J. Pharm. Pharmac., 1968, **20**, 497–504 Received December 22, 1967

The effects of drug-induced adrenocortical deficiency and of mineralocorticoid drugs on anaphylaxis in the guinea-pig

R. HICKS

Administration of adrenocortical inhibitors [metyrapone, amphenone-B, or 1,1-dichloro-2,2-di(*p*-chlorophenyl)ethane (DDD)] decreased anaphylactic preconvulsion times in guinea-pigs. Simultaneous corticotrophin treatment potentiated the effect of metyrapone. Metyrapone induced adrenocortical deficiency, and changed the character of corticosteroid secretion from predominantly glucocorticoid to mineralocorticoid. Mineralocorticoid treatment decreased preconvulsion times. Mepyramine prevented the effect of mineralocorticoids on anaphylaxis but did not modify the effect of metyrapone which probably resulted from glucocorticoid lack, but mineralocorticoid secretion may have contributed. Mineralocorticoids probably potentiated the histaminic component of anaphylaxis.

IN 1922, Kepinow claimed that an almost complete bilateral adrenalectomy increased the severity of guinea-pig anaphylaxis, while Banting & Gairns (1926) reported that adrenalectomy decreased the resistance of guinea-pigs to administered histamine. Gross & Haefeli (1952) used adrenalectomized animals maintained with deoxycortone, and demonstrated increased severity of anaphylactic reactions. On the other hand, Benaim, Feinberg & Sternberger (1955) were unable to show any increase in the severity of reactions to aerosols of either antigen or histamine, in hypersensitive guinea-pigs which were stated to have been completely, bilaterally, adrenalectomized. Moreover, Takaishi (1935) and Bongiovanni (1957) have claimed that adrenalectomy decreased the severity of anaphylactic and histamine shocks in this species.

A possible reason for the contradiction may lie in the technical difficulties of performing complete bilateral adrenalectomy in the guinea-pig. It is now possible to induce adrenocortical deficiency by administration of drugs which inhibit corticosteroid secretion. I have used some compounds of this nature to investigate the effects of adrenocortical deficiency on anaphylaxis in the guinea-pig.

Experimental

MATERIALS AND METHODS

Virgin female albino guinea-pigs of the Dunkin Hartley strain, weighing 300–500 g, and maintained on unrestricted supplies of water, and diet S.G.I. (Oxoid) supplemented by hay and green vegetables, were used. They were housed in well ventilated conditions, with the temperature regulated at 65° F.

Active hypersensitivity was induced by single intraperitoneal injections of crystalline egg albumen (50 mg/kg) in aqueous solution. Reactions were induced 28 days after sensitization.

Antigen-aerosol induced anaphylaxis. Sensitized animals were placed in

From the Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, 7, England.

a closed chamber and exposed to an aerosol of 5% egg albumen solution. The aerosol was produced by a "Wright" nebulizer using compressed air at a pressure of 10 lb/inch^2. The severity of the resultant anaphylactic reaction was assessed according to the method of Herxheimer (1952). Evaluation was in terms of the "preconvulsion time," i.e. the time of exposure required to elicit consistently recognizable signs of the onset of respiratory distress, such that the immediate removal of the animal from the aerosol would avert convulsions and death. Unless otherwise stated all experiments were made using groups of 5 animals, and results were taken as the mean values of individual preconvulsion times. Effects of drug treatment were evaluated by comparison with control groups treated with the inert vehicle used in drug administration.

Experimental and results

EFFECTS OF REPEATED DAILY ADMINISTRATION OF DRUGS INHIBITING ADRENOCORTICAL SECRETIONS

Daily intramuscular doses of either 1,1-dichloro-2,2-di(*p*-chlorophenyl)-ethane (DDD) (50 mg/kg), amphenone-B (200 mg/kg) or metyrapone (200 mg/kg) were administered to groups of sensitized guinea-pigs and the animals were exposed to the antigen aerosol after either 48 hr or one week of treatment. Control groups received corresponding treatment with arachis oil, or saline, and were exposed to the antigen aerosol after similar periods. Preconvulsion times were noted (Table 1).

