J. Pharm. Pharmacol., 1964, **16**, 108–114. Received August 29, 1963

Observations on the anti-anaphylactic activity of hydrocortisone and related steroids

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Hydrocortisone and some synthetic analogues had negligible anti-anaphylactic activity in actively sensitised guinea-pigs when administered alone but they potentiated the protection afforded by mepyramine. Hydrocortisone did not affect the yield of histamine from sensitised guinea-pig lung subjected to anaphylaxis *in vitro* but reduced the amount of SRS-A produced under these conditions.

SEVERAL authors have been unable to prevent death from experimental anaphylactic shock by pretreating animals with cortisone or ACTH (Leger, Leith & Rose, 1948; Dworetsky, Code & Higgins, 1950; Friedlander & Friedlander, 1950; Malkiel, 1951.)

Herxheimer & Rosa (1952) showed that a single injection of cortisone given before exposure of actively sensitised guinea-pigs to aerosolised antigen did not increase the time for onset of dyspnoea and cough. However, Feinburg, Malkiel & McIntire (1953) found that cortisone increased the time of production of dyspnoea and cough in passively sensitised animals when the drug was administered 18 hr before exposure to aerosolised antigen.

The anti-anaphylactic activity of hydrocortisone and related steroids in actively sensitised guinea-pigs exposed to aerosolised antigen is here reported.

Methods

ANAPHYLACTIC SHOCK *In Vivo*

Groups of 10 guinea-pigs were sensitised to commercial egg albumin by the intraperitoneal injection of 100 mg as a 5% solution in normal saline. Three weeks later the animals were subjected to anaphylactic shock using the technique of Herxheimer (1952) as modified by Smith (1961). Each animal was placed in a glass vessel and exposed to an aerosol of antigen produced by applying air at 10 lb/in^2 to a Riddostat inhaler (Riddell Products, London) containing a 1% w/v solution of egg albumin in distilled water. The animals were removed from the chamber at the onset of dyspnoea and cough. The time for onset of dyspnoea and cough was measured at weekly intervals and was relatively constant for each animal. The mean of the last two exposures was termed the "normal collapse time" (Smith, 1961). Table 1 shows the times to onset of dyspnoea and cough for a sample group with the calculated "normal collapse time" of each animal.

Drug pretreatment was carried out before the fourth weekly exposure to antigen. An increase in the time to onset of dyspnoea and cough on exposure to antigen following treatment ("treated collapse time") indicated a protective effect. The protection was expressed as a "protection

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TABLE 1. EVALUATION OF "NORMAL COLLAPSE TIMES" AND "PROTECTION RATIOS"

Animal No.	Collapse time (sec)			Normal collapse time (calculated from 2 and 3 weeks)	Treated collapse time Week 4	Protection ratio (calculated from cols. 5 and 6)
	Week 1	Week 2	Week 3			
1	40	38	34	36	63	1·75
2	34	39	55	47	80	1·70
3	22	34	30	32	69	2·16
4	30	49	49	49	62	1·27
5	28	45	41	43	105	2·44
6	15	20	18	19	48	2·53
7	31	36	42	39	138	3·53
8	20	22	30	26	40	1·54
9	33	27	41	34	75	2·20
10	26	30	42	36	82	2·28

Group Mean Protection Ratio = 2·14. Standard deviation = 0·64. Treatment = 50 mg hydrocortisone sodium hemisuccinate intramuscularly 18 hr before shock.

ratio", this being the ratio of the "treated collapse time" to the "normal collapse time". It was found that animals which tolerated antigen for more than twenty times their "normal collapse time" did not exhibit signs of dyspnoea and cough even though exposure to antigen was continued for much longer. Therefore an animal showing a protection ratio of 20 was considered to be fully protected from the effects of anaphylaxis.

PHARMACOLOGICAL ACTIVITY ON GUINEA-PIG ILEUM

The terminal ileum was removed from a freshly killed guinea-pig. The last 2 cm adjacent to the caecum were rejected and suitably sized segments of the remaining ileum were suspended in aerated Tyrode solution at 37° in a 2 ml isolated tissue bath. The drugs were applied as solutions in Tyrode solution.

The SRS–A (slow reacting substance of anaphylaxis) was prepared by the method of Brocklehurst (1960) and standardised by comparison with a laboratory standard (see Marquis & Smith, 1963).

The hydrocortisone was applied 30 sec before the addition of the stimulant drug. Histamine was allowed 30 sec contact with the tissue and SRS–A was allowed 90 sec contact.

PHARMACOLOGICAL ACTIVITY ON GUINEA-PIG TRACHEAL CHAIN

Tracheal chains from the guinea-pig (Castillo & de Beer, 1947) were mounted in Krebs Hensleit solution (1932) in a 15 ml isolated tissue bath. The hydrocortisone was added 1 min before the stimulant drug. Histamine was allowed 5 min contact with the preparation and SRS–A 10 min contact.

