Life Sciences Vol. 9, Part I, pp. 1081-1089, 1970. Pergamon Press
Printed in Great Britain

THE INTERACTION OF GLUCAGON WITH THEOPHYLLINE, PGE_1, ISOPROTERENOL, OUABAIN, AND $CaCl_2$ ON THE DOG ISOLATED PAPILLARY MUSCLE[1]

Michael J. Antonaccio and Benedict R. Lucchesi

Department of Pharmacology, The University of Michigan
Medical School, Ann Arbor, Michigan 48104

(Received 22 May 1970; in final form 3 August 1970)

Glucagon has been shown to be both a positive inotropic and chronotropic agent in many species including man (1-5). However, the positive inotropic effect of glucagon on dog papillary muscles can be reversed by previous exposure of the tissues to a relatively high concentration of ouabain (2). The purpose of this study was to determine the interactions of glucagon on dog isolated papillary muscles with other positive inotropic agents which presumably have a different mechanism of action.

Methods

Mongrel dogs, 6 to 14 kg in weight, were anesthetized with sodium pentobarbital (30 mg/kg i. v.) and one hour later the right ventricular papillary muscles were rapidly removed and suspended in an organ bath containing 30 ml of oxygenated Chenoweth-Koelle solution maintained at 37° C. One end of the muscle was attached by silk thread to a muscle holder and the other end to a Grass FT03 force displacement transducer. The muscles were placed under a resting tension of 2 g and stimulated through a punctate electrode with an AEL stimulator at a rate of 30 stimuli/minute. The applied stimuli were square-wave pulses of 2 msec duration at a threshold voltage. Isometric recordings were obtained to step-wise increases in the concentration of drugs added to the organ bath after the tension for each dose reached a steady level. When the interaction of glucagon and other drugs was studied, dose-response relationships were determined after the maximum response to the initial drug

[1] This study was supported by a grant from the Michigan Heart Association.

in the organ bath was obtained. The maximum response to a paired pulse was determined with each preparation either before the addition of a drug or after exposure to the first drug when combined drug effects were studied. All results were expressed as a percentage of the maximum response obtained with paired pulse stimulation. The maximum response to a second paired pulse at the completion of a dose-response curve also was determined.

The significance of differences was assessed by Student's t test; P values and standard errors of the mean are given in parentheses (6).

Drugs

The drugs used in this study were: l-isoproterenol hydrochloride, prostaglandin E_1 (PGE_1), theophylline, ouabain hydrochloride, $CaCl_2 \cdot 2H_2O$, and crystalline glucagon (supplied by Eli Lilly Research Laboratories, Indianapolis, Ind.). All drug solutions were prepared by dissolving the agents in Chenoweth-Koelle solution.

Results

I. Interaction of Glucagon with Theophylline: Glucagon increased the tension of the dog isolated papillary muscle as shown in FIG. 1. Theophylline (3×10^{-4}M) produced a 16.6 ± 4.7% increase in isometric tension and had no effect on the normal response of the tissues to glucagon or on the ability of the tissue to respond to a second paired pulse (TABLE 1). A higher concentration of theophylline (10^{-3}M) increased the tension of the muscles by 28.1 ± 4.8% and significantly reduced both the response of the tissues to glucagon (FIG. 1) and to a second paired pulse (TABLE 1).

II. Interaction of Glucagon with PGE_1: PGE_1 (2.5×10^{-7}M) had no effect on the muscle by itself (1.3 ± 1.4%, $P > 0.4$) nor did it have any effect on the responses of the tissues to glucagon (FIG. 2). Concentrations of PGE_1 (10^{-5}M) that increased the isometric tension of the papillary muscles by 12.9 ± 2.4% had no effect on the response of the muscle to glucagon (FIG. 2). Neither concentration of PGE_1 affected the ability of the tissues to respond to a second paired pulse (TABLE 1).

III. Interaction of Glucagon with Isoproterenol: Isoproterenol increased

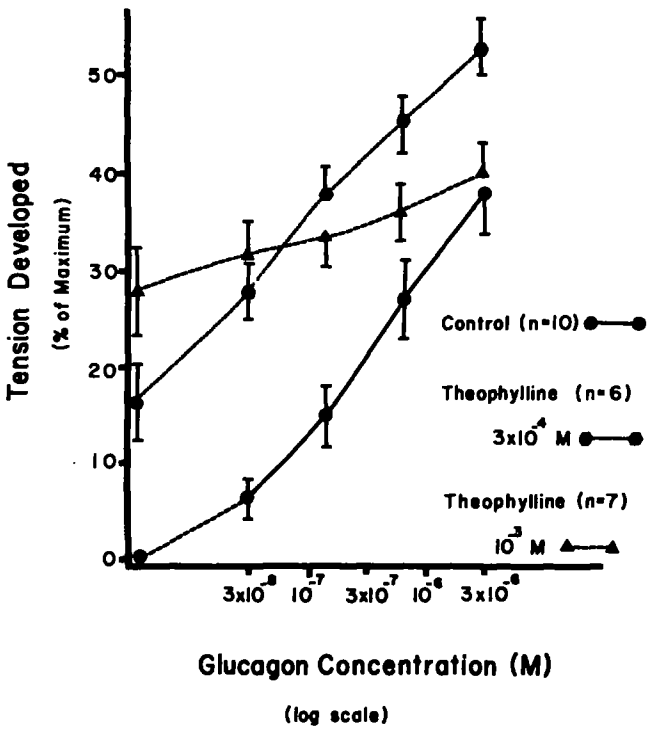

FIG. 1

Effect of theophylline upon the response of dog isolated papillary muscle to glucagon. Ordinate, change in tension developed, expressed as a percentage of the maximal response to a paired pulse. Shown are dose-response curves for glucagon on papillary muscles from at least 6 animals. Given are mean values. Vertical lines represent standard errors of the mean.