TABLE 1. EFFECTS OF REPEATED DAILY ADMINISTRATION OF INHIBITORS OF ADRENOCORTICAL SECRETION ON ANAPHYLACTIC PRECONVULSION TIMES IN THE GUINEA-PIG. Preconvulsion times expressed as means for groups of five animals ± standard errors.

Treatment (daily intramuscular injections)	Preconvulsion times (sec) (means ± s.e.)	
	48 hr	1 week
DDD (50 mg/kg)	35·0 ± 2·0	17·8 ± 2·9
Controls	46·2 ± 2·2*	49·2 ± 2·5*
Amphenone-B (200 mg/kg) ..	35·5 ± 4·2	39·8 ± 3·8
Controls	53·6 ± 4·0*	47·4 ± 2·7
Metyrapone (200 mg/kg) ..	42·8 ± 3·5	39·0 ± 3·8
Controls	63·4 ± 5·4*	56·4 ± 2·9*

* Denotes significant difference $P < 0.05$.

The repeated administration of either DDD, amphenone-B, or metyrapone over 48 hr caused significant decreases in anaphylactic preconvulsion times compared with the control groups. Preconvulsion times were significantly decreased after treatment with DDD or metyrapone for one week, but not after amphenone-B.

Guinea-pigs undergoing these treatments for one week were weighed initially and then immediately before exposure to the antigen aerosol. After induction of anaphylactic reactions the animals were killed and the adrenal glands removed and weighed. The effects of the treatment on body weight and upon adrenal gland weights are in Table 2. Animals treated with DDD had decreased body weight and weight of adrenal glands. The mean weights of adrenal glands of animals receiving either

TABLE 2. EFFECTS OF REPEATED DAILY ADMINISTRATION OF ADRENOCORTICAL INHIBITORS ON TOTAL BODY WEIGHT AND ADRENAL GLAND WEIGHT IN THE GUINEA-PIG. Each value represents the mean weight from a group of five animals.

Treatment (daily intramuscular injections)	Mean total body weight (g)			Adrenal weight (g)	
	Initial	Final	% change	Mean	% of control
DDD (50 mg/kg)	432	421	−2·5	0·43	87
Controls	445	469	+5·4	0·49	
Amphenone-B (200 mg/kg) ..	391	398	+1·8	0·54	110
Controls	421	444	+5·7	0·49	
Metyrapone (200 mg/kg) ..	426	427	+0·2	0·65	118
Controls	420	444	+5·7	0·52	

amphenone-B or metyrapone were greater than those of the controls, but body weight increases were less. When examined before exposure to the antigen aerosol, the animals treated with adrenal inhibitors gave the subjective impression of being in poor physical condition, showing muscular weakness by apparent lack of limb and posture control, with dirtiness and lack of sheen of the coat. This evidence was taken as a general indication that effective adrenocortical inhibitor action had been produced.

TIME-COURSE OF THE EFFECT OF REPEATED ADMINISTRATION OF METYRAPONE

Metyrapone (200 mg/kg, s.c.) was administered at 12-hrly intervals to groups of sensitized guinea-pigs, and the animals were exposed to the antigen aerosol at 6, 12, 24, 48 or 96 hr after treatment began. Corresponding saline-treated control groups were exposed after similar times. Preconvulsion times are in Table 3. Repeated administration of metyrapone produced a gradual decrease in anaphylactic preconvulsion times, significant differences being apparent after 48 and 96 hr.

TABLE 3. THE EFFECTS OF REPEATED ADMINISTRATION OF METYRAPONE (200 MG/KG, S.C. AT 12 HR INTERVALS) ON ANAPHYLACTIC PRECONVULSION TIMES OF SENSITIZED GUINEA-PIGS. Mean results from groups of five animals ± standard errors.

