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THE ACTION OF CARDIAC GLYCOSIDES ON AUTONOMIC GANGLIA

BY

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The decelerator action of the digitalis glycosides on the normal mammalian heart has been described as " practically entirely vagal " by Sollmann (1948), and attributed to three components—a vaso-sensory reflex arising from a raised blood pressure, a chemical excitation of the carotid body, and a sensitization of the myocardium to vagal stimulation, and hence to acetylcholine (ACh). Furthermore, Gremels (1937) suggested that the action might be, in part, due to an anticholinesterase activity. The potentiation by digitalis glycosides of the action of ACh on the heart has been examined by many workers but with conflicting results. Thus potentiation was found by Gremels (1935 and 1937) in the heart-lung preparation of the dog and in the isolated frog heart; by Abdon, Hammarskjöld and Nielsen (1938), and by Abdon and Nielsen (1938) in the isolated heart of the frog and rabbit and of the human foetus; by Mazella (1947) in the isolated heart of the toad; and by Baker (1953) in that of the human foetus. On the other hand, Wells, Dragstedt, Rall, and Ruge (1943) obtained no such potentiation in the dog heart *in situ* ; nor did Lendle and Wienke (1951), recording the bradycardia by electrocardiography, in rat, rabbit, guinea-pig, or cat hearts.

Largely as a result of the work of Cattell (1938), who showed that glycosides modify the exchange of potassium across the cell membrane of striated muscle, it has usually been assumed that the direct action of the glycosides on the myocardium also results from some ionic imbalance of this kind, thus leading to potentiation of the action of ACh on these cells.

The action of glycosides on the heart can, however, be explained in another way which has been overlooked by previous workers. The glycosides could potentiate the action of ACh on the heart by sensitizing the intracardiac vagal ganglion cells, rather than the myocardial cells, to its action. This possibility was envisaged by Konzett and Rothlin (1952). They found that the glycosides potentiated the effect of ACh and of preganglionic nerve stimulation on the perfused superior cervical ganglion of the cat, and suggested that the glycosides might have a similar action on the intracardiac vagal ganglion cells in the heart.

Recently, Perry and Talesnik (1953) showed that the bradycardia produced by small doses of ACh in a perfused cat's heart could be prevented by small doses of ganglionic blocking drugs. They concluded that ACh, in such small doses, acted on the intracardiac ganglion cells of the vagus, the direct action of ACh on the myocardium being produced only when the dose was increased. These results offered a means of testing whether any part of the action of glycosides on the heart was mediated through the intracardiac vagal ganglion cells, and this paper describes experiments carried out with this end in view. A preliminary account of some of these results has been published elsewhere (Perry and Reinert, 1954a).

METHODS

Superior Cervical Ganglia of the Cat

The superior cervical ganglion was perfused in cats anaesthetized with ethyl chloride and ether followed by intravenous chloralose (80 mg./kg.). The method used was that originally described by Kibjakow (1933) with some modifications introduced by Perry (1953). If not otherwise stated, the perfusion fluid was Locke's solution containing twice the usual content of glucose, thus making a final concentration of 200 mg./100 ml. In some experiments the ganglion was not perfused, and drugs were injected through a cannula in the tied external carotid artery, all branches of the common carotid artery except those to the ganglion and the occipital artery having been ligated. The preganglionic cervical sympathetic trunk was stimulated, at a frequency of 10/sec. for periods of 10 sec., with square pulses of supramaximal voltage and 0.5 msec. duration. Injections were made in volumes of 0.2–0.5 ml. into the perfusion stream.

Perfused Mammalian Hearts

The isolated mammalian heart was perfused through the coronary arteries by the Langendorff method. When necessary, both vagi were stimulated at a frequency

of 20/sec. for periods of 5 sec. with square pulses of supramaximal voltage and 0.1 msec. duration. Injections were made in volumes of 0.1–0.2 ml. into the perfusion stream just proximal to the heart.

Drugs

All doses are given in terms of acetylcholine chloride, tetramethylammonium (TMA) iodide, and pure crystalline *k*-strophanthin-γ, ouabain, digitoxin, and scillaren A.

RESULTS

Cat's Superior Cervical Ganglion

We have confirmed the finding of Konzett and Rothlin (1952) that small doses of the glycosides potentiate the action of ACh and of preganglionic stimulation on the perfused ganglion. Fig. 1 shows the graded responses obtained with doses of 2–10 μg. of ACh, followed by constant responses to doses of 2 μg. of ACh; 30–40 sec. after 30 μg. ouabain the response to 2 μg. of ACh is greatly augmented, but 10 min. later has returned to its original level.

The maximum potentiation is such that 2 μg. of ACh produces a response equivalent in height but of longer duration than the previous normal response to 5 μg. ACh. This is a potentiation of at least 150%, and represents about the maximum degree of potentiation which we were able to record.

