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Cat assay for the emetic action of digitalis and related glycosides (digitoxin, digoxin, lanatoside C, ouabain and calactin)

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Summary

1. A titration assay with two end points is described for comparison of the emetic and lethal potencies of digitalis-like drugs.

2. A drug was infused at constant rate to a conscious, unrestrained cat, through an indwelling venous cannula. At the moment of vomiting the cat was rapidly anaesthetized and infusion continued at the same rate until the moment of cardiac arrest.

3. With very slow and very fast infusions, the emetic and lethal doses tended to rise. In the range between these extremes (which varied from drug to drug) they were independent of time.

4. The observations could be accounted for by analogue computation, assuming that the drugs entered an initial pool and were distributed at finite rates to receptors in the CNS (vomiting centre) and heart.

5. Half times of metabolic loss derived from this computation for digitoxin, digoxin and ouabain (17, 9·9 and 1·8 h, respectively) were in the same ratio as the threefold longer half times reported for these drugs in man.

6. When measured with infusion rates in the time independent range, the ratio of lethal to emetic doses did not vary between the drugs studied. All caused vomiting at 40% of the lethal dose.

7. From a review of the literature, the emetic and cardiotoxic actions of digitalis-like drugs appear inseparable and probably share a common biochemical mechanism.

8. It is concluded that foreseeable improvements in digitalis-like drugs are small and would depend on the elimination of any local emetic effect on gut receptors which they may have.

Introduction

Digitalis-like drugs are given at doses closer to the toxic level than any other medicine in general use. Several have been claimed to have an advantage in the ratio of their therapeutic to toxic potency but such claims must be weakened by the fact that at least four have been recommended on these grounds (Fahr & La Due, 1941 ; Hoffman & Pomerance, 1952 ; Marriott, 1954 ; Owen, 1963). Differences in toxicity

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of digitalis-like drugs may be so small that they can be entirely explained by variations in presentation and dose schedule.

The early symptoms of digitalis poisoning are extracardiac, and in order to compare drugs objectively a titration assay has been developed, with separate extracardiac and cardiac end points. The first end point is the moment of vomiting, the earliest and most regular symptom of digitalis toxicity, and the second the moment of cardiac death, as in pharmacopoeial assays. Cats were used in this study since they closely resemble man in their sensitivity and pattern of response to digitalis. Vomiting is inhibited by anaesthesia and experiments were carried out in unrestrained conscious animals with indwelling venous cannulae.

Methods

Cats (2–4 kg) were prepared with indwelling polyvinyl venous cannulae 1–3 days before the experiment. The cannulae were made from 1–1·5 mm i.d. transparent grade polyvinyl tubing, Shore Hardness 85–95 (Portex Ltd., Hythe, Kent, size NT2—NT3. Bolab Inc., 359 Main Street, Reading, Mass. 01867, U.S.A., size V7–V9. Braun Apperatebau, Melsungen, Germany, size P3–P5). Polyvinyl tubing has a very smooth surface, is soft and elastic at body temperature, and remains patent for much longer than polythene tubing. Blunted needles were used for connexion to syringes and pumps but the tubing was undamaged by the repeated occlusion necessary to prevent air being drawn in.

The operation was carried out with aseptic precautions under anaesthesia with halothane. Cannulae were filled with sterile 0·9% saline, inserted and tied into the external jugular or saphenous vein so that the tip lay in the vena cava. The free end of the cannula was threaded through the eye of a large sacking needle which was passed subcutaneously around the waist or neck of the animal and pushed out through skin in the midline of the back. This area was previously shaved and sprayed with Nobecutane (Evans Medical) to provide an adhesive surface for sticking plaster. The sacking needle was removed and the skin incision overlying the cannulated vein closed with sutures. The cannula was then pulled through a hole in a piece of plaster. The plaster was smoothed on to the tacky skin, the cannula flushed, sealed with a pin, coiled and covered with another layer of plaster. This plaster was protected from chewing or licking by a coat of foul tasting compound (quinine, 10% ; chloral hydrate, 40% ; copper sulphate, 10% ; soap powder, 40% ; and glycerin to paste). Cannulae could be kept patent for up to 3 months provided they were flushed after use and the anchoring plaster maintained in good condition. Heparin was found unnecessary.

