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# Comparison of the inotropic response to glucagon, ouabain and noradrenaline

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**Summary**

1. The inotropic activity of glucagon was compared with catecholamines and cardiac glycosides by *in vitro* procedures which were able to differentiate between the activities of the latter two groups.

2. The frequency-force curve for glucagon resembled that of noradrenaline at low stimulation frequencies (1 and 2/min) and that of ouabain at more rapid frequencies of stimulation.

3. Noradrenaline and adrenaline increased the amplitude of contraction of cat papillary muscles and markedly shortened the time to reach peak tension. Ouabain and glucagon increased tension without any change in the time to peak tension.

4. Noradrenaline caused a rapid onset and rate of rise of contraction of cat aortic strips, whereas the response to ouabain was slow in onset and rate of development. Glucagon had no effect on this preparation, even at high concentrations.

5. Manganese ions caused a shift of the dose-response curve to ouabain and glucagon, but not to noradrenaline or calcium. In 0·5 mM Ca media, the response to ouabain was abolished and the curve to noradrenaline shifted.

6. When glucagon was added to an atrial preparation, the time to the initial increase in tension and the time to maximal tension was intermediate between that necessary for noradrenaline and that necessary for cardiac glycosides.

7. Propranolol blocked the inotropic response to noradrenaline, but not to either ouabain or glucagon.

8. A relative measure of contraction-dependency was described. Cardiac glycosides exhibited a greater degree of contraction-dependency than either noradrenaline or glucagon.

9. Adrenaline elevated the depressed plateau of the action potential from calf and sheep Purkinje fibres, but ouabain and glucagon were without effect.

10. Electrophysiological measurements demonstrated that moderate concentrations of glucagon exerted only a small effect in prolonging atrial and ventricular action potentials.

11. Several pharmacological blocking drugs and other inotropic agents did not potentiate or block the inotropic response to glucagon. Reserpine pretreatment increased the response to glucagon.

12. It was concluded that glucagon has its own spectrum of inotropic activity and does not completely mimic the effects of either ouabain or noradrenaline.

## Introduction

Glucagon is a polypeptide hormone primarily secreted by the $\alpha$-cells of the pancreas, whose major role as a hyperglycaemic agent is well known. Glucagon also elicits a positive inotropic response *in vitro* (Farah & Tuttle, 1960 ; Lucchesi, 1968) and *in vivo* (Parmley & Sonnenblick, 1969 ; Eddy, O'Brien & Singh, 1969 ; Parmley, Glick & Sonnenblick, 1968) and has been shown to be useful in the treatment of patients with a variety of cardiac diseases, including those patients refractory to digitalis therapy (Brogan, Kozonis & Overy, 1969). The mechanism by which glucagon causes a positive inotropic response has not been elucidated, but one factor may be the stimulation of adenyl cyclase which it elicits (Levey & Epstein, 1969 ; Mayer, Namm & Rice, 1970). The purpose of this study was to characterize the inotropic activity of glucagon by comparing its pharmacological behaviour with that of cardiac glycosides and catecholamines, primarily by procedures which differentiate between the activities of the latter two groups. Ouabain and noradrenaline were chosen as representative examples of those groups.

## Methods

Male guinea-pigs (300–500 g) were killed by a blow on the head and their hearts then rapidly excised and placed in a dish of oxygenated (95% oxygen ; 5% carbon-dioxide) Krebs-bicarbonate solution (NaCl 118·4 mM, KCl 4·7 mM, $CaCl_2$ 2·5 mM, $MgSO_4 \cdot 7H_2O$ 1·2 mM, $NaHCO_3$ 25·0 mM, $KH_2PO_4$ 1·2 mM, and dextrose 11·1 mM). Left atria were dissected free of other tissues and were mounted in filled and well-oxygenated 20 ml muscle chambers maintained at 25° C, 30° C or 37° C, depending upon the nature of the experiment. A fine thread was tied around the tip of the cut base of the tissue, which was then brought into close proximity to the stimulating electrode on the muscle holder by a second tie. Another thread was attached to the tissue opposite the first tie and then attached to the force displacement transducer (Ether Corp.) directly above the muscle chamber. A resting tension of 1 g was applied to all atria. Devices Mark IV stimulators were used for producing rectangular pulses of 2 ms duration with voltages 10–15% above threshold at a frequency of 60 or 120 pulses/min. Isometric contractions were recorded on a Devices Polygraph.

Cats of either sex (2·0–3·3 kg) were anaesthetized with sodium pentobarbitone (30 mg/kg) and papillary muscles from the right ventricle were set up as above. Aortic strips from cats were dissected and mounted according to the method of Furchgott (1960), except that a resting tension of 2 g was applied to each strip and all contractions were recorded isometrically.

Calf and sheep hearts were obtained immediately after slaughter and placed in Tyrode solution of the following composition: NaCl, 137 mM ; KCl, 2·7 mM ; $CaCl_2$, 2·7 mM ; $MgCl_2$, 0·5 mM ; $NaH_2PO_4$, 0·9 mM ; $NaHCO_3$, 24·0 mM ; dextrose, 5·5 mM. Studies in "low" $Ca^{2+}$ were performed with $Ca^{2+} = 0{\cdot}45$ mM.

Pieces of papillary muscle with attached Purkinje fibres were dissected from both ventricles and the muscles stimulated at a frequency of 60/min with rectangular pulses of 1 ms duration at voltages 10–15% above threshold. Guinea-pig left atria and papillary muscles were also mounted for electrophysiological recordings. Experiments were performed at both 30° C and 37° C.

