THE ANTITUSSIVE ACTIVITY OF GLYCYRRHETINIC ACID AND ITS DERIVATIVES

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The antitussive activity of 18β-glycyrrhetinic acid has been investigated using chemical stimulation in the unanaesthetised guinea-pig and electrical stimulation in the lightly anaesthetised cat. Derivatives of glycyrrhetinic acid were active in both types of experiment indicating a central antitussive effect. Several derivatives had approximately the same potency as codeine when given subcutaneously to guinea-pigs; one of these, dicholine glycyrrhetinic acid hydrogen succinate, exhibited the same degree of activity after oral administration.

LIQUORICE extracts have been included in cough mixtures for generations, although they are used to-day for their flavouring and demulcent properties rather than as active therapeutic constituents. An important constituent of liquorice is glycyrrhizin, the aglycone of which is glycyrrhetinic acid. We have examined 18β-glycyrrhetinic acid and a number of its derivatives for antitussive effect and here report activity of the same type and magnitude as that possessed by codeine.

MATERIALS AND METHODS

Materials

18β-glycyrrhetinic acid (G.A.), the ethanolamine salt of G.A., and the *N*-methylglucamine salt of G.A., were administered as suspensions in water. The choline salt of G.A. (choline G.A.), the piperazine salt of G.A., the disodium salt of G.A. hydrogen succinate, and the dicholine salt of G.A. hydrogen succinate (dicholine G.A.H.S.) were administered as solutions in water. G.A. and 3-keto G.A. were also administered as finely divided suspensions in 9 parts sesame oil : 1 part ethanol. Codeine was administered as the phosphate in solution in water and hydrocortisone hemisuccinate in solution in water or in suspension in water for high doses.

Method using Guinea-pigs

The compounds were investigated by a modification of the technique described by Winter and Flataker (1954). A guinea-pig was placed in a sealed glass chamber and coughs elicited by exposure to an aerosol of 3 per cent aqueous ammonia solution formed in a Riddostat inhaler (Riddell Products Ltd., London) using air at 10 lb./sq. in. Coughs were detected by means of a crystal microphone (Acos type Mic 35–1) connected by earthed co-axial cable to the upper beam of a Cossor double beam oscilloscope (Model 1049 Mk II). The lower beam was used for signals indicating the start and end of the period of exposure to the aerosol. Coughs could be recorded photographically with the aid of a standard camera attachment using a stationary signal spot and a film speed of 3 in./min. Examples of the records so obtained are shown in Fig. 1.

In all experiments, groups of ten animals were used for each dose of drug and the results were calculated as a percentage reduction in the mean cough counts obtained for 3 min. exposures to ammonia aerosol before and after drug administration.

Method using Cats

The technique used was that of Domenjoz (1952). Cats were lightly anaesthetised with pentobarbitone sodium intraperitoneally (usually 25 mg./kg.). Coughing was induced by electrical stimulation of the