For each experiment a cat was placed in an observation cage measuring 60 × 60 × 60 cm and connected to a constant speed infusion pump by a thin vinyl tube descending from the centre of the roof. Miniature peristaltic pumps were used of the type described by Bainbridge & Wright (1965). Cats did not require restraint and lay purring if a cloth to lie on and a warming lamp were provided. If necessary, interference with the tube could be prevented by the experimenter since the animal was never left unattended.

To study differences in the time course of emetic and cardiac action of the various drugs, the time taken to give a lethal dose was varied systematically. Pump speeds were varied from 4 ml/h in the longest to 30 ml/h in the shortest experiments, and the appropriate glycoside dilution was calculated from the predicted lethal dose.

In each experiment, glycoside was infused until vomiting occurred (sometimes preceded by licking of the lips and salivation). At this point the infusion was interrupted, the dose noted, and the cat quickly anaesthetized with vinyl ether or halothane (ethyl chloride was found to precipitate fibrillation in digitalized animals). The infusion was then resumed, chloralose (70 mg/kg) given intravenously as a 1% solution, volatile anaesthetic discontinued and the trachea cannulated.

To determine the point of cardiac arrest, lead II electrocardiograms were taken through subcutaneous needle electrodes in all but the shortest experiments (total duration of infusion less than 15 min). In these no anaesthesia was attempted and the animal was left unrestrained, ventricular arrest being signalled by a series of sharp inspiratory gasps.

Solutions

Stock solutions of glycosides (1 mg/ml) were made up in 50% aqueous ethanol. They were diluted in sterile 0·9% saline before use. To avoid supersaturation of solutions the concentration of digitoxin was never above 0·01 mg/ml and of digoxin not over 0·1 mg/ml. In short experiments high concentrations of digitoxin were needed to avoid infusion of unphysiologically large volumes. These high concentrations were made up in cotton seed oil emulsion prepared for intravenous feeding (Infonutrol, (Astra)/diluted 1:10 with saline) which, in control experiments, was found not to affect the emetic or lethal dose of the water soluble glycoside ouabain.

Results

Preliminary experiments showed that vomiting occurred 5–10 min after intravenous injection of half the calculated lethal dose of ouabain, while injection of half the lethal dose of digitoxin caused vomiting after 30–60 minutes. To be able to compare rapidly and slowly acting drugs under corresponding conditions, the effect of varying the rate of infusion was studied with three drugs.

The results obtained are shown graphically in Fig. 1, in which the emetic and lethal doses in mg/kg are plotted against the duration of infusion. In each experiment, since the infusion rate was constant, the two end points (vomiting and death) are represented by a pair of points which would lie on a straight line passing through the origin, its gradient corresponding to the rate of infusion in that particular experiment. Pairs of points lying on steep gradients correspond to fast rates of infusion and those on shallow gradients to slower rates.

At fast rates of infusion the amount of glycoside infused at the time of vomiting or death rose steeply. At slower rates, as foreshadowed in the preliminary experiments, the emetic and lethal doses fell to reach plateau values. The time at which the emetic dose became independent of the rate of infusion varied considerably between the three drugs. With ouabain the dose was more or less constant when infusions lasted more than 15 min and with digoxin when they lasted more than 30 minutes. With digitoxin, infusions lasting more than an hour were needed to make the emetic dose independent of time.

The lethal dose varied with infusion rate in a similar way to the emetic dose. The order in which the drugs approached their time independent doses was the same as in the emetic assay. With ouabain the lethal dose reached a plateau value with

infusions lasting longer than 1 h, with digoxin longer than 2 h and with digitoxin longer than 3·5 hours.

Ouabain was not only the fastest acting but also the most rapidly excreted glycoside. This was shown by a definite tendency for the emetic and lethal doses to rise when the infusions were given slowly.

Using only points which lie in the plateau regions of the curves in Fig. 1, mean values have been calculated for the emetic and lethal doses and the ratios of lethal to emetic doses of digitoxin, digoxin and ouabain. These are listed in Table 1 together with their standard errors, based on a direct estimate of variance.

