THE PHARMACOLOGICAL PROPERTIES OF GLYCYRRHETINIC ACID—A NEW ANTI-INFLAMMATORY DRUG

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A study of the pharmacological properties of glycyrrhetinic acid, or glycyrrhetic acid, a new anti-inflammatory drug from liquorice, shows it has an extremely low toxicity and is non-irritant to the skin. It has no adverse effects on the heart, circulation or respiration and shows no glucocorticoid-like activity. In large doses in animals it produces water retention, slight sodium retention and an increased excretion of potassium. These effects are not seen with smaller doses used in man.

GLYCYRRHETINIC or glycyrrhetic acid, a triterpenoid obtained from liquorice, has been proved to be an anti-inflammatory agent[1]. The toxicological properties and pharmacodynamics of this drug are now described. Previously there have been no detailed reports on the pharmacology of this compound, most of the published literature referring to liquorice extract and glycyrrhizin. Molhuysen and others[2] found that a liquorice extract had a deoxycortone-like action, promoting the retention of sodium and water and increasing the excretion of potassium in normal persons. Groen and others[3] reported that liquorice and glycyrrhizinic acid maintained two patients with Addison's disease in correct electrolyte balance and a similar result was obtained by Calvert[4]. Pelser and others[5] found that glycyrrhetinic acid was more effective than glycyrrhizinic acid in this condition.

Liquorice extract was ineffective in one severe case of Addison's disease which had previously shown no response to ACTH[2]. While glycyrrhetinic acid potentiated the action of cortisone[6], alone, it was unable to maintain adrenalectomised patients. Glycyrrhizin also was unable to effect adequate maintenance of the patient with bilateral adrenalectomy[7].

Although glycyrrhetinic acid was thought to have deoxycortone-like actions, Galal[8] has shown that its antidiuretic action differs from that of deoxycortone in rats. Glycyrrhetinic acid has no glucocorticoid-like activity and Hems[9] showed it to be inactive in the mouse liver glycogen test. Recently Atherden[10] found glycyrrhetinic acid to inhibit the metabolism of progesterone and 11-deoxycorticosterone by rat-liver homogenates.

Materials. The glycyrrhetinic acid (Fraction "S") used in these investigations was supplied by Biorex Laboratories Ltd. It was used in the form of tablets and as a saline suspension.

METHODS

Acute Toxicity

The acute toxicity of glycyrrhetinic acid was determined on albino mice of both sexes. Injections were made on a weight basis into animals

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weighing between 18 and 22 g. which had fasted overnight. For all routes of administration, where possible, the regression of mortality per cent as probits on the logarithm of the dose was found and the LD50 and limits of error (P = 0·95) calculated by the method of Finney[11].

Subacute Toxicity

The subacute toxicity of glycyrrhetinic acid was determined on young rats, which were injected intramuscularly three times a week for 4 weeks. Their weights were determined twice weekly and finally the rats were killed and examined pathologically. Histological sections of the major organs were prepared. The adrenal glands were weighed and frozen sections stained for lipid with Sudan III.

Dermal Toxicity

This was determined in rabbits as described under the "Procedures for the Appraisal of the Toxicity of Chemicals in Foods, Drugs and Cosmetics"[12]. The primary irritation of the skin was measured by an examination of the skin of an albino rabbit after treatment with glycyrrhetinic acid as follows. Small pellets of cotton-wool were saturated with 0·5 ml. of a suspension of glycyrrhetinic acid containing 100 mg./ml. Three of these saturated pellets were then fixed with adhesive plaster to the previously shaven skin of a rabbit's back. The trunk of the animal was then wrapped in a plastic film to minimise evaporation. The skin underneath the pellets was examined after 24 and 72 hours. In another rabbit the skin was abraded before the pellets were applied.

Pharmacodynamics

The pharmacological effects of glycyrrhetinic acid on the cardiovascular system, and on the central and autonomic nervous systems, were studied in mice, rats and anaesthetised cats. The cats were anaesthetised with chloralose (80 mg./kg.), the blood pressure was recorded from the carotid artery and the respiration was recorded from a tracheal cannula by the method described by Paton[13]. Glycyrrhetinic acid, having a low water solubility, could not be injected intravenously, therefore it was injected intraperitoneally or directly into the duodenum. The effects on gastrointestinal motility were studied *in vitro* on the isolated duodenum of the rabbit, and *in vivo* by the transport of a charcoal meal in mice as described by Bryant and others[14].