Duration of treatment (hr)	Preconvulsion times (sec) (mean ± s.e.)		% decrease
	Control	Test	
6	57·6 ± 2·8	56·8 ± 2·2	1·4
12	45·8 ± 1·4	40·8 ± 2·7	10·9
24	67·6 ± 4·3	61·2 ± 2·9	9·4
48	66·1 ± 4·7	52·8 ± 3·0	20·0*
96	48·5 ± 2·3	33·0 ± 4·0	32·0*

* Denotes significant differences $P < 0·05$

EFFECT OF METYRAPONE IN COMBINATION WITH CORTICOTROPHIN

With an interval of 12 hr, two subcutaneous doses of either metyrapone (200 mg/kg) alone, corticotrophin (10 u/kg) alone, or both simultaneously, were administered to groups of sensitized guinea-pigs. Twelve hr after the last injection, the animals were exposed to the antigen aerosol, and preconvulsion times were measured. Saline-treated control groups were similarly exposed. Results are in Table 4.

TABLE 4. THE EFFECTS OF METYRAPONE (200 MG/KG, 2 × 12 HRLY, S.C.) AND CORTICOTROPHIN (10 U/KG, 2 × 12 HRLY S.C.) ON ANAPHYLACTIC PRECONVULSION TIMES IN THE GUINEA-PIG. Preconvulsion times expressed as mean ± standard error.

Treatment	Number of animals	Preconvulsion times (sec) (mean ± s.e.)	
		Control	Test
Metyrapone	5	53·6 ± 3·8	47·0 ± 1·2
ACTH	6	46·1 ± 5·5	48·6 ± 5·1
Metyrapone + ACTH	5	53·6 ± 3·8	37·8 ± 4·9*

* Denotes significant difference $P < 0.05$.

No significant difference from the controls was observed in animals treated with metyrapone or with corticotrophin alone. However, animals treated with metyrapone and corticotrophin showed a further and significant decrease in preconvulsion times, thus indicating a potentiating effect of the combination.

EFFECTS OF MINERALOCORTICOID DRUGS

Groups of sensitized guinea-pigs treated with single doses of either deoxycortone (10 mg/kg, i.m.), cortexolone (5 mg/kg, i.m.) or aldosterone (1·0 mg/kg, i.m.), were exposed to the antigen aerosol after 4, 8, 12 or 24 hr. Preconvulsion times for these and for corresponding saline or arachis oil treated control groups are in Table 5.

TABLE 5. ANAPHYLACTIC PRECONVULSION TIMES OF SENSITIZED GUINEA-PIGS AT VARIOUS TIMES AFTER ADMINISTRATION OF SINGLE DOSES OF DEOXYCORTONE (10·0 MG/KG, I.M.), CORTEXOLONE (5·0 MG/KG, I.M.) ALDOSTERONE (1·0 MG/KG, I.M.) OR FLUDROCORTISONE (4·0 MG/KG, I.M.). Results are expressed as mean values for groups of five animals ± standard errors.

Treatment	Interval (hr)	Preconvulsion times (sec) (mean ± s.e.)	
		Control	Test
Deoxycortone	4	50·4 ± 2·4	36·0 ± 2·6*
	8	52·6 ± 3·8	40·1 ± 1·2*
	12	46·5 ± 7·8	44·1 ± 5·6
	24	49·2 ± 8·1	47·0 ± 9·8
Cortexolone	4	41·1 ± 3·4	26·5 ± 3·3*
	8	47·1 ± 3·2	38·2 ± 5·0
	12	50·5 ± 8·3	54·2 ± 8·3
Aldosterone	4	54·2 ± 4·1	35·8 ± 3·4*
	8	45·6 ± 1·8	44·7 ± 2·1
	12	37·0 ± 6·7	33·0 ± 1·8
	24	42·5 ± 3·2	43·8 ± 2·7
Fludrocortisone	4	32·2 ± 1·9	32·5 ± 3·1
	18	37·2 ± 3·1	58·2 ± 5·6*

* Denotes significant difference $P < 0.05$.

In animals exposed to the antigen 4 hr after these mineralocorticoid drugs, preconvulsion times were significantly lower. Such a decrease was also observed after 8 hr in deoxycortone-treated animals. No significant differences were observed after longer times.