Transmembrane potentials were obtained using glass capillary electrodes filled with 3 M KCl. Electrode resistances varied between 15 and 30 MΩ. The signal picked up by the microelectrode passed through an agar filled cell with tungsten wire and was amplified by neutralized input capacity preamplifier and then passed simultaneously through a d.c. input audio-indicator and to an oscilloscope, from which film records were taken.

Drugs used in this study include crystalline glucagon (Sigma), ouabain octahydrate (Sigma), noradrenaline bitartrate (Koch-Light), adrenaline bitartrate (Burroughs-Wellcome), propranolol hydrochloride (I.C.I.), calcium chloride dihydrate (Hopkin and Williams) and manganous chloride (B.D.H.). Concentrations are expressed in terms of g of the salt per ml of medium in the muscle chamber, unless otherwise specified.

## Results

Dose-response curves for glucagon in guinea-pig atria at 30° C demonstrate threshold activity at $1 \times 10^{-8}$ and maximal activity at $1{\cdot}5 \times 10^{-6}$ (Fig. 1). The $\beta$-adrenoceptor blocking agent propranolol, at a concentration ($5 \times 10^{-7}$ for 40 min) which effectively blocked the noradrenaline-induced increase in contractility, had no significant effect on the dose-response curves for glucagon, or on those for ouabain.

Frequency-force (interval-strength) curves were determined with ouabain, noradrenaline and glucagon (Figs. 2, 3 and 4). The frequency-force curve determined 40 min after ouabain ($1 \times 10^{-7}$) demonstrated an upward parallel shift at all stimulation frequencies tested up to 180/min (Fig. 2). The curve for noradrenaline ($5 \times 10^{-7}$), obtained 5 min after addition, was not significantly changed from control at low stimulation frequencies (1 and 2/min). Above those frequencies,

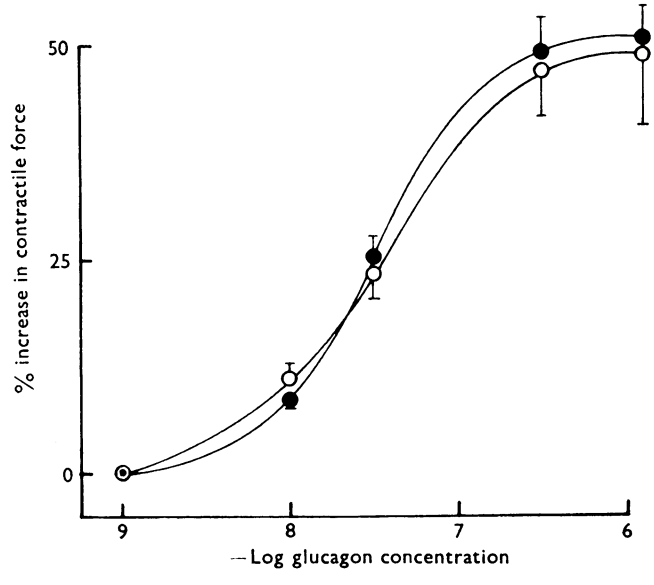


FIG. 1. Dose-response curves to glucagon before (●) and after (○) propranolol. Each value was the mean (±S.E.) of nine experiments. Concentration is expressed as −log g/ml.

noradrenaline abolished the triphasic nature of the curve, as there were no significant differences between contractile amplitudes recorded at frequencies between 24 and 180 beats/min (Fig. 3). Glucagon ($1 \times 10^{-6}$) had no significant effect on the frequency-force curve at frequencies of 1 and 2/min, but caused an upward parallel shift of the curve at faster stimulation frequencies (Fig. 4). No significant differences were obtained between the curves obtained five and forty min after the addition of glucagon. Therefore, the frequency-force curve for glucagon resembled that of noradrenaline at low stimulating frequencies and that of ouabain at more rapid frequencies of stimulation.

Cat aortic strips respond to noradrenaline and ouabain by a contraction of their smooth muscle fibres. The length of time which was necessary to reach maximal contraction was less for noradrenaline than for ouabain (Fig. 5). Glucagon ($10^{-8}$ to $10^{-3}$) was inactive in this preparation at both 30° C and 37° C ($n=3$). Glucagon ($10^{-8}$ to $10^{-4}$) was also inactive when tested on rat arterial rings and in the perfused

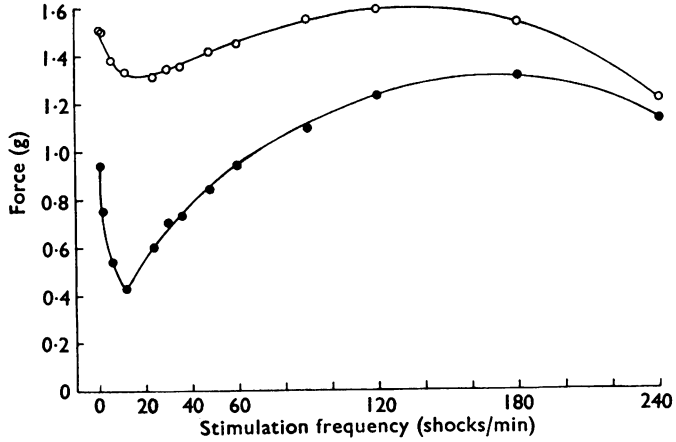


FIG. 2. Frequency force curves of guinea-pig left atria at 30° C, obtained before (●) and 40 min after (○) ouabain ($1 \times 10^{-7}$ g/ml) ($n=7$). Every S.E. was less than 10% of the value of each point.