Nine determinations of the ratio of lethal to emetic doses were also made for calactin, a glycoside which is not used therapeutically. In these experiments the drug was not infused but injected in small aliquots at 5 min intervals, as in the pharmacopoeial cat assay for digitalis. These results are also given in Table 1. On average, vomiting occurred after 45 min and death after 105 minutes.

In one experiment, lanatoside C was tested in the same way as calactin. Vomiting occurred after 45 min and death after 105 min, giving a ratio of lethal to emetic doses of 2·3.

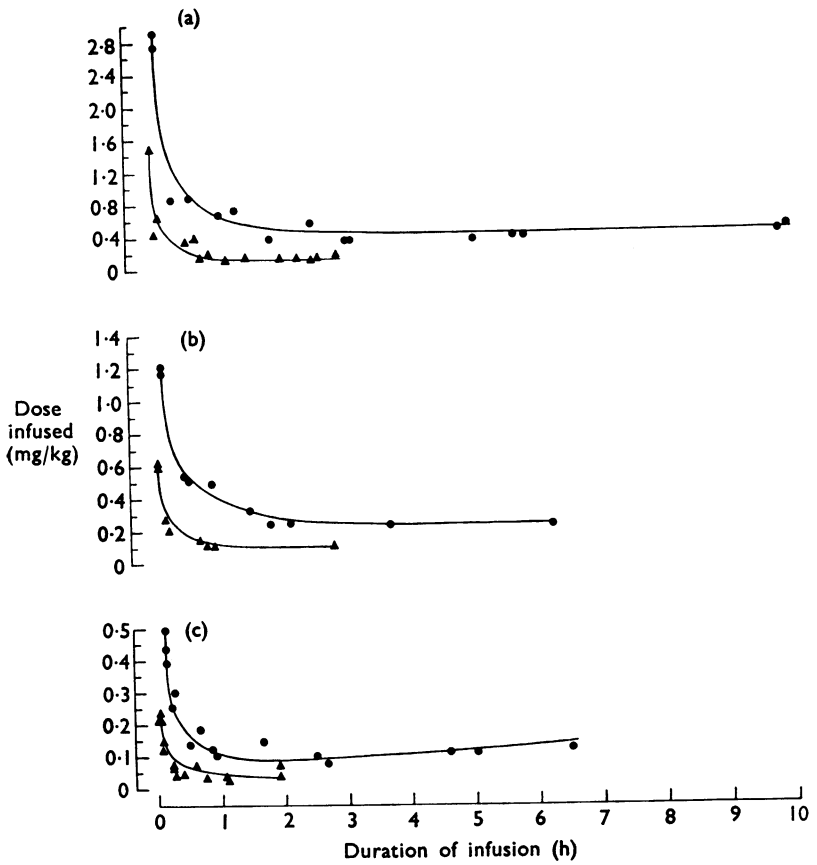

FIG. 1. Graph showing the effect of the duration of intravenous infusion on the doses of three glycosides required to cause vomiting (▲) and cardiac arrest (●). As described in the text, the lines are drawn by analogue computation according to a simple hypothesis of drug distribution and metabolic loss. (a), Digitoxin ; (b), digoxin ; (c), ouabain.

Statistical examination of the results in Table 1 showed no significant difference between the ratios of lethal to emetic doses of the four glycosides ($P>0{\cdot}2$ even for the comparison between the highest and lowest). The mean of all the ratios was 2·4, indicating that all these drugs caused vomiting at 40% of the dose causing cardiac arrest.

Analogue computation

It was believed that the results obtained by infusion at a series of different rates could be interpreted in terms of a simple mathematical model which is illustrated in Fig 2. The model assumes that the drug is initially distributed in a single compartment. This must represent not only the blood volume but also a proportion of the body water and tissue spaces into which cardiac glycosides have been shown to penetrate (Friedman, St. George & Bine, 1954 ; Okita, Talso, Curry, Smith & Geiling, 1955b ; Doherty & Perkins, 1966 ; Harrison, Brandenburg, Ongley, Orvis & Owen, 1966 ; van Zwieten, 1968).