The potentiation produced by such doses of ouabain was frequently followed by a pronounced long-lasting depression, such as has been described by Konzett and Rothlin (1952). Similar results were obtained using *k*-strophanthin-γ, digitoxin, and scillaren A. The potentiation of the ACh effect is also obtained in the ganglion with its normal blood circulation, but the effect is usually more transient.

TMA first stimulates and then blocks ganglia by virtue of its depolarizing action (Paton and Perry, 1953). Its stimulant effect, like that of ACh, is potentiated by ouabain (Fig. 2). The maximal recorded potentiation in this experiment was again of the order of 100%.

In further experiments it was shown that the effect of preganglionic stimulation, like that of injected ACh, was also potentiated by similar doses of ouabain.

When the Locke's solution perfusing the ganglion contained no potassium, it was never possible to demonstrate any potentiating effect of ouabain or of any of the cardiac glycosides tried, but they regularly caused depression of the responses to ACh and to preganglionic stimulation (Fig. 3).

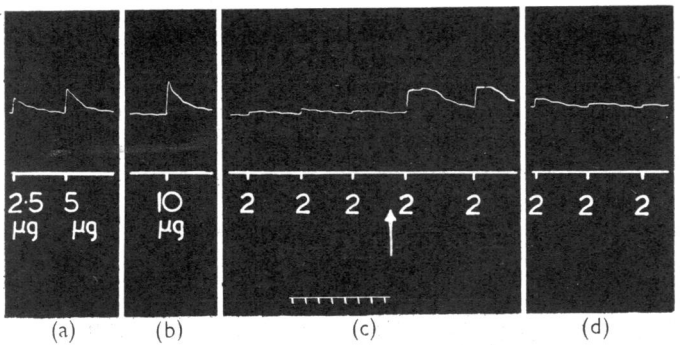

FIG. 1.—Cat, chloralose, ganglion perfusion with Locke's solution. Injections into perfusion stream. Upper trace: contractions of nicitating membrane. Lower trace: signal of ACh injections. Doses marked on record. At arrow ouabain 30 μg. into perfusion stream. Two minutes between (a) and (b) and (b) and (c); 8 minutes between (c) and (d). Time signal 30 sec.

Perfused Hearts

Cat.—Small doses (1.0 μg.) of ACh produce slight cardiac slowing and diminution of the amplitude of the contraction. Both effects are potentiated by a small dose of ouabain (0.5 μg.) which itself has no direct effect on the heart (Fig. 5a and b).

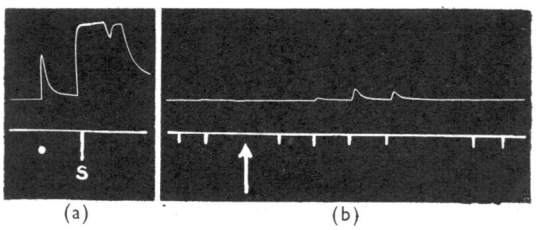

FIG. 2.—Cat, chloralose, ganglion perfusion with Locke's solution. Upper trace: contractions of nictitating membrane. Lower trace: signals. In (a), at dot, 20 μg. ACh injected into perfusion stream; at "S" supramaximal stimulation of preganglionic nerve for 5 min. (0·5 msec. pulse; frequency 10/sec.). In (b) at signal marks injections of 8 μg. TMA into perfusion stream. At arrow, 20 μg. ouabain. 2 min. between (a) and (b).

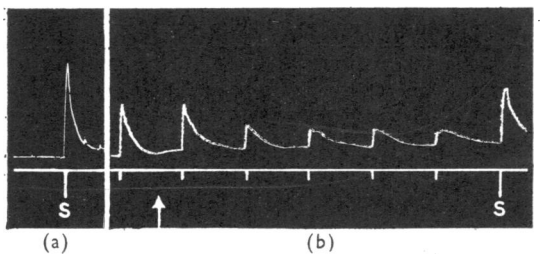

FIG. 3.—Cat, chloralose, ganglion perfusion with Locke's solution containing no KCl. Upper trace: contraction of nictitating membrane. Lower trace: signals of injections of 5 μg. ACh into perfusion stream. At "S" supramaximal stimulation of preganglionic nerve for 10 sec. (0·5 msec. pulse; frequency 10/sec.). At arrow 10 μg. ouabain. 2 min. between (a) and (b).

Similar results have been obtained with *k*-strophanthin-γ, and both of these glycosides potentiate the action of TMA in the same way as that of ACh (Fig. 4).

With larger doses of ouabain (10–20 μg.) the potentiating effect is usually less pronounced and is often followed by a period of cardiac depression. In addition, cardiac irregularity frequently occurs, and is usually initiated by a subsequent injection of ACh.