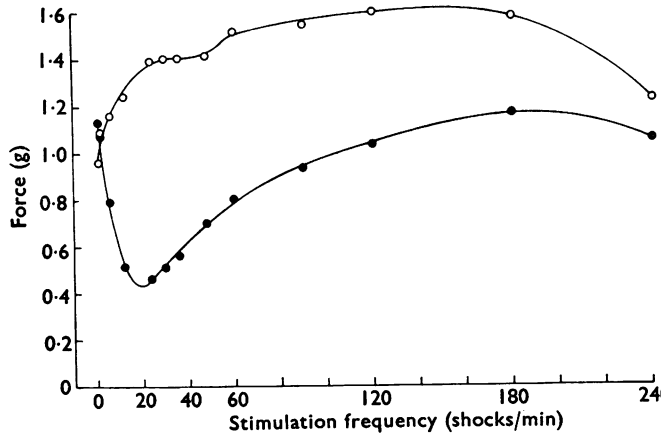


FIG. 3. Frequency-force curves of guinea-pig left atria at 30° C, obtained before (●) and 5 min after (○) noradrenaline ($5 \times 10^{-7}$ g/ml) ($n=6$). Every S.E. was less than 10% of the value of each point.

rabbit ear artery, which suggests that glucagon acts differently from ouabain and noradrenaline in these arterial smooth muscle preparations.

In cat papillary muscle preparations noradrenaline and adrenaline increased the strength of isometric tension while greatly shortening the time to reach peak tension (Fig. 6)—that is the rate of tension development was markedly increased while the duration of the active state was markedly decreased (Fig. 6). Ouabain also increased the rate of tension development, but had little or no effect on the duration of the active state (Fig. 6). Glucagon increased the rate of tension development, but did not alter the time to reach peak tension in cat papillary muscle preparations and behaved similarly to ouabain (Fig. 6). The time for relaxation to be completed

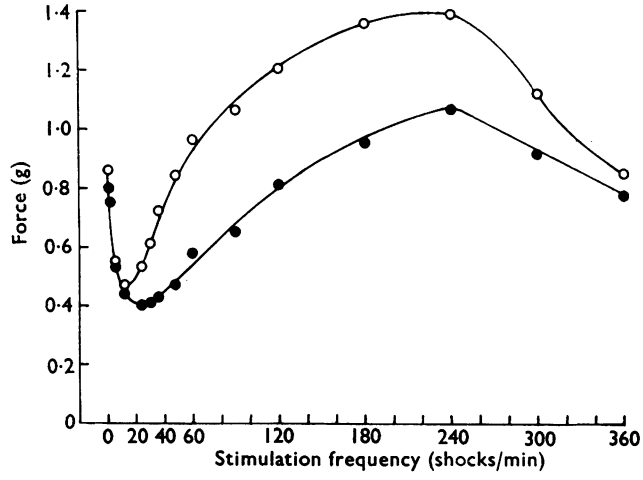


FIG. 4. Frequency-force curves of guinea-pig left atria at 30° C, obtained before (●) and 5 min after (○) glucagon ($1 \times 10^{-6}$ g/ml) ($n=7$). Every S.E. was less than 10% of the value of each point.

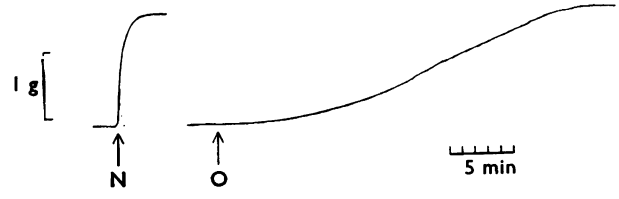


FIG. 5. Contractile responses of cat aortic strips at 30° C to noradrenaline (N) ($5 \times 10^{-6}$ g/ml) and to ouabain (○) ($1 \times 10^{-4}$ g/ml).

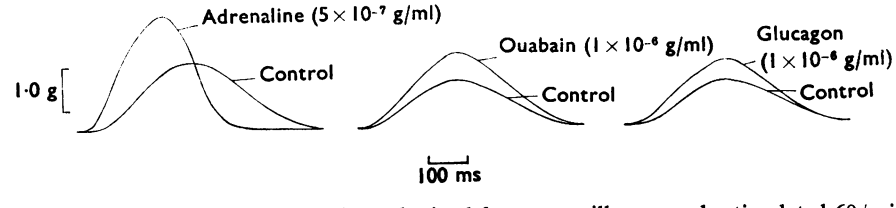


FIG. 6. Isometric contractile recordings obtained for cat papillary muscle stimulated 60/min at 30° C.

as well as the total duration of contraction were unaltered by ouabain and glucagon.

Manganese ions (Mn) have been alleged to compete with calcium ions (Ca) in heart muscle (Sabatini-Smith & Holland, 1969; Yanaga & Holland, 1969; Meinertz & Scholz, 1969), aortic smooth muscle (Sullivan & Briggs, 1968) and in barnacle muscle fibres (Hagiwara & Nakajima, 1966). Yanaga & Holland (1969) also demonstrated that Mn ($5 \times 10^{-3}$) depressed the plateau of the action potential in rabbit atria which was reversed by high Ca concentrations. Sabatini-Smith & Holland (1969) reported that Mn blocked the uptake of $^{45}Ca$, and competitively antagonized the positive inotropic response to ouabain. They concluded that a part of the Ca involved in activation of mammalian heart contractions moves inward through the membrane during depolarization, the remainder coming from cellular storage sites. Manganese and ouabain were said to act at the membrane and/or cellular storage sites to influence Ca movement. Meinertz & Scholz (1969) were unable to demonstrate any shift of the dose-response curve to digitoxigenin after Mn, even in low Ca media, and could not account for the discrepancy between their results and those of Sabatini-Smith & Holland (1969). They did, however, observe shifts in the dose-response curves to both noradrenaline and theophylline after Mn.