Urinary System

The effects of glycyrrhetinic acid on the secretion of urine and the excretion of sodium and potassium were studied in rats and anaesthetised cats. An experiment was also made in student volunteers. After an injection of glycyrrhetinic acid, rats were given 10 ml. of water orally per 100 g. body weight and randomly distributed into groups of three or six and placed into metabolism cages. Control groups were given saline. The urine was collected and measured hourly over 5 hours and the sodium and potassium estimated by flame photometry. Cats were anaesthetised

with chloralose and the bladder cannulated through the urethra for collection of the urine. After a 75 minute control period 100 mg./kg. of glycyrrhetinic acid was injected intraperitoneally. The urine was collected over another 60 minutes. The volumes of urine excreted were measured at 15 minute intervals and taken for estimation of the sodium and potassium present. Samples of blood were also taken at these times for estimation of sodium, potassium, total chloride and glycyrrhetinic acid in the serum.

Glycyrrhetinic acid was estimated in the serum by a modification of the method described by Van Katwijk and Huis in't Veld[15] for the determination of glycyrrhetinic acid in urine. Serum or plasma (0·2 ml.) was added to 0·1N sulphuric acid (1 ml.) and the mixture extracted three times with ether (3 × 2 ml.). The combined ethereal solution was then extracted with 0·5N ammonium hydroxide solution (2 ml.) and the ether layer discarded. The alkaline layer was acidified with 0·7 ml. of 2N sulphuric acid and extracted three times with ether. The combined ether extract was evaporated in a current of air and the residue dried over silica gel at 20° and 3 mm. The residue was dissolved in 3 ml. 95 per cent spectroscopically pure ethanol and the optical density measured at 248 mμ in a spectrophotometer. Serum from the same animal collected immediately before the administration of glycyrrhetinic acid was similarly extracted and the ethanolic solution of the final residue was used as a reference blank. A calibration curve was prepared by measuring the optical density of solutions of glycyrrhetinic acid in 95 per cent ethanol containing 1 to 40 μg./3 ml. The curve was linear throughout this range. The accuracy of the method was checked by adding known concentrations of glycyrrhetinic acid to a control sample of the serum. In a typical experiment in which 5·0 μg. and 10·0 μg. were added to 0·2 ml. serum samples, recoveries of 4·4 μg. (88 per cent) and 10·1 μg. (101 per cent) respectively were obtained. To ensure that the optical density at 248 mμ was specific for glycyrrhetinic acid, measurements were made over the wavelengths 230 to 270 mμ. Maximum absorption at 248 mμ was found unless the serum specimen was haemolysed, in which case non-specific absorption was observed.

The effects of an oral dose of glycyrrhetinic acid was determined in eight healthy male student volunteers in a blind cross over trial. Each student was given 0·2 g. or 0·5 g. of glycyrrhetinic acid or a dummy tablet and 30 minutes later drank 1500 ml. of water. The urine was collected at 30-minute intervals over 2½ hours and the volume was recorded.

Glucocorticoid Action

This was tested in adrenalectomised mice submitted to a cold stress[16]. Groups of 10 mice were adrenalectomised under ether anaesthesia. The following day one group was injected intraperitoneally with 170 mg./kg. of glycyrrhetinic acid and the other group with saline as the controls. Their survival times in a refrigerator at 4° were then recorded to the nearest half hour.

RESULTS

Acute Toxicity

Glycyrrhetinic acid had a low toxicity. Given orally to mice no deaths occurred following single doses as high as 610 mg./kg., which was the maximal dose that could be administered. Similarly by the subcutaneous route it was not possible to kill any mice at this dose level. By the intraperitoneal route deaths did occur over a period of 48 hours and the LD50 was 308 mg./kg. with fiducial limits of error (P = 0·95) from 279 to

TABLE I

ACUTE INTRAPERITONEAL TOXICITY OF GLYCYRRHETINIC ACID IN ALBINO MICE

Dose mg./kg.	No. of mice	Deaths (after 2 days)
216	20	3
263	20	6
320	20	10
390	20	16

LD50 = 308 mg./kg.   Fiducial limits (P = 0·95) 279 to 340 mg./kg.