The metabolic loss from the compartment is represented by a first order rate constant m. The simultaneous exchange of drug with two smaller compartments

TABLE 1

	Emetic dose mg/kg mean±S.E.	Lethal dose mg/kg mean±S.E.	Ratio of lethal to emetic doses mean±S.E.
Digitoxin $n=9$	0·15 ±0·011	0·38±0·023	2·72±0·23
Digoxin $n=4$	0·11 ±0·011	0·24±0·011	2·20±0·11
Ouabain $n=7$	0·044±0·003	0·11±0·007	2·55±0·11
Calactin $n=9$	0·049±0·002	0·11±0·005	2·35±0·14

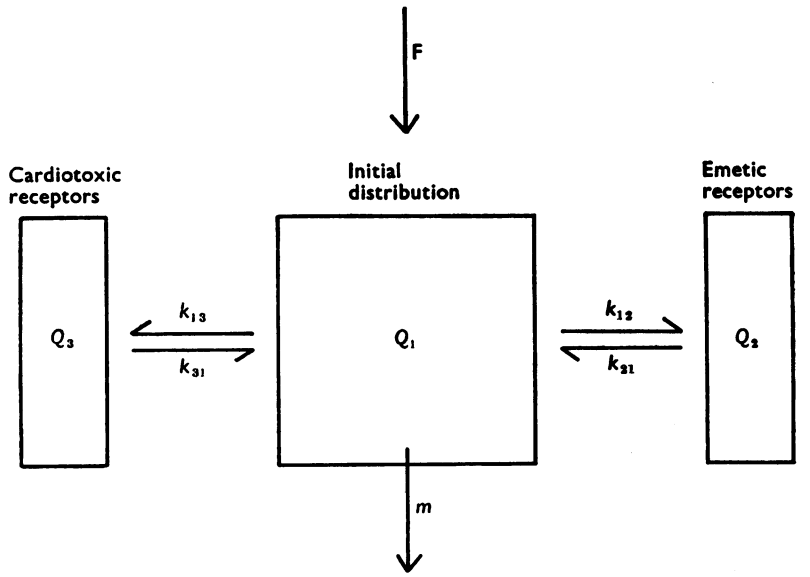

FIG. 2. Diagram illustrating the hypothesis of drug distribution and metabolic loss used for analogue computation of the data in Fig. 1. For explanation see text.

containing, respectively, emetic and cardiotoxic receptors is similarly represented by a set of first order rate constants k_{12}, k_{21}, k_{13} and k_{31}.

If the simplifying assumption is made that the quantities of drug taken up by the receptor compartments are negligible, equations can be derived for the quantities of drug in the receptor compartments (Q_2 and Q_3) as a function of time (t) and rate of infusion (F). The analytic solution of Q_2 is as follows: that for Q_3 is obtained simply by substituting the suffix 3 for the suffix 2 throughout.

$$Q_2 = \frac{k_{12}F}{k_{21}-m}\left[\frac{1-\exp(-mt)}{m} - \frac{1-\exp(-k_{21}t)}{k_{21}}\right] = \frac{k_{12}F}{k_{21}-m}\,\phi\,(t)$$

There are too many unknowns to permit a complete solution of these equations, but with the further assumption that vomiting and death occur when Q_2 and Q_3 reach critical levels ($\bar{Q}_2$ and $\bar{Q}_3$) which are constant for any one drug, it is possible to write two equations between any three pairs of experimental values of F and t. Since for each drug the value of the metabolic rate constant (m) should allow simultaneous agreement with triplets both from the emetic and the cardiotoxic data, these equations can be solved for m, k_{21} and k_{31}.

The values thus obtained were inserted in a simulation of the model on an analogue computer to obtain the solid curves drawn in Fig. 1. These fit the experimental results satisfactorily in the case of digitoxin and digoxin. For ouabain, however, the value of m which gives the best fit to the lethal doses does not account so well for the emetic doses ; observed values for the latter were clearly in the plateau region when infusions lasted longer than 15 minutes.

Overall, however, the analogue computations appear to account for the measurements surprisingly well, considering the simple nature of the model adopted. The time dependence of lethal doses of digitoxin and ouabain was earlier studied by Hildebrandt & Geppert (1942). They were able to fit their measurements empirically to hyperbolic curves, but this mathematical treatment corresponded to no physical model of the mechanism of drug distribution.