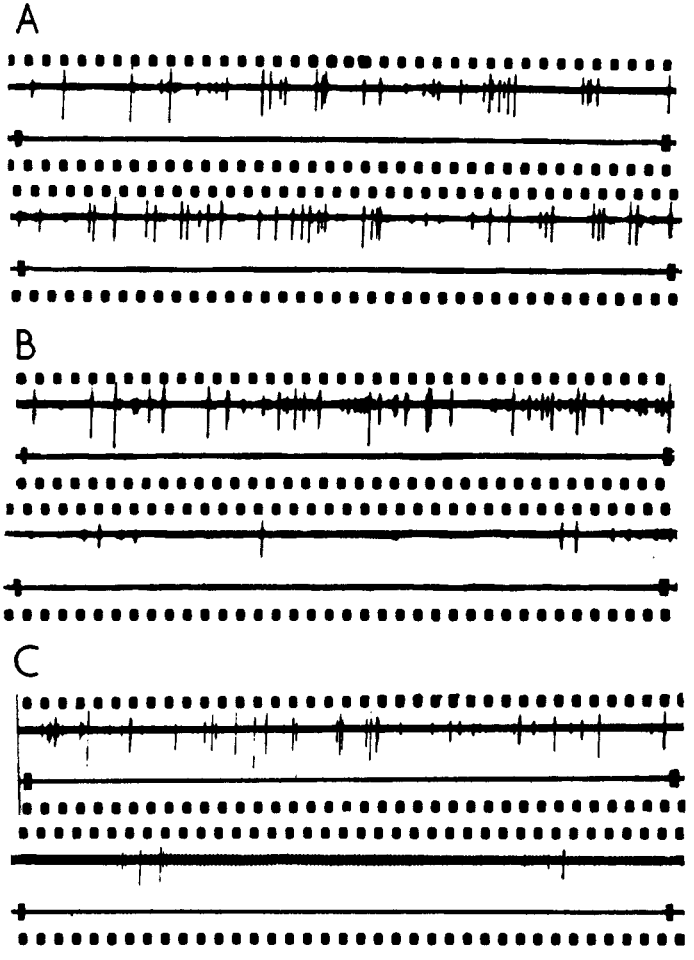

FIG. 1. Photographic records of antitussive effects in unanaesthetised guinea-pigs exposed to an ammonia aerosol.

(A) Normal saline s/c., (B) codeine 1 mg./kg. s/c., (C) Choline G.A. 1 mg./kg. s.c. In each example the upper record shows the coughs recorded before drug administration and the lower record the coughs recorded 1 hr. after drug administration. Coughs were recorded on the upper beam and the time interval on the lower beam of a standard double beam oscilloscope.

superior laryngeal nerve for 15 sec. at regular 3 or 5 min. intervals. The pulse width was fixed at 100 millisec., but the voltage (1 to 10 V.) and rate of stimulation (5 or 10 stimulations/sec.) were adjusted to obtain optimum responses from each animal. Coughing was recorded on a kymograph by a thread attached to the abdominal wall immediately below the inferior end of the sternum, and passed over pulleys to a Starling heart lever. Tension in the thread was adjusted so that cough responses were superimposed on a baseline of respiratory movement. Coughing in

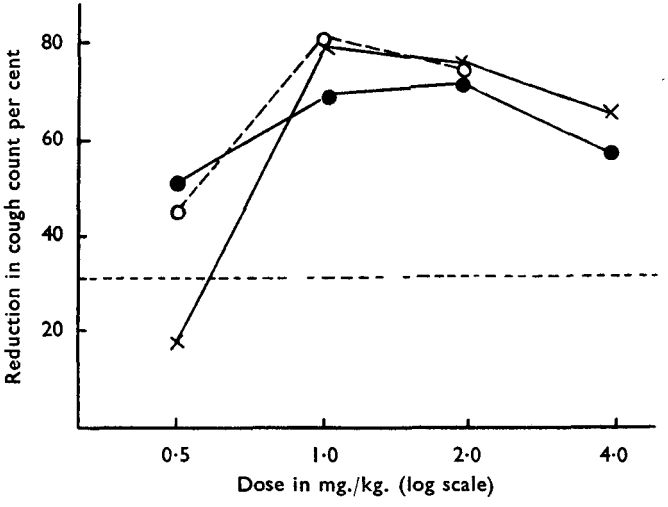

FIG. 2. The antitussive effects of codeine, choline G.A. and dicholine G.A.H.S. one hour after subcutaneous administration to unanaesthetised guinea-pigs. X—X Codeine. ●—● Dicholine G.A.H.S. ○- - -○ Choline G.A. The dotted line indicates the maximum effect (P = 0·95) expected after normal saline (see text).

response to stimulation culminated in an expiratory gasp recorded as a large upward excursion of the lever; its abolition was considered to be indicative of antitussive effect.

RESULTS

Subcutaneous Administration to Guinea-pigs

Control experiments. The effects of normal saline administration were first determined. In five groups of animals the highest reduction in cough count observed was 24 per cent. The mean value for these groups was 10·4 per cent with a standard deviation of 10·6 per cent and it was calculated that the maximum reduction in cough count to be expected at a probability level of 0·95 was 30·1 per cent. Only reductions in cough count in excess of this figure were subsequently considered indicative of antitussive activity.