340 mg./kg. (Table I). High doses caused sedation, palor of the extremities, and respiratory depression. Death was usually delayed, the mice generally dying on the second day after the administration. Pathologically there was evidence of peritonitis, probably caused by the presence of the insoluble glycyrrhetinic acid in the peritoneal cavity. Glycyrrhetinic acid could not be given intravenously because of its low solubility in water.

Subacute Toxicity

The growth of young rats was not depressed by intramuscular injections of 10 and 20 mg. of glycyrrhetinic acid three times a week. The treated rats maintained good health, ate well and grew as well as the untreated

TABLE II

THE EFFECT OF GLYCYRRHETINIC ACID ON THE EXCRETION OF WATER IN RATS

Treatment	Volume of urine excreted in ml. Hours after water administration				
	1	2	3	4	5
1. Saline controls	3·0*	7·0	8·3	8·7	9·3
2. Glycyrrhetinic acid 125 mg./kg. Water 30 min. after injection	0·8	4·2	7·2	7·3	8·0
3. Glycyrrhetinic acid 125 mg./kg. Water 2 hours after injection	3·7	7·8	8·3	8·6	9·5
4. Glycyrrhetinic acid 125 mg./kg. Water 4 hours after injection	2·5	9·2	9·5	9·8	10·0

* Each figure represents the mean volume from three rats.

controls. When killed after 4 weeks there was no evidence at postmortem of any gross pathological effects and histological sections of the major organs showed no abnormalities. There was no adrenal atrophy, as occurs with cortisone, and sections of the glands were normal except for a slight thinning of the lipid in the zona glomerulosa. This was by no means as severe as occurs with deoxycortone.

Dermal Toxicity

There was no evidence of oedema or erythema of the normal or abraded skin, proving that glycyrrhetinic acid has no primary irritant action on the skin of the rabbit.

Pharmacodynamics

Glycyrrhetinic acid had no untoward effects on the central or autonomic nervous systems, nor on the heart and circulation.

The central nervous system. This was affected only by extremely large doses of glycyrrhetinic acid. In the mouse a dose of 25 mg. (1250 mg./kg.)

TABLE III

THE EFFECT OF GLYCYRRHETINIC ACID ON THE URINARY EXCRETION OF SODIUM AND POTASSIUM IN RATS

Treatment		Sodium excretion		Potassium excretion	
		mg./5 hr.	Per cent controls	mg./5 hr.	Per cent controls
1. Saline controls		3·2*	—	3·8	—
2. Glycyrrhetinic acid 125 mg./kg.	Water 30 min. after injection	0·9	29	4·8	125
3. Glycyrrhetinic acid 125 mg./kg.	Water 2 hours after injection	3·8	117	7·7	200
4. Glycyrrhetinic acid 125 mg./kg.	Water 4 hours after injection	2·8	86	6·0	157

* Each figure represents the mean from three rats.

intraperitoneally caused sedation, hypnosis, hypothermia and respiratory depression.

The autonomic nervous system. In the mouse intraperitoneal and oral doses of 25 mg. or 12 mg. subcutaneously did not stimulate or depress
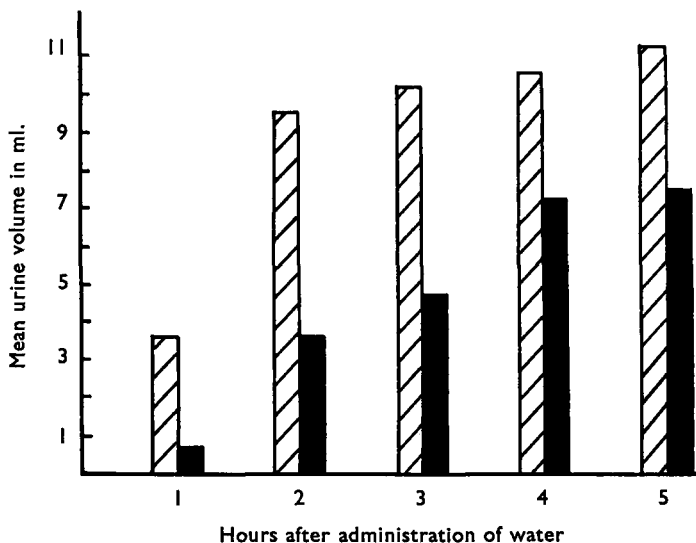