The values of m obtained from the analogue computation can be converted into half times of metabolic loss for the three drugs. The values are for digitoxin 17 h, for digoxin 9·9 h and for ouabain 1·8 hours. Precise rates of disappearance of these drugs appear not to have previously been measured in the cat, but the present figure for digitoxin is consistent with the results of an approximate method used by Hatcher in 1912 (titration to cardiac arrest at intervals after a sublethal dose). It is noteworthy that these half times in the cat are in almost exactly the same ratio to one another as the 3-fold longer half times observed for digitoxin, digoxin and ouabain in man (Okita, Talso, Curry, Smith & Geiling, 1955a ; Doherty & Perkins, 1962 ; Marks, Dutta, Gauthier & Elliot, 1964).

Discussion

The drugs included in this study differ widely in physical and pharmacodynamic properties. Digitoxin is relatively fat soluble and one of the most persistent glycosides, widely prescribed in the USA and continental Europe. It is totally absorbed from the alimentary tract and strongly bound to plasma proteins. Ouabain represents the other extreme, being relatively very water soluble, fast acting and rapidly excreted in the urine. It is virtually unabsorbed by mouth and not bound to plasma proteins. Digoxin was chosen because it has intermediate properties and is the

glycoside most widely prescribed in this country. A single experiment on lanatoside C was included because it has been claimed to have exceptionally low toxicity (Fahr & La Due, 1941 ; Rothlin & Bircher, 1954). Calactin, which is not used therapeutically, was included in the study because of its apparent biological role as an emetic toxin. It is stored by several insects feeding on Asclepiad plants and appears to protect them against some of their predators (Parsons, 1965 ; von Euw, Fishelson, Parsons, Reichstein & Rothschild, 1967 ; Reichstein, von Euw, Parsons & Rothschild, 1968).

Digitalis does not act specifically on the heart ; at appropriate concentrations it probably affects every living tissue in the body (Withering, 1785 ; Koppe, 1875 ; Shrager, 1957 ; Von Capeller, Copeland and Stern, 1959 ; Somlyo, 1960 ; Lendle and Mercker, 1961). Wherever it has been found to act, a digitalis sensitive ion transport mechanism has been identified (Bonting, Caravaggio & Hawkins, 1962 ; Bonting, 1964) and there is now a body of evidence that an ion transport effect mediates its therapeutic actions. For instance Repke (1964) showed that the therapeutic potency of a wide range of digitalis-like drugs was correlated with their potency as inhibitors of sodium and potassium transport. The mechanism of the improvement in myocardial contractility is still uncertain, but may involve changes in the distribution and mobilization of calcium, perhaps secondary to a change in the intracellular sodium concentration (Klaus, 1963 ; Repke, 1964).

Similarly, tissues responsible for the symptoms of digitalis toxicity (for instance the vomiting centre, intestines, skeletal muscle and retina) have digitalis-sensitive ion transport systems. Here too there is evidence to suggest that it is the partial inhibition of transport which mediates the effects. Therapeutic doses of drugs which have the characteristic properties of digitalis, even though chemically unrelated, inhibit membrane transport. Without exception, at slightly higher doses they cause the same spectrum of toxicity. These drugs include the bufadienolides (Chen & Ling Chen, 1933), erythrophleum alkaloids (Chen, Hargreaves & Winchester, 1938 ; Cotten, Goldberg & Walton, 1952 ; Bonting, Hawkins & Canady, 1964) and bisguanylhydrazones (Kroneberg & Stoepel, 1964).

Thus the biochemical evidence suggests that after absorption, the therapeutic and toxic actions of digitalis are inseparable and share a common mechanism. Among this wide range of compounds with apparently inseparable effects a therapeutic advantage might be found if a drug existed with a favourable ratio of affinities for different tissues. The early symptoms of digitalis toxicity are extracardiac and if, for instance, a drug had special affinity for cardiac receptors the dose required for effective therapy might be low enough to avoid toxic symptoms. These experiments have been designed to examine drugs for variation of this type and none has been found. However, it cannot be concluded that there are no differences between glycosides in normal oral therapy. Experiments which extended over many years from the work of Eggleston & Hatcher (1913) to that of Borison and his colleagues (Borison & Wang, 1953 ; Gaitondé, McCarthy & Borison, 1965) established that the principal receptors of the emetic action of digitalis are in the central nervous system, located in the area postrema of the medulla. Accordingly, in this study, drugs were administered by intravenous infusion in order to eliminate differences in rate and completeness of absorption and also to eliminate a local emetic effect on gut receptors (see Appendix).