Since the subcutaneous injection of the disodium salt of glycyrrhetinic acid hydrogen succinate had been reported by Finney and Tarnoky (1960)

to have a necrotic action in rat skin, two groups of animals were injected with a strongly necrotic solution which was not expected to have antitussive activity (tartar emetic 1 per cent in normal saline) to see whether or not the resultant irritation interfered with the cough counts of the animals. The observed reductions in cough count were 14 per cent and 1 per cent and well within the range obtained with normal saline.

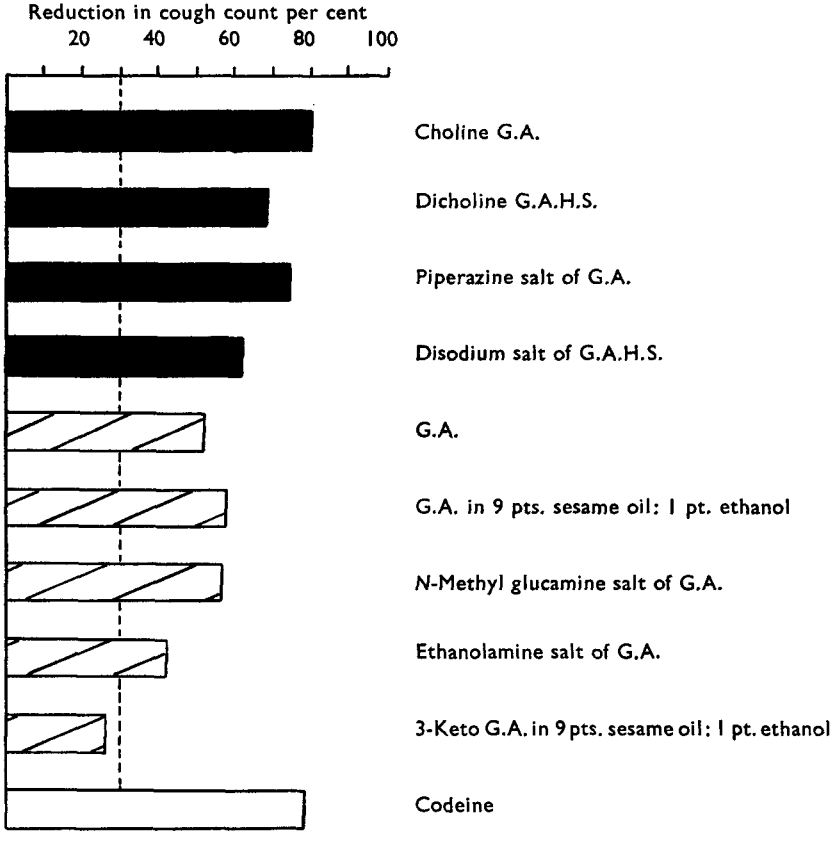

FIG. 3. The antitussive effect of a number of glycyrrhetinic acid derivatives one hr. after subcutaneous administration of 1 mg./kg. to unanaesthetised guinea-pigs. Solid columns indicate solution injected. Shaded columns indicate suspension injected. Last column—codeine 1 mg./kg. s/c. The dotted line indicates the maximum effect (P = 0·95) expected after normal saline (see text).

The activity of codeine, choline G.A., *and dicholine* G.A.H.S. These three substances were injected within the dose range of 0·25 to 4·0 mg./kg., and antitussive effects recorded 1 hr. later. Regression lines were obtained and are shown in Fig. 2.

Peak activity occurred with all three substances at about 1 mg./kg. At that dose all had a comparable activity.

The activity of other derivatives of 18β*-glycyrrhetinic acid.* A number of other derivatives as well as glycyrrhetinic acid itself were then examined

for activity at the 1 mg./kg. dose level. The results are shown in Fig. 3. All the compounds examined except 3-keto G.A. had activity in excess of the control level and several had an activity approaching that of codeine.