Mn itself elicited a negative inotropic dose-response curve in electrically driven (120 beats/min) guinea-pig left atria (Table 1) at 26° C. A concentration of $6 \times 10^{-4}$M, which depressed contractile amplitude by $24{\cdot}5 \pm 5{\cdot}6\%$, was chosen to

TABLE 1. *Effect of Mn on contractile tension in guinea-pig left atria*

| Concentration (M) | % decrease in contractile tension ±S.E.* |
|---|---|
| $6 \times 10^{-5}$ | 0 |
| $1 \times 10^{-4}$ | 1·5±3·5 |
| $3 \times 10^{-4}$ | 9·5±4·4 |
| $6 \times 10^{-4}$ | 24·5±5·6 |
| $1 \times 10^{-3}$ | 44·2±5·9 |
| $3 \times 10^{-3}$ | 77·1±2·2 |

* $n = 6$ for each value.

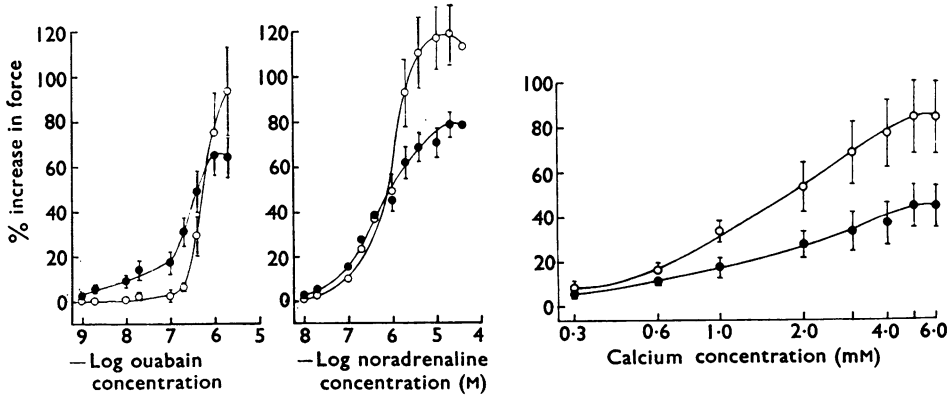


FIG. 7. Dose-response curves to ouabain (left), noradrenaline (centre) and calcium (right), before (●) and after (○) Mn ($6 \times 10^{-4}$M) at 26° C. Each value is the mean of six experiments ±S.E. All values for ouabain up to and including $3 \times 10^{-7}$, after Mn, are significantly less than control ($P<0{\cdot}02$). The four highest values for noradrenaline after Mn are significantly greater than control ($0{\cdot}05>P$).

determine whether it would block the inotropic response of the agonists (ouabain, noradrenaline, glucagon and Ca).

A dose-response curve was obtained for ouabain, followed by the addition of Mn ($6 \times 10^{-4}$M) which was allowed to fully equilibrate with the tissue. A second dose-response curve for ouabain was then determined. The second curve was shifted to the right (Fig. 7). Mn also caused a marked shift to the right for the dose-response curve of glucagon (Fig. 8). The second dose-response curves for noradrenaline and Ca, however, were not shifted to the right by Mn (Fig. 7). Two dose-response curves for ouabain determined in the absence of Mn served as a control and were essentially identical. Two successive dose-response curves for glucagon, noradrenaline and Ca were also essentially identical when Mn was not present.

Although Mn ($6 \times 10^{-4}$M) depressed contractile responses by 25%, it is conceivable that the concentration of Ca in the bathing medium (2·5 mM) was too high to allow Mn to compete successfully and to shift the curves for noradrenaline and Ca. In order to test this possibility, Krebs-bicarbonate solution was prepared with one-fifth of the normal Ca concentration and dose-response curves determined for ouabain, noradrenaline and Ca in the presence of Mn (Fig. 9). The dose-response curves for Ca in the presence of Mn were the same as in normal Ca-Krebs (Fig. 8) —that is Mn had no depressant effect on the dose-response curve to Ca. The apparent potentiation of Ca by Mn was due to the lower control values after Mn and the same increase in g tension after Ca. However, Mn completely abolished the response to ouabain and caused some shift to the right of the dose-response curve for noradrenaline (Fig. 9).

The present experiments as well as those of Sabatini-Smith & Holland (1969), which showed a shift of the dose-response curve for ouabain after Mn, were performed at 26° C. However, the experiments of Meinertz & Scholz (1969), which did not demonstrate a shift of the dose-response curve for digitoxigenin after Mn, were performed at 35° C. In order to determine whether temperature might account for the conflicting observations, the present experiments were repeated at 37° C.