ANAPHYLACTIC SHOCK *In Vitro*

Twelve sensitised guinea-pigs whose "normal collapse times" had been determined as described earlier were divided into two groups of approximately equal sensitivity to antigen.

One week after the third exposure to antigen, the animals of one group were injected intramuscularly with 50 mg of sodium hydrocortisone hemisuccinate as a 5% solution in Water for Injection B.P.

The animals of the second group received an equal volume of normal saline by the same route. Eighteen hr later the animals were killed, their lungs excised, perfused with Tyrode solution and subjected to anaphylaxis *in vitro* as described by Brocklehurst (1960).

The released histamine and SRS–A were assayed on segments of guinea-pig ileum (Marquis & Smith, 1963). The assays of SRS–A were made in Tyrode solution without sodium bicarbonate since this increased the sensitivity of the ileum to SRS–A (Firth, unpublished).

DRUGS

Cortisone acetate, hydrocortisone, prednisolone and triamcinolone were administered as fine suspensions and hydrocortisone sodium hemisuccinate as a solution in Water for Injection B.P. The dosage used throughout was 50 mg per animal intramuscularly 18 hr before exposure to antigen. (Feinberg & others, 1953.)

Mepyramine: 1 mg/kg, given intramuscularly 1 hr before shock was used in the *in vivo* experiments. This dose gave the peak effect against the symptoms of anaphylaxis in the study reported by Smith (1961).

Results

PROTECTIVE EFFECT OF CORTICOSTEROID DRUGS AGAINST ANAPHYLAXIS

In Vivo

Each drug was administered to a group 18 hr before the fourth weekly exposure to antigen. Table 2 shows the mean "protection ratio" obtained for each drug using groups of 10 guinea-pigs. It can be seen that none of the compounds tested had marked anti-anaphylactic activity. The greatest activity was shown by hydrocortisone sodium hemisuccinate, the only soluble derivative used.

TABLE 2. ANTI-ANAPHYLACTIC EFFECT OF CORTICOSTEROID DRUGS (50 MG) GIVEN INTRAMUSCULARLY 18 HR BEFORE SHOCK

Drug	Group Protection Ratio	Standard deviation
Cortisone acetate	1·58	0·52
Hydrocortisone	1·38	0·19
Prednisone	1·36	0·42
Prednisolone	1·60	0·41
Triamcinolone	1·48	0·48
Hydrocortisone sodium hemisuccinate	2·14	0·64

POTENTIATION OF ANTI-ANAPHYLACTIC EFFECTS OF MEPYRAMINE BY CORTICOSTEROIDS

Each drug was tested in a group of ten animals. The protective effect of mepyramine administered 1 hr before anaphylaxis *in vivo* was determined for each group. One week later the animals were exposed to antigen without pretreatment to ascertain that the mepyramine had had no lasting effects and then another week was allowed before the determination of the effects of the double pretreatment with mepyramine (1 hr) and the corticosteroid drug (18 hr).

TABLE 3. COMPARISON OF ANTI-ANAPHYLACTIC ACTIVITY OF CORTICOSTEROIDS (50 MG) GIVEN 18 HR BEFORE SHOCK PLUS MEPYRAMINE (1 MG/KG) GIVEN 1 HR BEFORE SHOCK

Drug	Mepyramine only		Drug plus mepyramine		
	Group Protection Ratio	Standard deviation	Fully protected animals	Group Protection Ratio of remainder	Standard deviation
Cortisone acetate	4·42	1·44	3	7·13	2·93
Hydrocortisone sodium hemisuccinate	2·47	1·14	3	5·35	1·96
Prednisolone	4·53	1·55	2	6·44	1·41
Prednisone	4·02	1·38	2	5·98	2·13
Triamcinolone	3·41	1·02	1	5·20	2·13
Hydrocortisone	3·42	1·06	1	4·36	1·39

Table 3 shows the mean "protection ratio" obtained with mepyramine in each group, together with the mean "protection ratio" obtained after pretreatment with steroid and mepyramine.

Most of the compounds potentiated the effects of mepyramine. Those showing the greatest activity were cortisone acetate and hydrocortisone sodium hemisuccinate.

DETERMINATION OF OPTIMUM TIME BETWEEN PRETREATMENT WITH HYDROCORTISONE SODIUM HEMISUCCINATE AND EXPOSURE TO ANTIGEN

Twenty animals whose "normal collapse times" had been determined, were divided into five groups of four so that sensitivity to antigen was approximately the same for each group. The groups were pretreated with hydrocortisone sodium hemisuccinate 1, 6, 12, 18 and 24 hr respectively before exposure to antigen. All animals were also treated with mepyramine. The mean "protection ratio" for each group was respectively 4, 5·2, 7·4, 9·5 and 4·2. Thus the potentiation of mepyramine reached a maximum in animals pretreated with hydrocortisone 18 hr before exposure to antigen.

