the time of entry into the chamber for exposure to aerosolised antigen. The effect of varying dosage was achieved by altering the concentration of the drug solution and keeping the other conditions constant.

The histamine aerosol used to study the irritant effects of ethanolamine and hydrocortisone aerosols was produced by applying air at 15 lb/in^2 to a Wright nebuliser containing a 0·1% solution of histamine (as acid phosphate). The rate of aerosolisation was 8·0 ml/hr. Groups of ten unsensitised guinea-pigs were used. Partial protection was obtained by giving 0·05 mg/kg adrenaline, intramuscularly, 15 min before exposure to the histamine aerosol. Hydrocortisone and ethanolamine aerosols were given 12 hr and 45 min respectively before exposure to histamine aerosol.

Results

The results are expressed as the mean "protection ratio" ($\pm$ s.d.) for the group of ten animals. An animal which tolerated antigen for twenty times its "normal collapse time" was considered to be fully protected. The number of fully protected animals in a group is shown separately from the mean "protection ration" ($\pm$ s.d.) of the remainder.

Table 1 shows that potentiation of the anti-anaphylactic effects of mepyramine was maximal when ethanolamine was given 1 hr before

TABLE 1. TIME SEQUENCE OF THE PROTECTIVE EFFECTS OF ETHANOLAMINE AND HYDROCORTISONE IN GROUPS OF TEN ANIMALS

Group mean "Normal collapse time" (sec)	Mepyramine[1] pretreatment	Mepyramine + ethanolamine[2] pretreatment		Hr between ethanolamine pretreatment and shock	Group mean "normal collapse time" (sec)	Mepyramine[1] pretreatment	Mepyramine + hydrocortisone[3] preatment		Hr between hydrocortisone pretreatment and shock
	Group mean protection ratio ± s.d.	fully protected animals	Mean protection ratio of remainder ± s.d.			Group mean protection ratio ± s.d.	Fully protected animals	Mean protection ratio of remainder ± s.d.	
90·1	4·38 ± 1·28	0	6·27 ± 3·65	0·25	83·9	3·43 ± 1·23	1	4·33 ± 1·78	1
82·9	4·28 ± 1·65	3	7·34 ± 0·81	0·50	88·2	5·30 ± 1·88	2	6·44 ± 1·79	6
92·1	4·72 ± 1·18	3	5·63 ± 2·31	0·75	84·9	4·21 ± 1·27	3	7·36 ± 4·60	12
99·8	4·15 ± 2·23	5	11·30 ± 4·58	1·0	64·8	3·14 ± 1·98	4	5·45 ± 1·88	18
93·6	5·31 ± 2·50	1	6·62 ± 1·88	2·0	80·5	4·54 ± 1·81	0	4·97 ± 1·12	24
93·1	6·15 ± 1·98	2	8·19 ± 3·31	4·0					

[1] 1 mg/kg mepyramine (as maleate) intramuscularly 1 hr before shock. [2] 10 mg/kg ethanolamine (as hydrochloride) intramuscularly.
[3] 100 mg/kg hydrocortisone (as sodium hemisuccinate) intramuscularly.

TABLE 2. DOSE-EFFECT RELATIONSHIP OF ETHANOLAMINE AND HYDROCORTISONE IN GROUPS OF TEN ANIMALS

Group mean "Normal collapse time" (sec)	Mepyramine[1] pretreatment	Mepyramine + ethanolamine[2] pretreatment		Dose of ethanolamine (mg/kg)	Group mean "normal collapse time" (sec)	Mepyramine[1] pretreatment	Mepyramine + hydrocortisone[3] preatment		Dose of hydrocortisone (mg/kg)
	Group mean protection ratio ± s.d.	Fully protected animals	Mean protection ratio of remainder ± s.d.			Group mean protection ratio ± s.d.	Fully protected animals	Mean protection ratio of remainder ± s.d.	
89·4	3·74 ± 1·10	0	6·27 ± 1·69	5	87·5	4·87 ± 0·93	0	5·24 ± 1·37	25
99·8	4·15 ± 2·23	5	11·30 ± 4·58	10	89·8	5·13 ± 2·26	0	4·41 ± 1·56	50
74·7	3·81 ± 1·98	5	5·93 ± 3·27	20	64·8	3·14 ± 1·98	4	5·45 ± 1·88	100
86·9	3·76 ± 1·29	2	6·94 ± 3·60	40	94·7	3·85 ± 2·06	3	4·39 ± 2·23	200