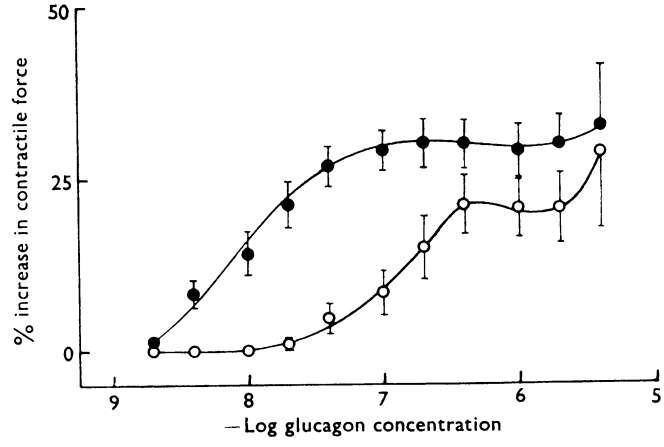


FIG. 8. Dose-response curves to glucagon in guinea-pig left atria at 26° C, obtained before (●) and after (○) Mn ($6 \times 10^{-4}$M). Each value is the mean of six experiments ± S.E. All values from $6 \times 10^{-9}$ to $10^{-7}$ after Mn were significantly less than control ($P < 0{\cdot}01$). The value for $3 \times 10^{-7}$ is significantly less ($0{\cdot}05 > P > 0{\cdot}02$).

The results with ouabain at 37° C show a similar shift to the right as in Fig. 7 ($n=6$). The dose-response curve to noradrenaline was not shifted to the right at 37° C and results were similar to those shown in Fig. 7 ($n=6$).

When catecholamines were added to atrial muscle preparations *in vitro*, the onset of the positive inotropic response was almost immediate at both 26° C and 37° C, whereas the onset of activity, after cardiac glycosides were added to the tissue bath, occurred after a latent period of several minutes (Table 2). In most conditions the latent period was not markedly affected by the rate of stimulation of the tissue but was decreased as the temperature was increased and as the concentration of ouabain was increased. The onset of the positive inotropic response to glucagon was more rapid than to ouabain but slower than to noradrenaline.

The time to reach maximal inotropic activity from the onset of the positive inotropic response was greater for cardiac glycosides than for catecholamines (Table 3). The peak response to glucagon was achieved more rapidly than to ouabain but slower than to noradrenaline. Nonetheless, the response to glucagon reached its

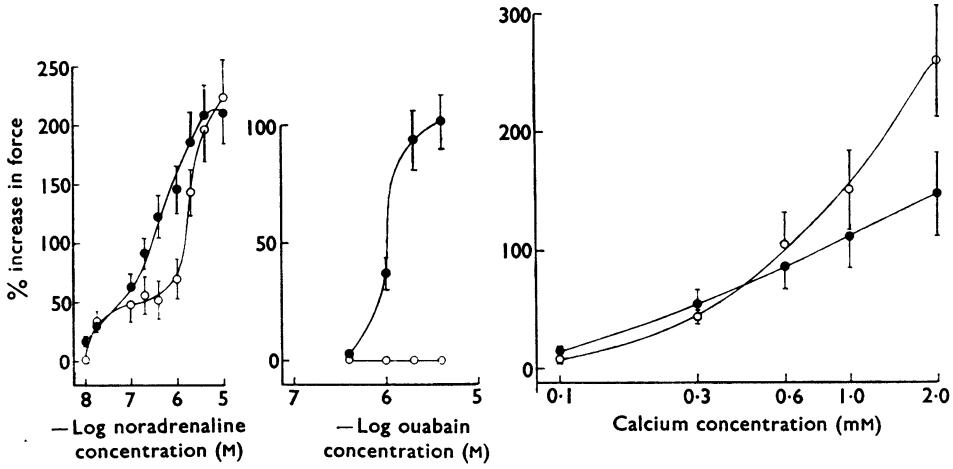


FIG. 9. Dose-response curves to noradrenaline (left), ouabain (centre) and calcium (right), before (●) and after (○) Mn ($6\times10^{-4}$M) at 26° C in 0·5 mM Ca Krebs-bicarbonate solution. Each value is the mean of six experiments ± S.E. None of the values for noradrenaline or calcium after Mn are significantly less than control.

TABLE 2. *Time to onset of positive inotropic activity in guinea-pig atria after noradrenaline, ouabain and glucagon*

| Agonist and concentration (g/ml) | Time to initial positive inotropic response in min ± S.E.* | | | |
|---|---|---|---|---|
| | 26° C Stimulating frequency | | 37° C Stimulating frequency | |
| | 15/min | 120/min | 15/min | 120/min |
| Noradrenaline $3\times10^{-7}$ | 0·30±0·03 | 0·33±0·09 | 0·21±0·01 | 0·11±0·01 |
| Ouabain $1\times10^{-7}$ | 9·25±0·93 | 3·53±0·48 | 6·46±1·20 | 7·96±0·63 |
| $1\times10^{-6}$ | 4·83±0·31 | 4·07±0·47 | 3·40±0·59 | 1·51±0·32 |
| Glucagon $1\times10^{-6}$ | 1·57±0·12 | 1·46±0·31 | | |
| $1\times10^{-5}$ | | | 1·36±0·16 | 0·84±0·11 |

* $n$ = 6–9.

maximal effect rapidly and more closely resembled noradrenaline than ouabain. Increasing the temperature from 26° C to 37° C decreased the time necessary for all three agonists to attain their maximal inotropic activity (Table 3).

The maximal inotropic response to cardiac glycosides has been shown to occur after a specific number of contractions have occurred, regardless of the frequency of stimulation. The phenomenon has been referred to as contraction dependency (Moran, 1963, 1967).