Single intramuscular injections of fludrocortisone (4 mg/kg) were administered to sensitized guinea-pigs and anaphylactic reactions were

induced 4 or 18 hr later. Preconvulsion times (Table 5) were compared with those from sensitized animals treated with the injection vehicle.

No significant difference in preconvulsion times resulted from fludrocortisone treatment 4 hr before the reaction. In the guinea-pigs exposed 18 hr after fludrocortisone there was a significant increase in preconvulsion times.

EFFECTS OF MEPYRAMINE ON ANIMALS TREATED WITH METYRAPONE OR CORTICOSTEROIDS

Six sensitized guinea-pigs were treated with 4 subcutaneous doses of metyrapone (200 mg/kg) at 12 hrly intervals, and each animal was exposed to the antigen aerosol 12 hr after the last dose. Other groups of six animals were treated with single doses of aldosterone (1·0 mg/kg, i.m.), or cortexolone (5·0 mg/kg, i.m.), and exposed to the antigen aerosol 4 hr after treatment. One group of animals was treated with fludrocortisone (4 mg/kg, i.m.), and anaphylactic reactions were induced 18 hr later. In all of these animals a single subcutaneous dose of mepyramine (1·0 mg/kg) was administered 1 hr before exposure to the aerosol. Preconvulsion times were recorded, and compared with those from corresponding mepyramine-treated control animals (Table 6).

TABLE 6. THE EFFECTS OF METYRAPONE AND CORTICOSTEROIDS ON ANAPHYLACTIC PRECONVULSION IN MEPYRAMINE-TREATED GUINEA-PIGS (1·0 MG/KG, S.C., 1 HR). Six animals per group.

Treatment	Preconvulsion times (means ± s.e.) (sec)		% change
	Control mepyramine alone	Test	
Metyrapone (4 × 12 hrly) 200 mg/kg, s.c. ..	189 ± 17	125 ± 15	33·9 decrease*
Aldosterone (4 hr) 1·0 mg/kg	151 ± 23	133 ± 15	12·0
Deoxycortone (4 hr) 10·0 mg/kg	151 ± 23	118 ± 13	21·8
Cortexolone (4 hr) 5·0 mg/kg	151 ± 23	168 ± 18	11·2
Dexamethasone (18 hr) 4·0 mg/kg	177 ± 14	409 ± 49	131·8* prolongation
Fludrocortisone (18 hr) 4·0 mg/kg	177 ± 14	340 ± 34	92·2* prolongation

* Denotes significant difference $P < 0.05$.

Preconvulsion times of animals treated with aldosterone, cortexolone or deoxycortone, followed by mepyramine, were not significantly different from animals receiving mepyramine alone. In metyrapone-treated guinea-pigs given mepyramine, preconvulsion times were significantly decreased. On the other hand, the mean preconvulsion time of the groups treated with mepyramine and fludrocortisone was significantly longer than that of the control group treated with mepyramine alone. In this case the prolongation of preconvulsion time by fludrocortisone previously administered alone, was much enhanced by mepyramine.

Thus mepyramine prevented the decreases in preconvulsion time caused by aldosterone, cortexolone and deoxycortone, but did not modify the effect of metyrapone. It potentiated the prolongation of preconvulsion times by fludrocortisone.

Discussion

A survey of the literature indicated that a protective effect of corticosteroid drugs may be demonstrated using preconvulsion-time methods for evaluation of aerosol-induced anaphylaxis (Herxheimer & Rosa, 1952; Mendes, 1957; Goadby & Smith, 1964; Gorog & Szporny, 1965).

Adrenalectomy results in increased severity of anaphylactic reactions in many species, including the rabbit, dog, rat and mouse (Rose, 1959). The lack of clear-cut evidence that adrenalectomy has a similar result in the guinea-pig is therefore anomalous. It would be expected that a lack of adrenocortical secretion, a restraining influence on anaphylaxis in the normal animal, should similarly potentiate anaphylactic severity.