The conditions of actual clinical use are much more complex and it is easy to see how variations in diet, dose schedules and timing of doses in relation to food may

account for contradictory reports on the emetic tendency of present-day digitalis-like drugs. For instance, Hoffman & Pomerance (1952) reported that in a series of twenty-six patients 'a definite decrease in the number and severity of toxic reactions was noted during the period of study with digoxin therapy as compared with other digitalis preparations.' Yet Owen (1963) has found that 'nausea and vomiting caused by therapeutic doses of digoxin almost invariably subside when equivalent amounts of digitoxin are substituted.'

We suggest that emetic assays of the present type can serve as a more objective guide to the development of less emetic glycosides. The reproducibility of titration assays is indicated by comparison of the lethal doses reported here with those published from other laboratories. The figure which we have obtained for digitoxin is identical with that given by Lendle (1935) and in three later papers (cited by Hoch, 1961) and the values for digoxin and ouabain agree almost equally well. Previous methods of assessing emetic potency have not utilized the titration principle and have therefore been less precise, but our values are in broad agreement with those for digitoxin reported by Chen, Chen & Anderson (1936) and for ouabain by Chen, Anderson & Worth (1948). These authors studied vomiting after single intravenous injections. The apparent differences in emetic tendency of glycosides and genins which they report are hard to interpret because no account was taken of the wide variation in rates of distribution, inactivation and excretion of the drugs used.

Clinical opinion of the significance of vomiting in digitalis therapy ranges from serious concern to almost total disregard. It is true that if it were abolished the earliest sign of overdose might be the development of cardiac arrhythmia. But many patients must be digitalized to the verge of toxicity to obtain maximum benefit and the frequent anorexia and vomiting may have to be borne during many years of treatment.

To summarize the situation, therefore, one possibility is that methods might be developed to sensitize the heart to subemetic doses of digitalis. But foreseeable improvements in digitalis-like drugs are small and would depend on the elimination of any local emetic effect on gut receptors which they may have. For example, penta-acetylgitoxin is a semisynthetic glycoside with very weak digitalis-like properties which has little or no action on the alimentary canal but is broken down after absorption to yield active compounds (Haustein, Markwardt & Repke, 1966).

The emetic assay used in this study could be modified by infusing drugs directly into the alimentary canal. This would permit the study of many factors responsible for the different effects of intestinal and intravenous administration.

Appendix

It is probable that gut receptors do play a part in causing vomiting (Gold, Kwit, Cattell & Travell, 1942), particularly in the case of those glycosides which are poorly absorbed when given by mouth (in general, the more polar and water soluble ones). Yet the experiments which have been held to show this are far from conclusive. Gold and his colleagues (1952) found that when digitoxin, digoxin and lanatoside C were given to their patients by mouth in the doses necessary for a certain level of cardiac action (judged by ECG changes), they had a greater tendency to produce vomiting than when given by injection in the smaller doses needed to produce the same effect.

An action on gut receptors is not the only explanation for such a finding. After oral dosage a drug will pass via the portal vessels to the liver before entering the general circulation and may have undergone almost complete alteration by enteral and hepatic enzymes (Repke, 1963; Hermann & Repke, 1964) before reaching central receptors. Thus the patterns of its metabolic transformation and distribution to receptors may be quite different when given by the oral and intravenous routes.

Haustein, Markwardt & Repke (1966) studied the action of glycosides on the isolated guinea-pig ileum at concentrations in the therapeutic range. Their experiments are compatible with a local emetic action on the gut *in vivo*, but they too are inconclusive. It can be quite misleading to compare the concentrations at which drugs act on isolated organs perfused by salt solution with concentrations at which they are active *in vivo*. For purely physico-chemical reasons a drug such as digitoxin, of which about 90% of the plasma content is protein bound, will appear 10 times too active if perfused in physiological saline solution at the nominal plasma concentration. On the other hand a glycoside such as ouabain, which is not protein bound, may be comparably potent in the two situations.

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