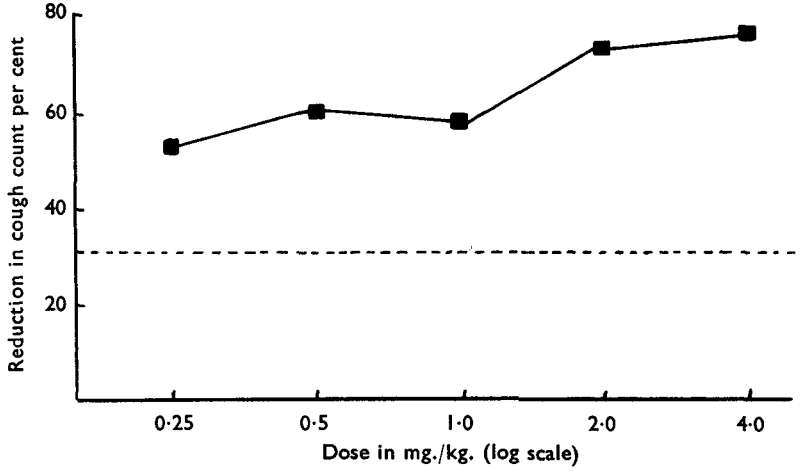

FIG. 4. The antitussive effect of hydrocortisone hemisuccinate one hour after subcutaneous administration to unanaesthetised guinea-pigs.
The dotted line indicates the maximum effect (P = 0·95) expected after normal saline (see text).

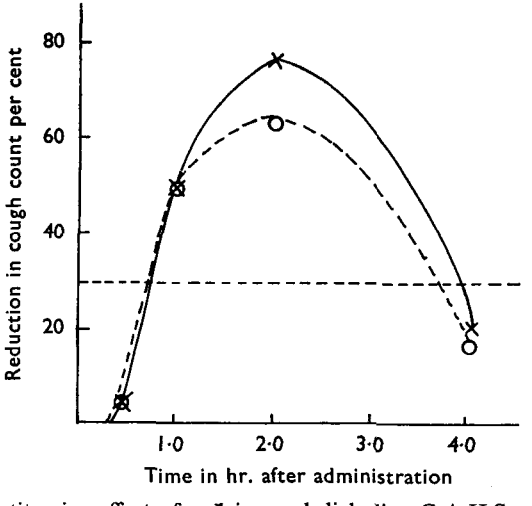

FIG. 5. The antitussive effect of codeine and dicholine G.A.H.S. at various times after oral administration of 5 mg./kg. to unanaesthetised guinea-pigs.
X—X Codeine. ○ – – ○ Dicholine G.A.H.S. The dotted line indicates the maximum effect (P = 0·95) expected after normal saline (see text).

The activity of hydrocortisone. Since glycyrrhetinic acid and some of the derivatives examined have been compared with hydrocortisone and reported to possess anti-inflammatory activity (Finney and Somers, 1958; Finney and Tarnoky, 1960), we examined hydrocortisone for antitussive

effect. It was observed to possess significant activity over the same dose range as codeine, choline G.A. and dicholine G.A.H.S. as shown in Fig. 4. But, the regression line obtained was different in slope from those obtained with the other three substances.

Oral Administration to Guinea-pigs

Preliminary experiments were conducted with codeine and dicholine G.A.H.S. to determine the time after oral administration that was coincident with maximum effect at the 5 mg./kg. dose level. The results are shown in Fig. 5. Maximum effect occurred 2 hr. after administration so this time interval between drug administration and measurement of antitussive effect was used in experiments with codeine, choline G.A. and dicholine