ANTAGONISM OF HISTAMINE AND SRS–A BY HYDROCORTISONE SODIUM HEMISUCCINATE

The effects of hydrocortisone sodium hemisuccinate on the responses of the guinea-pig ileum to histamine and SRS–A are shown in Figs 1 and

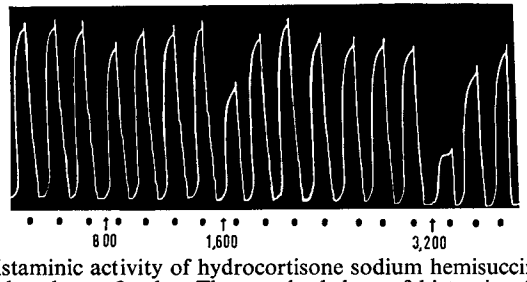

FIG. 1. Antihistaminic activity of hydrocortisone sodium hemisuccinate on guinea-pig ileum. Bath volume, 2 ml. The standard dose of histamine (●) was 0·2 μg The amounts below arrows are μg/ml of hydrocortisone sodium hemisuccinate added 30 sec before the next dose of histamine. Contact time for histamine = 30 sec and for hydrocortisone = 60 sec. Drum speed = 16 mm/min. Dose interval = 3 min.

2. The inhibitory effects of hydrocortisone sodium hemisuccinate on the responses to histamine and SRS–A of the guinea-pig tracheal chains are shown in Figs 3 and 4.

From the graphical relationship of the dose and the percentage inhibition of a reproducible response it was concluded that on the ileum there were indications of a preferential antagonism of SRS–A. There was no such preferential antagonism on the tracheal chain preparations.

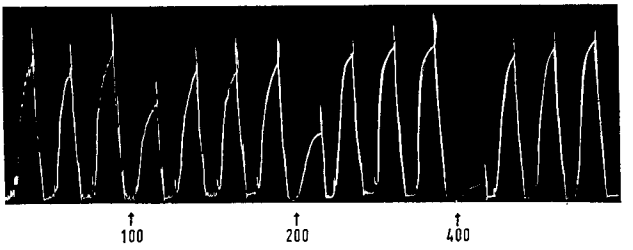

FIG. 2. Anti-SRS-A activity of hydrocortisone sodium hemisuccinate on guinea-pig ileum. The standard dose of SRS-A was two units. The amounts below arrows are mg/ml of hydrocortisone added 30 sec before the next dose of SRS–A. Contact time for SRS-A = 90 sec and for hydrocortisone = 120 sec. Drum speed = 8 mm/min. Dose interval = 5 min.

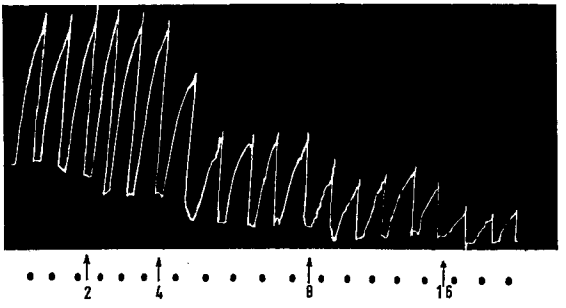

FIG. 3. Antihistaminic activity of hydrocortisone sodium hemisuccinate on the guinea-pig tracheal chain. Standard dose of histamine = 2 μg. The amounts below arrows are mg/ml of hydrocortisone added 1 min before the next dose of histamine. Contact time for histamine = 5 min and for hydrocortisone = 6 min Drum speed = 2 mm/min. Dose interval = 10 min.

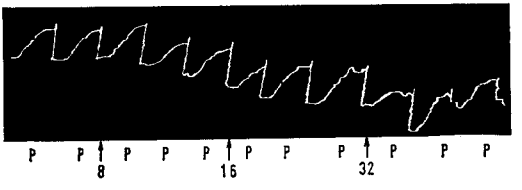

FIG. 4. Anti-SRS-A activity of hydrocortisone sodium hemisuccinate on the guinea-pig tracheal chain. P = 80 units of SRS–A. Amounts below arrows are mg/ml of hydrocortisone added 1 min before the next dose of SRS-A. Contact time for SRS-A = 10 min and for hydrocortisone = 11 min. Drum speed = 1 mm/min. Dose interval = 30 min.

EFFECTS OF HYDROCORTISONE SODIUM HEMISUCCINATE ON RELEASE OF CHEMICAL MEDIATORS OF ANAPHYLAXIS

The mean yields of histamine and SRS–A of the control and pretreated subgroups are shown in Fig. 5.