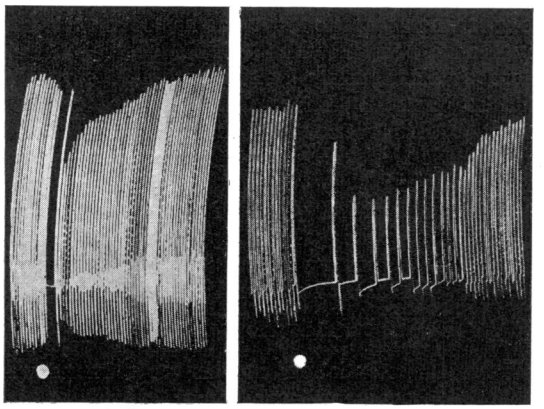

FIG. 4.—Isolated cat heart perfused with Locke's solution through coronary arteries. Injections into perfusion stream. At signals 4 μg. TMA. Between (a) and (b) 2·5 μg. ouabain.

In the presence of hexamethonium, perfused in a concentration sufficient to block the ganglionic effects of ACh on the heart (Perry and Talesnik, 1953), ouabain no longer potentiates the action of ACh or of TMA. In Fig. 5 the effect of ouabain is shown before and during the action of hexamethonium on the response to 1 μg. ACh. Before hexamethonium the ACh response is potentiated by 0.5 μg. ouabain; during perfusion with 300 μg./ml. hexamethonium, 1.0 μg. ACh no longer caused bradycardia but still caused a slight reduction in the amplitude of the contraction, and ouabain, in twice the previous dose (1.0 μg.), was now devoid of any potentiating effect.

In some experiments nicotine was used instead of hexamethonium to block the vagal ganglion cells; similar results were obtained.

Guinea-pig.—The guinea-pig heart behaved in most respects like the cat heart, and a typical experiment is shown in Fig. 6. This figure also illustrates the potentiation of vagal stimulation by 5 μg. of

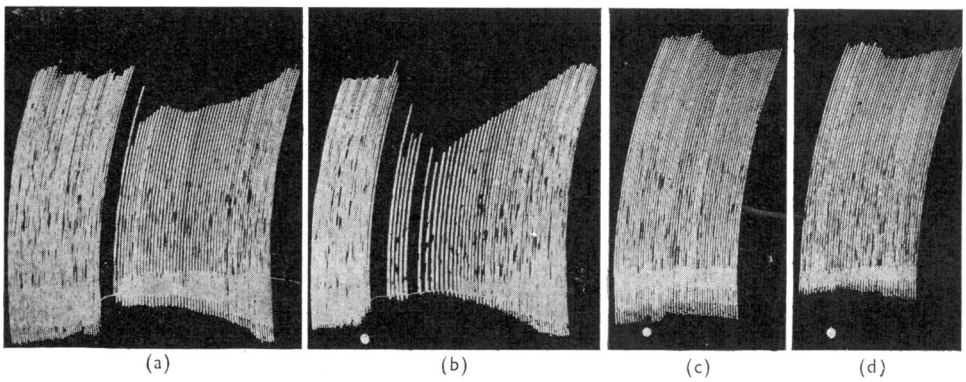

FIG. 5.—Isolated cat heart perfused through coronary arteries. Injections into perfusion stream. At signals 1 μg. ACh. Between (a) and (b) and between (c) and (d) 0·5 μg. ouabain. Between (b) and (c) perfusion fluid was changed from Locke's solution to Locke's solution containing 300 μg./ml. hexamethonium.

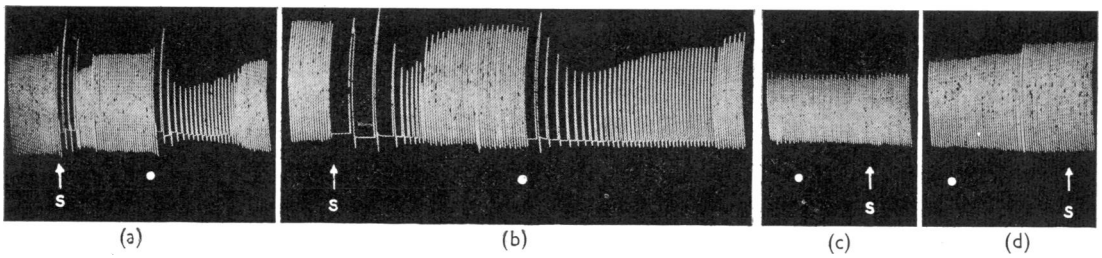

FIG. 6.—Isolated guinea-pig heart perfused through coronary arteries. Injections into perfusion stream. At "S" supramaximal stimulation of both vagi for 5 sec. (0·1msec. pulse; frequency 20/sec.). At signals 1 μg. ACh. Between (a) and (b) and between (c) and (d) 5 μg. ouabain. Between (b) and (c) perfusion fluid changed from Locke's solution to Locke's solution containing 500 μg./ml. hexamethonium.