An agent that exhibited contraction dependency would take eight times as long to reach its maximal response at a stimulating frequency of 15 beats/min as at 120 beats/min ; however, the ratio of the number of contractions necessary to reach the maximal inotropic response at 120 beats/min over the number of contractions necessary to reach the peak response at 15 beats/min would equal 1·0. The experimentally determined value of this ratio:

$$\frac{\text{(120 beats/min) (number of min to reach 100\% of the elicited response at a stimulating frequency of 120/min)}}{\text{(15 beats/min) (number of min to reach 100\% of the elicited response at a stimulation frequency of 15/min)}}$$

may be used as a relative degree of contraction dependency for any inotropic agent. The stimulating frequencies of 15 and 120/min were arbitrarily chosen because they were the extreme frequencies used by Moran (1963, 1967) in his demonstration of contraction dependency in rabbit atria. Although the ratio is based on the time to reach 100% of the total response, a similar ratio may be determined for 10, 20, 50 or any percentage of the maximal response. Since cardiac glycosides illustrate contraction dependency, they would be expected to take eight times as long to reach their maximal response at 15 beats/min as at 120/min, yielding a final ratio of 1·0. If an agent did not exhibit any degree of contraction dependency then the time to maximal effect would be the same whether the tissue was stimulated at 15 or 120/min and the ratio would be 8·0. The ratios for ouabain, noradrenaline and glucagon at 26° C and 37° C are presented in Tables 4 and 5.

Neither noradrenaline nor glucagon illustrated the phenomenon of contraction dependency. The contraction dependency ratio for ouabain was greater than the theoretical value of 1·0, but indicated that ouabain does exhibit at least partial contraction dependency in guinea-pig atria at these temperatures.

TABLE 3. *Time from the onset of positive inotropic activity to maximal inotropic response after noradrenaline, ouabain and glucagon*

| Agonist and concentration (g/ml) | Time from initial response to peak inotropic response ± S.E.* | | | |
|---|---|---|---|---|
| | 26° C Stimulating frequency | | 37° C Stimulating frequency | |
| | 15/min | 120/min | 15/min | 120/min |
| Noradrenaline $3\times10^{-7}$ | 4·20±0·11 | 1·11±0·08 | 2·65±0·47 | 1·46±0·24 |
| Ouabain $1\times10^{-7}$ | 113·2±0·71 | 36·8±2·39 | 36·44±4·39 | 27·7±0·80 |
| $1\times10^{-6}$ | 54·1±5·88 | 9·53±1·82 | 25·55 | 7·18 |
| Glucagon $1\times10^{-6}$ | 7·96±0·73 | 3·44±0·32 | | |
| $1\times10^{-5}$ | | | 4·03±0·26 | 2·16±0·39 |

* $n$ = 6–9 experiments, except for ouabain ($1\times10^{-6}$) where $n$ = 4 because several other tissues became spontaneously active at this concentration at 37° C.

In order to determine whether the inotropic response to glucagon would be affected by other inotropic drugs or by pharmacological blocking agents, several substances were studied. The inotropic response to glucagon ($5 \times 10^{-7}$) was neither blocked nor potentiated by the following agents: atropine ($1 \times 10^{-7}$), triprolidine (antihistamine; $3{\cdot}4 \times 10^{-6}$), imidazole ($3 \times 10^{-3}$), potassium (14·8 mM), 5-hydroxytryptamine ($5 \times 10^{-7}$), theophylline ($5 \times 10^{-4}$), histamine ($5 \times 10^{-7}$), carbamylcholine ($5 \times 10^{-7}$), noradrenaline ($1 \times 10^{-7}$), dimethylsulphoxide (5% v/v), dimethylformamide (2% v/v), $CaCl_2$ (5 mM) or ouabain ($5 \times 10^{-7}$); nor did glucagon affect the responses to any of these agonists ($n=3$ for each experiment).

Reserpine pre-treatment (1 mg/kg intraperitoneally for each of 3 days), however, did cause a greater response to glucagon in guinea-pig atria (Fig. 10).

Electrophysiological recordings were made in guinea-pig left atria and papillary muscles at 30° C and 37° C before and after glucagon ($5 \times 10^{-7}$ and $1 \times 10^{-6}$). Although the increase in tension was less marked at 37° C there was a small (14–18%) prolongation of the action potential at both temperatures. All other electrophysiological parameters measured (resting membrane potential amplitude, action potential amplitude, maximal rate of depolarization and time to 10% repolarization) were unchanged.

TABLE 4. *Contraction dependency ratios and % increase in contractile force for noradrenaline, ouabain and glucagon at* 26° *C*

| Agonist and concentration (g/ml) | Ratio of beats at a stimulation frequency of 120 min over 15/min for: 50% maximal inotropic response | Ratio of beats at a stimulation frequency of 120 min over 15/min for: 100% (total) inotropic response | % increase in contractile force at stimulating frequencies of: 15/min | % increase in contractile force at stimulating frequencies of: 120/min |
|---|---|---|---|---|
| Noradrenaline $3 \times 10^{-7}$ ($n=6$) | 3·60 | 2·57 | 154·8 | 54·0±10·5 |
| Ouabain $1 \times 10^{-7}$ ($n=6$) | 1·75 | 2·62 | 122·9± 23·5 | 32·3±3·14 |
| $1 \times 10^{-6}$ ($n=7$) | 2·41 | 1·80 | 152·5± 13·8 | 40·9±6·45 |
| Glucagon $1 \times 10^{-6}$ ($n=6$) | 4·14 | 4·11 | 29·1± 3·69 | 23·5±3·62 |