[1] 1 mg/kg mepyramine (as maleate) intramuscularly 1 hr before shock. [2] Ethanolamine (as hydrochloride) intramuscularly 1 hr before shock.
[3] Hydrocortisone (as sodium hemisuccinate) intramuscularly 18 hr before shock.

TABLE 3. TIME SEQUENCE OF PROTECTION OBSERVED AFTER AEROSOL ADMINISTRATION IN GROUPS OF TEN ANIMALS

Group mean "Normal collapse time" (sec)	Mepyramine[1] pretreatment	Mepyramine + ethanolamine[2] pretreatment		Hr between ethanolamine pretreatment and shock	Group mean "normal collapse time" (sec)	Mepyramine[1] pretreatment	Mepyramine + hydrocortisone[3] preatment		Hr between hydrocortisone pretreatment and shock
	Group mean protection ratio ± s.d.	Fully protected animals	Mean protection ratio of remainder ± s.d.			Group mean protection ratio ± s.d.	Fully protected animals	Mean protection ratio of remainder ± s.d.	
65·9	5·57 ± 2·18	0	5·94 ± 2·60	0·25	81·3	4·02 ± 1·41	0	4·13 ± 1·55	1
94·2	2·82 ± 1·26	2	4·69 ± 2·86	0·50	90·4	3·94 ± 1·28	1	5·95 ± 2·41	6
92·9	4·67 ± 1·09	6	7·01 ± 3·97	0·75	98·7	4·41 ± 2·14	4	4·78 ± 2·35	12
96·0	4·07 ± 1·67	1	5·35 ± 3·79	1·0	88·7	4·02 ± 1·59	1	7·50 ± 4·34	18
90·0	3·61 ± 1·96	2	5·53 ± 2·15	2·0					

[1] 1 mg/kg mepyramine (as maleate) intramuscularly 1 hr before shock. [2] Ethanolamine aerosol 5% given for 15 min ending at time quoted before shock.
[3] Hydrocortisone aerosol 5% given for 15 min ending at time quoted before shock.

TABLE 4. EFFECT OF INCREASING THE CONCENTRATIONS OF ETHANOLAMINE AND HYDROCORTISONE ON THE PROTECTION OBSERVED AFTER AEROSOL ADMINISTRATION IN GROUPS OF TEN ANIMALS

Group mean "Normal collapse time" (sec)	Mepyramine[1] pretreatment	Mepyramine + ethanolamine[2] pretreatment		Conc. ethanolamine aerosol %	Group mean "normal collapse time" (sec)	Mepyramine[1] pretreatment	Mepyramine + hydrocortisone[3] preatment		Conc. of hydrocortisone aerosol %
	Group mean protection ratio ± s.d.	Fully protected animals	Mean protection ratio of remainder ± s.d.			Group mean protection ratio ± s.d.	Fully protected animals	Mean protection ratio of remainder ± s.d.	
99·0	3·08 ± 1·21	1	3·67 ± 1·38	2·5	95·8	4·21 ± 3·20	3	6·44 ± 3·28	2·5
92·9	4·67 ± 1·09	6	7·01 ± 3·97	5	98·7	4·41 ± 2·14	4	4·78 ± 2·33	5
90·6	4·46 ± 1·42	1	5·72 ± 3·85	10	100·8	4·05 ± 1·93	2	6·55 ± 3·96	10

[1] 1 mg/kg mepyramine (as maleate) intramuscularly 1 hr before shock. [2] Ethanolamine aerosol for 15 min ending 45 min before shock.
[3] Hydrocortisone aerosol for 15 min ending 12 hr before shock.

exposure to antigen though some protection was observed at five of the six time intervals used. The potentiation of mepyramine by hydrocortisone was maximal when the animals were exposed to antigen 18 hr after administration of the steroid. Some protection was observed at four of the five time intervals tested.