ouabain. Hexamethonium is not such a potent ganglion-blocking agent in the guinea-pig as it is in the cat, and it was not possible to block the effect of small doses of ACh with concentrations of less than 500 μg./ml. With smaller concentrations the effect of ACh was partially blocked, and during this partial block ouabain still produced a potentiation of the response. When, however, the response to small doses of ACh and to vagal stimulation was completely blocked, ouabain ceased to have any potentiating action (Fig. 6). In this experiment 5 μg. ouabain had itself the effect of increasing the amplitude of the heart beat both before and during the action of hexamethonium.

Rat.—Ouabain potentiates the action of ACh on the rat heart; we were unable, however, to block this action by hexamethonium, even in large doses. This may be due to insensitivity of the ganglia of the rat to hexamethonium, or, more probably, to the fact that in the rat heart, which is very small, even small doses of ACh have a direct action on the myocardium as well as a ganglionic action.

Rabbit.—The results obtained from rabbit hearts were virtually identical with those obtained from guinea-pig hearts.

Discussion

Our results on the superior cervical ganglion of the cat confirm the findings of Konzett and Rothlin (1952), that cardiac glycosides potentiate the action of ACh and of preganglionic stimulation; furthermore, they show that the glycosides have the same effects on the ganglion perfused with Locke's solution as on a ganglion with its normal blood supply. Moreover, the finding that the glycosides potentiate the ganglion stimulant action not only of ACh but also of TMA makes it unlikely that the effects are due to an anticholinesterase activity of the glycosides, since TMA is not destroyed by cholinesterases.

The sensitization of the ganglion cells to the action of ACh produced by glycosides is very similar to the sensitization produced in denervated ganglia by the methonium compounds (Perry and Reinert, 1954b), which is probably associated with the transfer of potassium ions across the cell membrane. Thus, the failure of the glycosides to exert their sensitizing effect in the absence of external potassium may be significant, in that the action of ouabain may render the cell membrane more permeable to potassium ions, thus enhancing the effect of ACh. This action of the glycosides would resemble their action on striated muscle (Cattell, 1938), in which they also increase the permeability of the cell membrane to potassium; it would also resemble the dependence on the presence of external potassium of their negative inotropic effect on the isolated frog heart (Scarinci, 1953). Furthermore, the observation by Konzett and Rothlin (1952), that the glycosides may occasionally themselves exert a stimulant effect on the ganglion cells, could be accounted for by the same mechanism. The fact that large doses depress the action of ACh may be due to a second independent depressant action or to the fact that the continued leakage of intracellular potassium finally renders the cells relatively inexcitable.

The block of small doses of ACh produced by hexamethonium was interpreted by Perry and Talesnik (1953) as implying that these doses of ACh produced their action by stimulating the vagal ganglion cells and not the myocardium. In support of this contention they showed that hexamethonium did not exert any blocking action to the effect of stimulation of the post-ganglionic parasympathetic fibres supplying the pupil. We have now shown that the action of small doses of ACh is potentiated by the glycosides only when the vagal ganglia are intact, i.e. in the absence of blocking concentrations of hexamethonium; the action of larger doses of ACh, presumably directly on the myocardium, is not blocked by hexamethonium, nor is it potentiated by ouabain in the experiments which we report. We have not, however, attempted to investigate this last factor in detail, since it appears to be delayed in onset (Baker, 1953), and we have considered only the relatively short-term effects of this drug.

The doses which we have used would almost certainly, if given in dilute solution over a prolonged period, have other effects on the myocardium. It may well be that the administration of a large dose directly into the coronary artery is not a comparable situation to the clinical use of these compounds. Nevertheless, we consider it probable that the glycosides do have a direct action in sensitizing the vagal ganglion cells to ACh, and it should be borne in mind that this action may contribute in part to their clinical activity, and almost certainly to their toxic effects.

Summary

1. Small doses of cardiac glycosides potentiate the effects of preganglionic stimulation and of ACh and TMA on the superior cervical ganglion of the cat. Large doses of cardiac glycosides depress the action of ACh and TMA.

2. In the absence of external potassium, the glycosides exert only a depressant action on the superior cervical ganglion.

3. The glycosides potentiate the effect of vagal stimulation and of ACh and TMA on the isolated mammalian heart.

4. These actions are not present when the intracardiac vagal ganglion cells are blocked by hexamethonium or nicotine.

5. The rôle of the ganglion-sensitizing action of the cardiac glycosides is discussed in relation to their clinical activity and toxic effects and to their mode of action.

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