The experiments have shown that the repeated daily administration of either DDD or metyrapone for 48 hr or one week increased the severity of anaphylaxis, as indicated by significantly shortened preconvulsion times. The degree of adrenocortical deficiency was not evaluated but during these treatments signs consistent with such deficiency were observed: poor condition, possible muscular weakness, and particularly the failure to gain body weight. Furthermore, the mean weights of adrenal glands in DDD-treated animals were less than, whereas those from metyrapone-treated animals were more than, the corresponding controls. Inhibition of corticosteroid secretion by DDD treatment is associated with adrenal cortical atrophy (Nelson & Woodward, 1947; Nichols & Sheehan, 1952), whereas inhibition of corticosteroid secretion by metyrapone treatment is associated with adrenal hypertrophy (Chart & Sheppard, 1959).

A decrease in anaphylactic preconvulsion times was also observed after treatment with amphenone-B for two days, but was less marked after its administration for one week. This compound is a potent inhibitor of adrenocortical secretion in most species, but its effects are less potent in the guinea-pig (Chart & Sheppard, 1959) and it has a wide spectrum of biological activity. It is possible, therefore, that its effect on adrenocortical secretion is masked for instance by its potent antithyroid properties, which may be expected to decrease anaphylactic severity.

The one common property of DDD, metyrapone and amphenone-B is the inhibition of corticosteroid secretion, and it is therefore probable that this is the cause of the decreased anaphylactic preconvulsion times.

The present findings have shown that corticotrophin potentiated the effects of metyrapone, whereas corticotrophin administered alone had no such effect. Chart & Sheppard (1959) have shown that the inhibition of secretion of all 17-hydroxycorticoids by metyrapone, and the consequent increase in adenohypophysial corticotrophin secretion, results in adrenal hypertrophy. The increased corticotrophin levels also further stimulate corticosteroid biosynthesis. Metyrapone basically inhibits 11β-hydroxylation of corticosteroids in the final stages of biosynthesis, so that under the influence of corticotrophin an accumulation and secretion of steroid precursors, the 11-desoxysteroids cortexolone and deoxycortone, occurs. The largely glucocorticoid nature of the normal adrenocortical secretion is thus replaced by steroids with a predominantly mineralocorticoid nature.

The effects of administration of mineralocorticoid substances on anaphylaxis were therefore investigated, because an increase in this type of activity could contribute to the potentiation of the effects of metyrapone by corticotrophin. Mineralocorticoids were found to potentiate anaphylaxis.

Investigations of the normal secretion from the guinea-pig adrenal cortex indicate that the predominant secretion is hydrocortisone (Bush, 1962) and resting blood concentrations of corticosteroids in this species are higher than most other mammals (Done, Ely, & others, 1952). Thus the decreased anaphylactic preconvulsion times caused by metyrapone in the present work could be explained by a deficiency of glucocorticoid secretion.

Under the influence of metyrapone the anaphylaxis-potentiating effect of the mineralocorticoids which replace the predominantly glucocorticoid hydrocortisone secretion, may be expected to contribute further to the effect on anaphylaxis. It is of considerable interest that a large dose of the potent mineralocorticoid fludrocortisone failed to decrease anaphylactic preconvulsion times after 4 hr. But this compound, which also possessed marked glucocorticoid and anti-inflammatory properties, significantly prolonged preconvulsion times of reactions induced 18 hr after its administration. Significant anti-anaphylactic effects of potent anti-inflammatory steroids are observed when these are administered an optimal 18 hr before anaphylaxis (Goadby & Smith, 1964; Feinburg & Malkiel, 1952).

The combined effects of fludrocortisone and mepyramine much prolonged anaphylactic preconvulsion times, and the result was greater than that of a summation of their individual effects. This is comparable with the findings of Winter & Flataker (1955), and Goadby & Smith (1964), who showed that cortisone and related compounds had only small protective effects against anaphylaxis in the guinea-pig, but markedly potentiated mepyramine. This is further evidence that the observed effects of fludrocortisone are related to its glucocorticoid-anti-inflammatory activity rather than its mineralocorticoid properties. In contrast mepyramine pretreatment prevented the decrease in preconvulsion times caused by the mineralocorticoids.