FIG. 1. The antidiuretic action of glycyrrhetinic acid (125 mg./kg. i.p.) in rats given 100 ml./kg. of water orally. Each value is the mean from six rats. Shaded area, controls; black area glycyrrhetinic acid.

either the sympathetic or parasympathetic branches of the autonomic nervous system. Similarly in the cat, an intraperitoneal dose of 125 mg./kg. did not alter the blood pressure or affect the normal responses to stimulation of the sympathetic or parasympathetic nerves. The responses

to an intravenous injection of acetylcholine, nicotine or adrenaline were normal.

The cardiovascular system. In the anaesthetised cat very large doses (125 mg./kg.) administered intraperitoneally or injected directly into the duodenu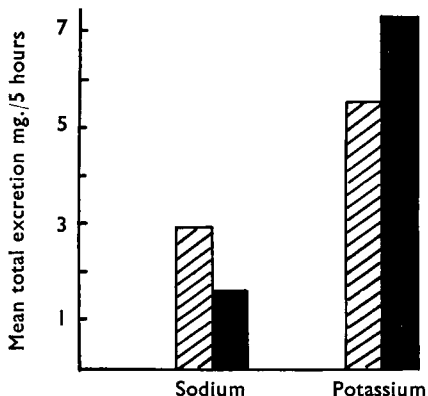m did not affect the blood pressure or the heart beat. Intravenous administration of glycyrrhetinic acid was precluded because of its low solubility.

The respiratory system. In the anaesthetised cat doses as high as 125 mg./kg., injected intraperitoneally, did not affect the depth or rate of respiration. In mice respiratory depression was only seen after toxic doses of glycyrrhetinic acid (610 mg./kg.) were given intraperitoneally.

The gastrointestinal tract. Glycyrrhetinic acid did not affect the motility of the gastrointestinal tract. *In vitro* the addition of 1 mg. of glycyrrhetinic acid to a 15 ml. bath did not affect the tone or contractions of the isolated duodenum of the rabbit. The normal responses to acetylcholine, adrenaline and barium chloride were unchanged, showing the absence of a spasmolytic action. *In vivo* the rate of transport of carbon through the stomach and intestine of the mouse was not affected when compared with untreated controls. The oral administration of glycyrrhetinic acid in rats and mice did not have a constipating action, or cause diarrhoea.

FIG. 2. The effect of glycyrrhetinic acid (125 mg./kg. i.p.) on the urinary excretion of sodium and potassium in rats. Each value is the mean from six rats. Shaded area, controls; black area, glycyrrhetinic acid.

TABLE IV

THE EFFECTS OF GLYCYRRHETINIC ACID ON THE EXCRETION OF URINE, SODIUM AND POTASSIUM IN THE CAT

Urine sample (15 min. intervals)	Volume ml.	Sodium		Potassium	
		m. eq./l.*	m. eq./15 min.	m. eq./l.	m. eq./15 min.
1	4·8	210	—	30	—
2	3·2	205	0·66	26·5	0·085
3	2·6	205	0·53	26	0·068
4	2·3	195	0·45	25·5	0·059
5	3·4	185	0·63	27·5	0·063
	Injection of 200 mg./kg. of glycyrrhetinic acid				
6	3·5	170	0·60	31·5	0·110
7	2·0	180	0·36	32	0·064
8	1·7	175	0·30	28	0·048
9	1·2	185	0·22	28	0·038

* Milliequivalents per litre urine.

The urinary system. Glycyrrhetinic acid did have an effect on kidney function in the rat. This has been examined in some detail. There was a marked antidiuretic action, which confirms the work of Galal[8], a retention of sodium and an increase in the urinary potassium excretion.