Ethanolamine in the presence of mepyramine was found to confer greater protection from the effects of anaphylaxis than mepyramine alone at three of the four dose levels used (see Table 2). The same number of animals was fully protected by both 10 mg/kg and 20 mg/kg, but of those animals not fully protected a greater level ($P > 0.05$) of protection was seen at 10 mg/kg. Hydrocortisone potentiated the anti-anaphylactic effects of mepyramine at two of the three dose levels tested. The maximum effect was shown by 100 mg/kg, four animals being fully protected. The response of the animals not fully protected did not differ from that of the mepyramine controls.

Ethanolamine aerosol showed the greatest potentiation of the anti-anapylactic effects of mepyramine when it was administered 45 min before exposure to antigen (Table 3). Six animals were fully protected and the remaining four showed a greater protection ($P > 0.05$) than the mepyramine controls. Hydrocortisone aerosol was most effective when given 12 hr before exposure to antigen, four animals being fully protected. The remaining animals did not differ significantly from the mepyramine controls.

Ethanolamine showed marked potentiation of the anti-anaphylactic effects when administered as an aerosol of a 5% solution (Table 4). Very little protection was observed with aerosols of 2·5% and 10% solutions. Hydrocortisone also showed maximum protection as a 5% solution. Those animals not fully protected gave responses which were not significantly different from the mepyramine controls. Some protection was observed with 2·5% and 10% solutions.

Aerosols of 10% solutions of hydrocortisone and ethanolamine did not affect the collapse times of unsensitised animals exposed to histamine aerosol (Table 5). Ethanolamine 10% did reduce the protection afforded by 0·05 mg/kg adrenaline against the histamine aerosol although hydrocortisone 10% did not.

TABLE 5. EFFECT OF PREVIOUS EXPOSURE TO AEROSOLS OF CONCENTRATED SOLUTIONS OF ETHANOLAMINE AND HYDROCORTISONE ON GROUPS OF TEN ANIMALS EXPOSED TO HISTAMINE AEROSOL, 0·1%

Group mean "normal collapse time" (sec)	Pretreatment	Fully protected animals	Protection ratio of remainder ±s. d.
66·7	Ethanolamine aerosol 10%, 45 min before shock	0	0·91 ± 0·35
100·7	Hydrocortisone aerosol 10%, 12 hr before shock	0	1·64 ± 0·53
83·3	Adrenaline 0·05 mg/kg intramuscular 15 min before shock ..	3	4·91 ± 2·36
99·4	Adrenaline 0·05 mg/kg i.m. + ethanolamine aerosol 10% ..	0	2·04 ± 0·97
99·6	Adrenaline 0·05 mg/kg i.m. + hydrocortisone aerosol 10% ..	2	3·96 ± 1·89

Discussion

The experiments confirm the previous results (Smith, 1961; Goadby & Smith, 1963) that whilst hydrocortisone and ethanolamine do not protect

actively sensitised guinea-pigs from the effects of aerosolised antigen, they potentiate the anti-anaphylactic effects of mepyramine in some animals. There are, however, differences in the pattern of activity of the two drugs.

The onset of the protective effect of ethanolamine was between 15 and 30 min and the effect was maximal 1 hr after intramuscular administration. The effect of hydrocortisone developed slowly and was not maximal until 18 hr after intramuscular injection. The time to onset of the effect of ethanolamine is probably that required for absorption, transport and binding of the drug at the site of action. The slow development of the effect of hydrocortisone probably involves an alteration of tissue metabolism, as suggested by Goadby & Smith (1962). It was not possible to obtain full protection from anaphylaxis by pretreatment with ethanolamine and mepyramine (as reported by Smith, 1961). With ethanolamine, some animals were fully protected and some others were partly protected, whilst with hydrocortisone some animals were protected but the remainder were not.