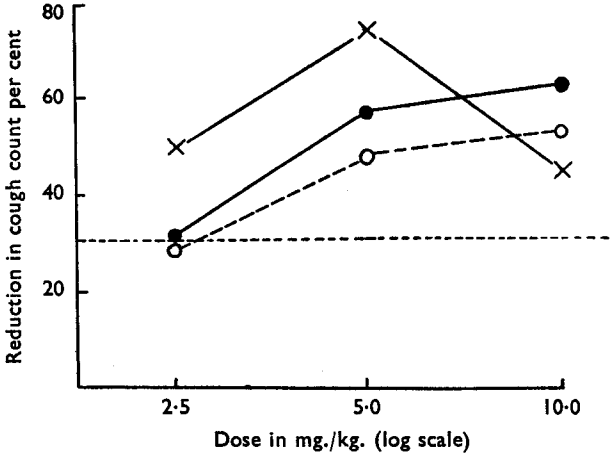

FIG. 6. The antitussive effects of codeine, choline G.A. and dicholine G.A.H.S. 2 hr. after oral administration to unanaesthetised guinea-pigs.
X—X Codeine. ●—● Dicholine G.A.H.S. ○ – – – ○ Choline G.A. The dotted line indicates the maximum effect (P = 0·95) expected after normal saline (see text).

G.A.H.S. The regression lines obtained are shown in Fig. 6. At the 5 mg./kg. dose level, the relative potency of dicholine G.A.H.S. to codeine was found to be the same as that observed after subcutaneous administration. Choline G.A., however, which had the same activity as codeine after subcutaneous administration showed only about half the activity of codeine when given orally. This might be due to poor absorption of choline G.A. from the gastrointestinal tract.

Intravenous Administration to Cats

The effects of hydrocortisone, codeine and choline G.A. in a single preparation are shown in Fig. 7. Doses of 1 mg./kg. and 2 mg./kg. of hydrocortisone were found to be inactive. After 1 mg./kg. of codeine the cough reflex was inhibited for 8 min. but returned after 11 min. A similar effect was observed with 1 mg./kg. of choline G.A. These

observations have been repeated in five preparations and found to be independent of the order in which the drugs were administered.

Fig. 8 shows the duration of the antitussive effects of codeine and choline G.A. within the dose range 1 to 4 mg./kg. Codeine and choline G.A. were active at the same dose levels but the duration of action of choline G.A. was approximately twice that of codeine.

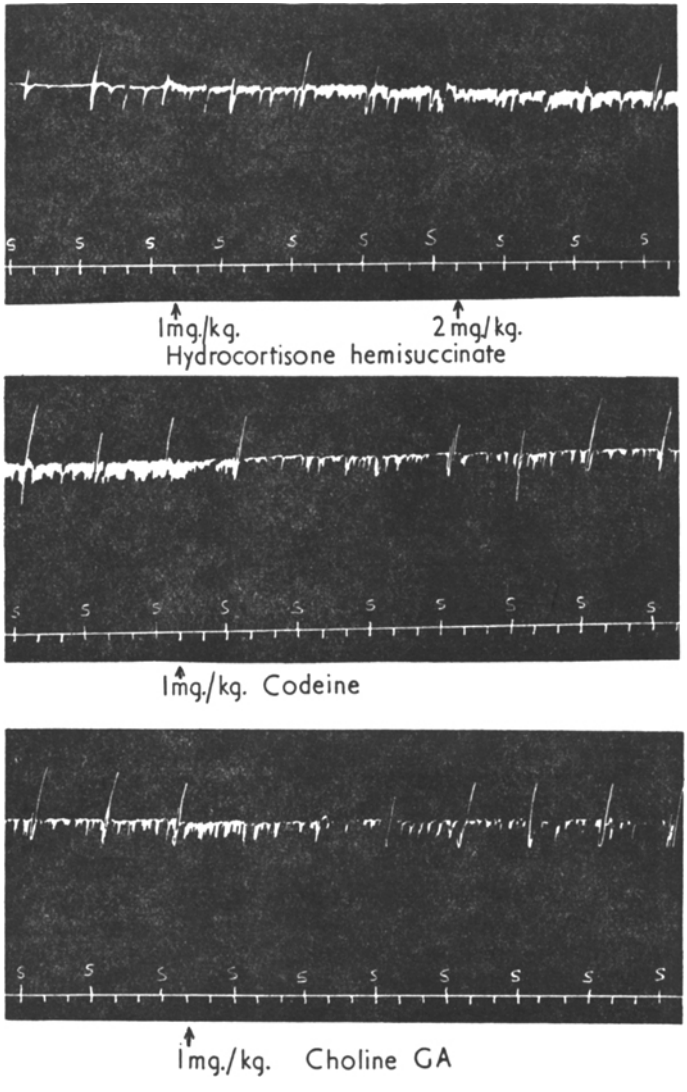

FIG. 7. A comparison of the antitussive effects of codeine, choline G.A. and hydrocortisone hemisuccinate after intravenous administration in the lightly anaesthetised cat. Coughs were recorded as large deflections upwards from a base line of respiratory movement. Time interval 1 min. S = stimulation.