In the mepyramine-treated animals it may be assumed that the contribution of histamine to the total anaphylactic response is so reduced that the observed reaction is mediated largely by the non-histamine component (Goadby & Smith, 1964). Thus any effects of corticosteroid treatment in such animals may be considered to arise from an influence on the residual response induced by SRS-A and probably other active materials. As pretreatment with mepyramine prevented the decrease in preconvulsion times resulting from mineralocorticoid administration, it is suggested that these steroids influenced mainly the histaminic component of the anaphylactic reaction. Such effects may be related to the increase in tissue histamine levels accompanying the administration of mineralocorticoids in the guinea-pig (Hicks, 1965).

Treatment of the guinea-pigs with repeated injections of metyrapone, followed by a single dose of mepyramine, resulted in a significant decrease

in anaphylactic preconvulsion times, and it would thus appear that at least part of the effect of metyrapone is exerted on the residual non-histaminic component. This evidence is consistent with a suggestion that the main effect of metyrapone results from deficiency of the normal predominantly glucocorticoid secretion. The relative dimensions of any contribution due to the replacement of this secretion by mineralocorticoids is difficult to assess, without a more precise knowledge of actual levels of secretion. The potentiation of the metyrapone action by corticotrophin suggests that the mineralocorticoid contribution could be an important component of the overall effect. It is of interest to note that administration of metyrapone provoked an increase in tissue histamine levels in the guinea-pig (Kovacs, 1965; Hicks, 1965), and that this effect is potentiated by simultaneous administration of corticotrophin.

Acknowledgements. I wish to express my gratitude to Professor G. D. H. Leach for help and encouragement received during this work. I also wish to acknowledge valuable technical assistance provided by Miss L. M. Taylor and Messrs. B. Emmett and K. Wilkinson.

References

Banting, F. G. & Gairns, S. (1926). *Am. J. Physiol.*, **77**, 100–112.
Benaim, C., Feinberg, S. M. & Sternberger, L. A. (1955). *J. Allergy*, **26**, 481–489.
Bongiovanni, M. (1957). *Ormonologia*, **17**, 285–299.
Bush, I. (1962). *Pharmac. Rev.*, **14**, 317–445.
Chart, J. J. & Sheppard, H. (1959). *J. mednl pharm. Chem.*, **1**, 407–441.
Done, A. K., Ely, R. S., Raile, R. B. & Kelley, V. C. (1952). *Proc. Soc. exp. Biol. Med.*, **81**, 667–669.
Feinberg, S. M. & Malkiel, S. (1952). *Ibid.*, **81**, 104–105.
Goadby, P. & Smith, W. G. (1964). *J. Pharm. Pharmac.*, **16**, 108–114.
Gorog, P. & Szporny, L. (1965). *Ibid.*, **17**, 250–251.
Gross, F. & Haefeli, H. (1952). *Int. Archs Allergy appl. Immun.*, **3**, 44–53.
Herxheimer, H. (1952). *J. Physiol., Lond.*, **117**, 251–255.
Herxheimer, H. & Rosa, L. (1952). *Ibid.*, **118**, 7P.
Hicks, R. (1965). *Br. J. Pharmac. Chemother.*, **25**, 664–670.
Kepinow, L. (1922). *C.r. Séanc. Soc. Biol.*, **87**, 327–329.
Kovacs, E. M. (1965). *Br. J. Pharmac. Chemother.*, **24**, 574–578.
Mendes, E. (1957). *Acta allerg.*, **11**, 181–187.
Nelson, A. H. & Woodward, G. (1949). *Archs Path.*, **48**, 387–394.
Nichols, J. & Sheehan, H. L. (1952). *Endocrinology*, **51**, 362–377.
Rose, B. (1959). In *Mechanisms of Hypersensitivity*, Boston, Mass: Little Brown & Co.
Takaishi, T. (1935). *Sei-i-Kwai med. J.*, **53**, 7–8.
Winter, C. A. & Flataker, L. (1955). *J. exp. Med.*, **101**, 17–24.