Pretreatment with hydrocortisone sodium hemisuccinate did not alter the amount of histamine but the same pretreatment reduced the amount of SRS–A released during anaphylaxis *in vitro*.

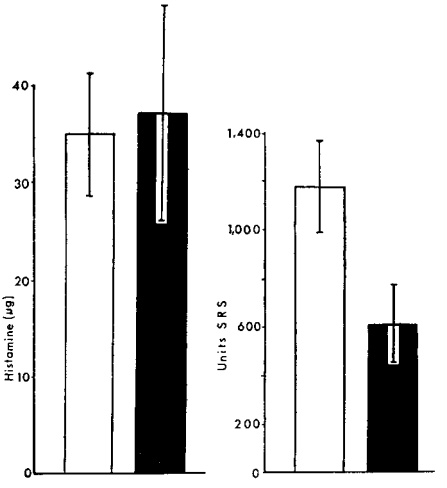

FIG. 5. Effect of pretreatment with hydrocortisone sodium hemisuccinate on the release of chemical mediators of anaphylaxis. The columns represented the mean yields of histamine and SRS-A from sensitised guinea-pig lungs shocked *in vitro*. Open columns, mean yields from untreated animals. Shaded columns, mean yields from animals pretreated 18 hr before shock with 50 mg/animal of hydrocortisone sodium hemisuccinate intramuscularly. The standard deviation of each mean is shown.

Discussion

The observation that corticosteroid drugs have little anti-anaphylactic activity when administered as a single injection to actively sensitised guinea-pigs before exposure to aerosolised antigen is in agreement with the findings of Herxheimer & Rosa (1952) for cortisone. However these results do not invalidate those of Feinberg & others (1953) who elicited a less severe reaction and who expressed results in a manner which emphasised a small degree of protection. Since the time at which maximum potentiation of the effects of mepyramine by hydrocortisone coincides with the optimum time of administration of cortisone as found by Feinberg & others, it is possible that mepyramine may magnify a reaction which is already present.

None of the drugs used in this investigation produced a greater level of protection than cortisone despite reports of greater anti-inflammatory potency. Thus it would appear that anti-inflammatory potency is not related to the anti-anaphylactic activity of the drugs. In view of the

difference between hydrocortisone administered as a fine suspension and as an aqueous solution of its sodium salt, water solubility would seem to be an important factor influencing anti-anaphylactic activity as studied under these conditions.

Although hydrocortisone can antagonise the smooth muscle stimulant actions of both histamine and SRS–A, large doses are needed and it is unlikely that its anti-anaphylactic activity observed 18 hr after intramuscular administration can be the result of such an antagonism.

The same dose of hydrocortisone as that used *in vivo* reduced the release of SRS–A during subsequent anaphylaxis *in vitro* to a level corresponding to about 50% of that found in the untreated animals without influencing the amount of histamine released. This observation may provide an explanation for the *in vivo* results, if it is assumed that the depression of SRS–A release was not apparent until the actions of histamine had been suppressed with mepyramine.

That the anti-anaphylactic action of hydrocortisone reached a maximum 18 hr after intramuscular injection suggests that the action may be due to an alteration in tissue metabolism. Goadby & Smith (1962) have reported that hydrocortisone, in the same dosage and under the *in vivo* conditions quoted in this paper, protected actively sensitised guinea-pigs from changes in the lipid metabolism of their lung tissue which were normally induced by anaphylaxis. Thus it is possible that the depression of the release of SRS–A may be related to such a metabolic action.

References

Brocklehurst, W. E. (1960). *J. Physiol.*, **151**, 416–435.
Castillo, J. C. & de Beer, E. J. (1947). *J. Pharmacol.*, **90**, 104–109.
Dworetsky, M., Code, C. F. & Higgins, G. 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.
Friedlander, S. & Friedlander, A. S. (1950). *Ibid.*, **21**, 303–309.
Goadby, P. & Smith, W. G. (1962). *J. Pharm. Pharmacol.*, **14**, 739–745.
Herxheimer, H. (1952). *J. Physiol.*, **177**, 251–255.
Herxheimer, H. & Rosa, L. (1952). *Ibid.*, **118**, 7P.
Krebs, A. H. & Hensleit, K. (1932). *Z. Phys. Chem.*, **210**, 33.
Leger, J., Leith, W. & Rose, B. (1948). *Proc. Soc. exp. Biol. N.Y.*, **69**, 529–531.
Malkiels, S. (1951). *J. Immunol.*, **66**, 379–384.
Marquis, V. O. & Smith, W. G. (1963). *J. Pharm. Pharmacol.*, **15**, 652–659.
Smith, W. G. (1961). *Ibid.*, **13**, 1–11.
Smith, W. G. (1962). *Life Sciences*, **1**, 133–140.