TABLE 5. *Contraction dependency ratios and % increase in contractile force for noradrenaline, ouabain and glucagon at* 37° *C*

| Agonist and concentration (g/ml) | Ratio of beats at a stimulation frequency of 120/min over 15/min for: 50% maximal inotropic response | Ratio of beats at a stimulation frequency of 120/min over 15/min for: 100% (total) inotropic response | % increase in contractile force at stimulating frequencies of: 15/min | % increase in contractile force at stimulating frequencies of: 120/min |
|---|---|---|---|---|
| Noradrenaline $3 \times 10^{-7}$ ($n=8$) | 5·50 | 4·10 | 144·1± 37·3 | 104·3±17·9 |
| Ouabain $1 \times 10^{-6}$ ($n=4$) | 2·55 | 2·45 | 300·3 | 70·7 |
| Glucagon $1 \times 10^{-5}$ ($n=6$) | 4·55 | 4·38 | 44·0× 11·6 | 29·4±3·9 |

The plateau of calf or sheep Purkinje fibre action potentials is sensitive to external Ca concentrations (Reuter, 1967). Lowering the Ca levels to 0·45 mM depressed the amplitude of the plateau at both 30° C and 37° C. This depression was reversed by the addition of Ca or adrenaline ($5 \times 10^{-7}$; $n=5$) (Fig. 11). Ouabain ($5 \times 10^{-7}$ and $1 \times 10^{-6}$) had no effect upon the depressed plateau at either 30° C or 37° C (Fig. 11, $n=5$). Mn ($6 \times 10^{-4}$M) did not affect the plateau of calf Purkinje fibres in normal Tyrode solution (2·7 mM Ca) at 30° C ($n=4$). Glucagon ($5 \times 10^{-7}$) and Mn also had no effect on the plateau of calf Purkinje fibres conducted in " low " Ca (0·45 mM) at 30° C (n=4). Similar results were obtained in Purkinje fibres isolated from sheep.

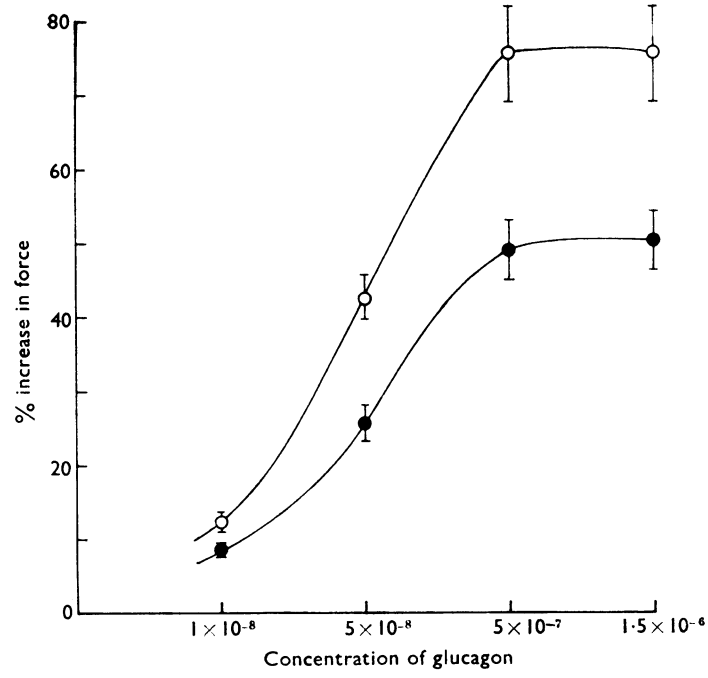


FIG. 10. Dose-response curves to glucagon (1 mg/kg, 3 days) in normal (●) ($n=12$) and in reserpine-pretreated (○) guinea-pig preparations ($n=9$).

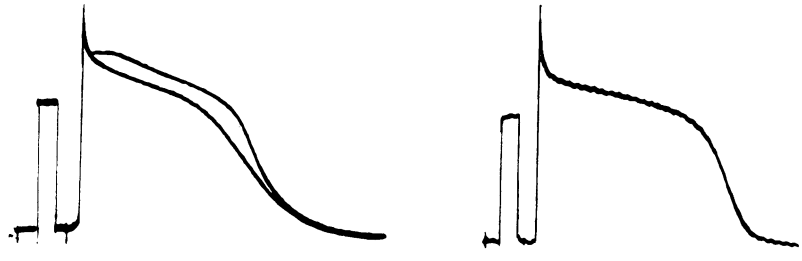

FIG. 11. Left: Two superimposed action potentials recorded in calf Purkinje fibres in 0·45 mM Ca Tyrode solution, obtained before (lower trace) and after (upper trace) adrenaline ($5 \times 10^{-7}$ g/ml). Right: Two superimposed and identical action potentials recorded in calf Purkinje fibres in 0·45 mM Ca Tyrode solution, obtained before and after ouabain ($1 \times 10^{-6}$ g/ml). The calibration signal represents 60 mV and 50 ms.

**Discussion**

Positive inotropic responses to glucagon were studied by *in vitro* procedures, many of which differentiate between the actions of cardiac glycosides and catecholamines on heart and aortic smooth muscle tissue.

The dose-response curves for glucagon in the present experiments are in general agreement with those previously reported (Glick, Parmley, Wechsler & Sonnenblick, 1968) as is the finding that the response of glucagon is not blocked by propranolol (Glick *et al.*, 1968 ; Lucchesi, Stutz & Winfield, 1969).