Typical results are shown in Tables II and III. In this experiment group 1, the controls, were given normal saline, while groups 2, 3 and 4 were injected intraperitoneally with 125 mg./kg. of glycyrrhetinic acid. The corresponding groups of rats were given an oral dose of 10 ml./100 g. of water either half, 2 or 4 hours after the administration of glycyrrhetinic acid. The effects on sodium and water metabolism occurred in group 2,

TABLE V

THE EFFECTS OF GLYCYRRHETINIC ACID ON SODIUM, POTASSIUM AND CHLORIDES IN THE SERUM OF THE CAT

Treatment	Glycyrrhetinic acid mg./100 ml.	m. eq./l. serum		
		Sodium	Potassium	Chlorides
A. Control(70 min. before glycyrrhetinic acid)	—	150	3·4	124
B. Control (5 min. before glycyrrhetinic acid) ..	—	151	3·7	124
C. 35 min. after glycyrrhetinic acid	0·25	154	3·6	121
D. 75 min. after glycyrrhetinic acid	0·65	153	3·8	126

TABLE VI

EFFECTS OF GLYCYRRHETINIC ACID ON URINE SECRETION IN EIGHT MALE VOLUNTEERS AFTER DRINKING 1500 ML. OF WATER

Treatment	Time in hours after drinking water				
	½	1	1½	2	2½
Placebo controls	69*	294	609	782	904
Glycyrrhetinic acid 0·2 g.	65	286	608	828	936
Glycyrrhetinic acid 0·5 g.	124	406	731	931	1096

* Average cumulative total excretion in ml.

TABLE VII

GLUCOCORTICOID ACTIVITY OF GLYCYRRHETINIC ACID IN THE MOUSE SURVIVAL TEST

Treatment (dose 20 g. mouse)	Mean survival time in hours	Standard error
Controls saline	4·9*	± 0·45
Glycyrrhetinic acid 1·56 mg. ..	3·55	± 0·40
,, ,, 6·25 mg. ..	2·65	± 0·26
,, ,, 25 mg. ..	2·65	± 0·36

* Each value is the mean of ten mice.

where the administration of glycyrrhetinic acid preceded the water loading by 30 minutes, and not in groups 3 and 4 where the time interval was longer. Potassium excretion was increased in all groups.

These results were confirmed in a second experiment using two groups of six rats. The first group received 0·25 ml. of saline and the second group 125 mg./kg. of glycyrrhetinic acid intraperitoneally. 10 ml. of water per 100 g. rat was given orally 30 minutes later. The urine excretion is shown in Figure 1 and the excretion of sodium and potassium in Figure 2.

In the anaesthetised cat an intraperitoneal injection of 100 mg./kg. of glycyrrhetinic acid caused a reduction in the urine flow, a slight decrease in the urinary excretion of sodium and a slight increase in the excretion of potassium (Table IV). There were no significant changes in the serum concentrations of sodium, potassium and total chlorides (Table V).

Estimation of glycyrrhetinic acid in the serum showed that absorption occurred from the peritoneal cavity.

In eight student volunteers oral doses of 0·2 g. and 0·5 g. of glycyrrhetinic acid before drinking 1500 ml. of water did not produce an antidiuretic effect (Table VI).

Glucocorticoid action. Glycyrrhetinic acid did not increase the survival time of adrenalectomised mice submitted to a cold stress (Table VII). This confirms the observations of Wenzel and others[17] and D'Arcy and others[16]. Hems[9] was unable to find a glucocorticoid action with glycyrrhetinic acid when tested by the liver glycogen test.

CONCLUSIONS

Glycyrrhetinic acid is seen to have a remarkably low toxicity and therefore can be applied to the skin with complete safety in dermatological conditions. So far it has been little used internally, although liquorice extract has been taken orally for years. We have confirmed that exceptionally large doses of glycyrrhetinic acid in animals have an antidiuretic action associated with changes in the metabolism of sodium and potassium; but do not cause kidney damage. Water retention was not seen with small doses used in human volunteers. The low solubility of glycyrrhetinic acid in body fluids has so far precluded parenteral administration in man, but this will be possible with development of more soluble derivatives which may prove of value in rheumatic diseases. An important property of glycyrrhetinic acid is its complete freedom from glucocorticoid-like actions, a serious disadvantage with the corticosteroids. While it has been shown that little absorption of these steroids occurs through normal skin this cannot be assumed in dermatological conditions where the protective dermal layers may be broken. Much remains to be discovered about the mode of action of glycyrrhetinic acid, but it offers a new approach to the treatment of inflammatory conditions free from the disadvantages of corticoids which have claimed so much attention and disproves the concept that an anti-inflammatory agent must of necessity have a concomitant corticoid-like action.

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