Discussion

The evaluation of antitussive activity in a pharmacological laboratory presents certain difficulties. Although many methods are available and coughing can be induced by chemical, mechanical or electrical means, it is questionable whether the experiments imitate the type of cough stimulus for which antitussives are used in man. The methods used in the present study were selected because in our hands they proved both reliable and easy. In combination, they provided results from which it was possible to draw conclusions regarding the site of action of antitussive drugs. The guinea-pig technique is sensitive to peripheral actions on sensory

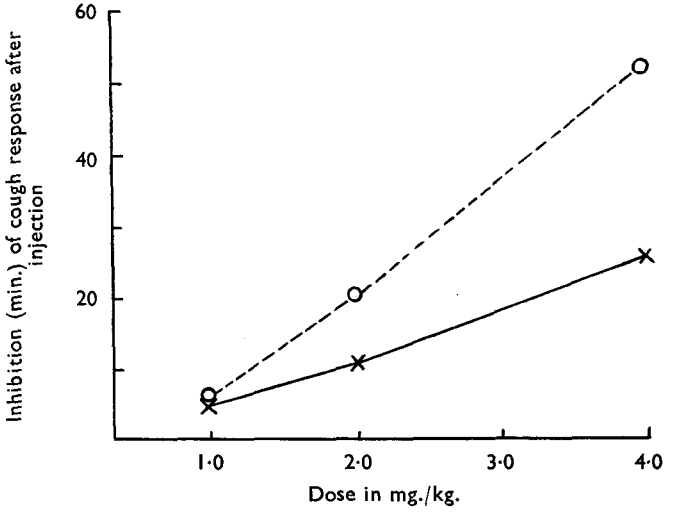

FIG. 8. A comparison of the duration of antitussive effect observed after various doses of codeine and choline G.A. administered intravenously to lightly anaesthetised cats. X—X Codeine. ○ - - - ○ Choline G.A.

nerve endings in the trachea as well as central actions on other parts of the cough reflex. In the cat method, sensory nerve impulses are derived from electrodes placed on the central portion of the sectioned superior laryngeal nerve so that only centrally acting antitussives can be detected. Thus hydrocortisone was found to be active in guinea-pigs but not in the cat, whereas codeine was active in both preparations. Choline G.A. was also active in both preparations, and it can therefore be concluded that it has central antitussive actions.

The guinea-pig technique could be criticised on the grounds that chemically induced coughing is artificial. Nevertheless it provides a rapid method of screening a number of compounds for antitussive activity if positive results are accepted with some reservation. The regression lines obtained with codeine, choline G.A. and dicholine G.A.H.S., using this technique are worthy of comment in that although antitussive activity increased with increasing dose, it then passed a peak value and declined. The reasons for this are not known, but the effect has been observed with

pholcodeine by Anderson and Smith (1959 unpublished) and opiate antitussives (Green, 1961, private communication). Although the fall in activity with high dosage might perhaps be indicative of an acute toxic response, no evidence for this has been found.

The discovery of antitussive activity in the glycyrrhetinic acid molecule is an interesting one. Some of its derivatives have activity of the same magnitude as codeine when given subcutaneously to guinea-pigs and one of these, dicholine G.A.H.S. is approximately equiactive when given orally. If dicholine G.A.H.S. has activity of this magnitude in man, the traditional use of liquorice for cough therapy can be considered to have some rational basis. Liquorice extracts, however, have too small a glycyrrhizin content for the full realisation of their antitussive potentialities.

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