Frequency-force curves determined after glucagon were different from those determined after ouabain or noradrenaline. Glucagon did not alter contractile responses at low stimulation frequencies and elevated the curve at faster stimulation frequencies. The former effect is similar to that shown by noradrenaline and the latter effect similar to that shown by ouabain at moderate concentrations.

Mn has been alleged to compete with Ca since it has been shown to block the uptake of $^{45}Ca$ and to cause a shift to the right of the dose-response curve for ouabain in rabbit atria (Sabatini-Smith & Holland, 1969). The present experiments confirmed this latter finding with ouabain and demonstrated a similar result with glucagon in guinea-pig atria. However, no shift of the dose-response curve for Ca or noradrenaline was observed after the addition of Mn. In low (0·5 mM) Ca Krebs, dose-response curves to Ca were not affected by Mn, but the dose-response curve to ouabain was abolished and that to noradrenaline shifted to the right.

Meinertz & Scholz (1969), however, did not observe any shift of the dose-response curve to digitoxigenin in the presence of Mn, but observed a shift to the right of the dose-response curve for noradrenaline. The reason for the discrepancy between the results for ouabain and digitoxigenin was shown in the present experiments not to be related to the temperature of the media, but was presumably due to differences between the two agents. When the results of Meinertz & Scholz (1969) for noradrenaline were plotted as % increase in force instead of $\Delta$ g/g dry weight as they did, similar curves to the present results (Fig. 7) were obtained. Thus it appears that the discrepancy between their results and the present experiments for noradrenaline is primarily due to the manner of presenting the data.

The time to peak contractile tension in isometrically beating papillary muscles is a measure of the duration of the active state (Sonnenblick, 1962). Noradrenaline increased tension in this preparation, while markedly shortening the time to reach peak tension. Ouabain also elicited an increase in tension, but did not affect the time to reach peak tension. These findings have been well documented (Sonnenblick & Parmley, 1967). Glucagon was found to act similarly to ouabain in that the increased amplitude of contraction was not accompanied by a change in the time to peak tension.

The technique for differentiating between the actions of ouabain and noradrenaline with cat aortic strips on the basis of time required to reach maximal activity has been previously reported (Thorp & Cobbin, 1967). The lack of contractile activity after glucagon is not completely surprising, because glucagon has been shown to have various effects in the vasculature. Ross (1969) reported that glucagon (1–10 $\mu$g) dilated mesentery resistance vessels, constricted the hepatic arterial vascular bed and had no effect on the renal and femoral vasculature and

concluded that these vasomotor changes were probably not mediated by β-adrenoceptors. Glucagon was also without effect when perfused or injected into the *in vitro* rabbit ear artery and in the rat aortic ring preparation, both of which responded to noradrenaline and ouabain.

A relative measure of contraction dependency was obtained by the determination of a ratio which varied from 1·0 for total contraction dependency to 8·0 for a complete lack of contraction dependency. It was possible on this basis to differentiate between the activities of ouabain and noradrenaline. The ratios were calculated for both the number of beats to reach 50% and 100% of the maximal response, although Moran (1963, 1967) reported that the ratio for 50% maximal activity was the most accurate indicator of contraction dependency. The ratio for ouabain was greater than the predicted value of 1·0, which is in agreement with the results of Vincenzi (1967), who only observed a partial contraction dependency with ouabain on guinea-pig atria. He stated that temperature may be the most important factor responsible for observing contraction dependency since strict contraction dependency was reported in rabbit atria at relatively low temperatures (30° C or less). The results of Moran (1967) agree with this observation in that the ratios to 50% increase in force were greater at 37° C than at 30° C. We also observed lower ratios for ouabain (a greater degree of contraction dependency) at lower temperature, but found that the ratios for noradrenaline were also lowered, which made the separation of their actions more difficult than at 37° C.

The plateaux of calf and sheep Purkinje fibre action potentials were sensitive to extracellular Ca concentrations. In low (0·45 mM) Ca Tyrode solution the amplitude or height of the plateau was decreased. This decrease has been shown to be elevated by adrenaline or Ca (Reuter, 1967) and in the present experiments these results were confirmed. It was also observed that neither ouabain nor glucagon had any effect on the depressed plateau. Reuter (1967) and Reuter & Beeler (1969) have shown that Ca is responsible for a slow inward current during membrane depolarization which is distinct from the sodium inward current. Reuter & Beeler concluded that the "$Ca^{2+}$ inward current serves primarily to fill up some intracellular stores from which $Ca^{2+}$ can be released by moderate depolarization".

Adrenaline has been shown to increase $^{45}Ca$-influx in contracting guinea-pig atria (Reuter, 1965) as well as to increase the Ca-dependent slow inward current in Purkinje fibres (Reuter, 1967). Since glucagon had no effect on the depressed plateau of the Purkinje fibre action potential it would indicate that it was acting by a different mechanism than the catecholamines.

The only significant effect of glucagon on guinea-pig atrial and papillary muscle action potentials, at a concentration which yielded the maximal inotropic response, was to cause a small prolongation in the time for repolarization to be complete. Both ouabain and noradrenaline can cause either a small prolongation or shortening of the action potential, depending on the experimental conditions, so that the observations with glucagon were not considered especially important.

It is concluded that glucagon has its own spectrum of inotropic activity and does not completely mimic the effects of either ouabain or noradrenaline, and thus cannot be said to exert its effect by the stimulation of cardiac glycoside or β-adrenoceptors.

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