These results contrast with those of Herxheimer & Stresemann (1965). The differences between their method and the one reported here are small but important. Herxheimer & Stresemann did not use the same animals both for the mepyramine control and for the ethanolamine plus mepyramine study. Also, when our animals received mepyramine plus ethanolamine they were receiving their second protective pretreatment in three weeks. Other differences have been reviewed by Smith (1965). Administration of the drugs by aerosol showed the same general pattern as that for intramuscular injection, except the time to onset of the effect was shorter in both instances. No greater level of protection was obtained but the amount of drug to obtain protection is smaller. Animals were exposed to 100 mg of drug in 90 litres of air. The minute volume of a guinea-pig is 0·16 litres (Spector, 1956). Therefore, without allowing for losses on the apparatus, in 15 min the maximum dose it received is 2·66 mg.

The failure to produce increased protection by increasing the concentration of the aerosolised solutions above 5% has received further investigation. Aerosols of 10% solutions of both drugs were found to be irritant to the respiratory passages of guinea-pigs and caused a characteristic scratching of the nose with the front paws. This might account for the result obtained with 10% ethanolamine aerosol since, although this solution did not affect the collapse times of sensitised guinea-pigs subjected to aerosolised antigen or unsensitised guinea-pigs exposed to histamine aerosol, it reduced the protection afforded by a small dose of adrenaline against histamine aerosol. However, it is unlikely that an irritant effect would explain the result obtained with hydrocortisone 12 hr after administration. Also, 10% aerosols of hydrocortisone failed to reduce the collapse times of sensitised guinea-pigs subjected to aerosolised antigen, of unsensitised guinea-pigs exposed to histamine aerosol and of guinea-pigs partially protected by adrenaline against the effects of histamine aerosol.

Ethanolamine produces a similar protection to that of hydrocortisone in

sensitised guinea-pigs subjected to aerosolised antigen. The onset of action of ethanolamine is quicker and its duration is shorter. The dose of ethanolamine is less critical than that of hydrocortisone when the drug is given parenterally, but the irritant nature of concentrated solutions should be considered when using aerosol administration.

References

Bordley, J. E., Carey, R. A., Harvey, A. McG., Howard, J. E., Kattus, A. A. N., Newman, E. V. & Winkwerder, W. L. (1949). *Bull. John Hopkins Hosp.*, **85**, 396–398.

Carryer, H. M., Koelsche, G. A., Prickman, L. E., Maytum, C. K., Lake, C. F. & Williams, H. L. (1950). *Proc. Mayo Clinic*, **21**, 282–287.

Dworetsky, M., Code, C. F. & Higgins, C. M. (1950). *Proc. Soc. exp. Biol. N.Y.*, **75**, 201–206.

Feinberg, S. M., Malkiel, S. & McIntyre, F. C. (1953). *J. Allergy*, **24**, 302–308.

Foulds, W. S., Greaves, D. P., Herxheimer, H. & Kingdom, L. G. (1955). *Lancet*, **18**, 234.

Friedlander, S. & Friedlander, A. S. (1950). *J. Allergy*, **21**, 303–309.

Goadby, P. & Smith, W. G. (1962). *J. Pharm. Pharmacol.*, **14**, 739–745.

Goadby, P. & Smith, W. G. (1964). *Ibid.*, **16**, 108–114.

Helm, W. H. & Heyworth, F. (1958). *Brit. med. J.*, **2**, 765–767.

Herxheimer, H. & Stresemann, E. (1965). *J. Pharm. Pharmacol.*, **17**, 125.

Leger, J., Leith, W. & Rose, B. (1948). *Proc. Soc. exp. Biol. N.Y.*, **69**, 559–531.

M.R.C., Report by Sub-committee on clinical trials in asthma (1956), *Lancet*, **2**, 798–803 and 803–806.

Smith, W. G. (1961). *J. Pharm. Pharmacol.*, **13**, 1–11.

Smith, W. G. (1962). *Biochem. Pharmacol.*, **11**, 183–186.

Smith, W. G. (1965). *J. Pharm. Pharmacol.*, **17**, 247.

Spector, W. S. (1956). In *Handbook of Biological Data*, p. 267, London: W. B. Saunders and Co.

Wright, B. M. (1958). *Lancet.*, **2**, 24–35.