the tension of the isolated dog papillary muscle as shown in FIG. 3. Exposure of the tissues to glucagon (2. 6 μg/ml; 3. $3x10^{-8}$M) increased the tension of the muscles by 24. 0 ± 3. 5% and reduced the responses to lower concentrations of isoproterenol. The response to a second paired pulse was unaltered (TABLE 1). A larger concentration of glucagon (10. 6 μg/ml; 3. $5x10^{-6}$M) increased the isometric tension by 34. 5 ± 4. 1% and reduced the responses both to isoproter-

TABLE 1

Effects of Drug Treatment upon Responses of Dog Papillary Muscles to Paired Pulse Stimulation*

Drug(s)	% of maximum response to paired pulse stimulation
Glucagon 3.5×10^{-6}M	101.7 ± 6.6
PGE_1 1×10^{-5}M	98.3 ± 3.4
Isoproterenol 3×10^{-4}M	97.4 ± 1.2
Theophylline 3×10^{-4}M + Glucagon 3.5×10^{-6}M	93.5 ± 2.8
Theophylline 1×10^{-3}M + Glucagon 3.5×10^{-6}M	83.3 ± 2.2
PGE_1 2.5×10^{-7}M + Glucagon 3.5×10^{-6}M	102.5 ± 1.9
PGE_1 1×10^{-5}M + Glucagon 3.5×10^{-6}M	99.0 ± 3.2
Glucagon 3.3×10^{-8}M + Isoproterenol 3×10^{-4}M	96.2 ± 5.1
Glucagon 3.5×10^{-6}M + Isoproterenol 3×10^{-4}M	50.6 ± 11.7
Ouabain 3.3×10^{-7}M + Glucagon 3.5×10^{-6}M	97.2 ± 2.1
Ouabain 6.9×10^{-7}M + Glucagon 3.5×10^{-6}M	92.6 ± 13.3
Ouabain 1×10^{-6}M + Glucagon 3.5×10^{-6}M	72.7 ± 4.2
Ouabain 1.3×10^{-6}M + Glucagon 3.5×10^{-6}M	52.6 ± 5.7
Ouabain 2×10^{-6}M + Glucagon 3.5×10^{-6}M	47.5 ± 9.5
$CaCl_2$ 3×10^{-3}M + Glucagon 3.5×10^{-6}M	100.3 ± 4.0
$CaCl_2$ 6×10^{-3}M + Glucagon 3.5×10^{-6}M	98.0 ± 1.7
$CaCl_2$ 9×10^{-3}M + Glucagon 3.5×10^{-6}M	99.6 ± 3.5
$CaCl_2$ 1.2×10^{-2}M + Glucagon 3.5×10^{-6}M	93.9 ± 4.3

*The maximum response to paired pulse stimulation was determined with each preparation either before the addition of a drug to the bath medium or after exposure to the first drug when combined drug effects were studied.

enol (FIG. 3) and to a second paired pulse (TABLE 1).

IV. Interactions of Glucagon with Ouabain: Control responses to four cumulative concentrations of glucagon are shown by the open panels on the far left of FIG. 4. After exposure of the papillary muscles to increasing concentrations of ouabain, the positive inotropic responses of the tissues to glucagon

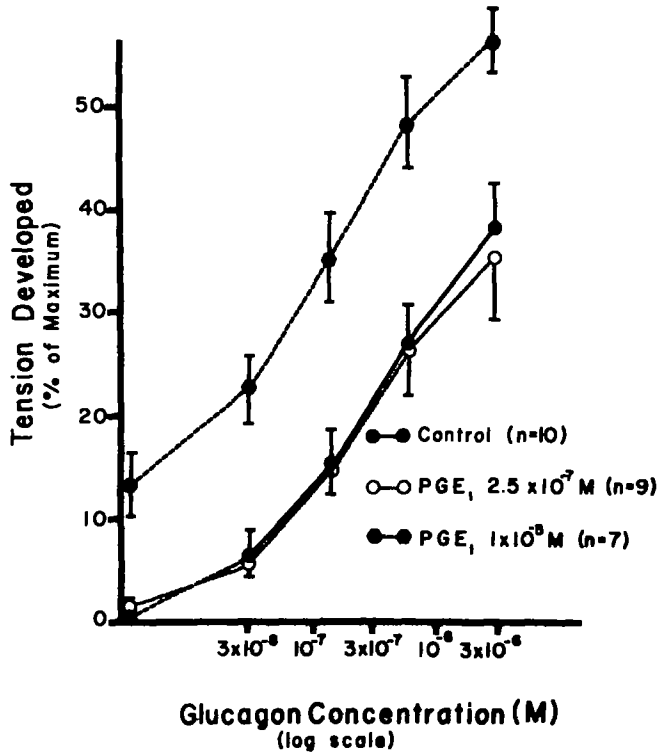

FIG. 2

Effect of Prostaglandin E_1 (PGE_1) upon the response of dog isolated papillary muscle to glucagon (See FIG. 1 for legend).

is attenuated until it is finally reversed by previous exposure to 1.5 μg/ml ($2x10^{-6}$M) of the glycoside (FIG. 4). Low concentrations of ouabain in combination with glucagon did not affect the ability of the muscles to respond maximally to a second paired pulse but as the concentration of ouabain was increased, the ability of the muscles to respond was concomitantly reduced (TABLE 1).

V. Interaction of Glucagon with $CaCl_2$: $CaCl_2$ (320 μg/ml; $3x10^{-3}$M) increased the isometric tension of the dog isolated papillary muscles by 33.2 ± 1.8% and did not alter the response of the tissues to glucagon. Further

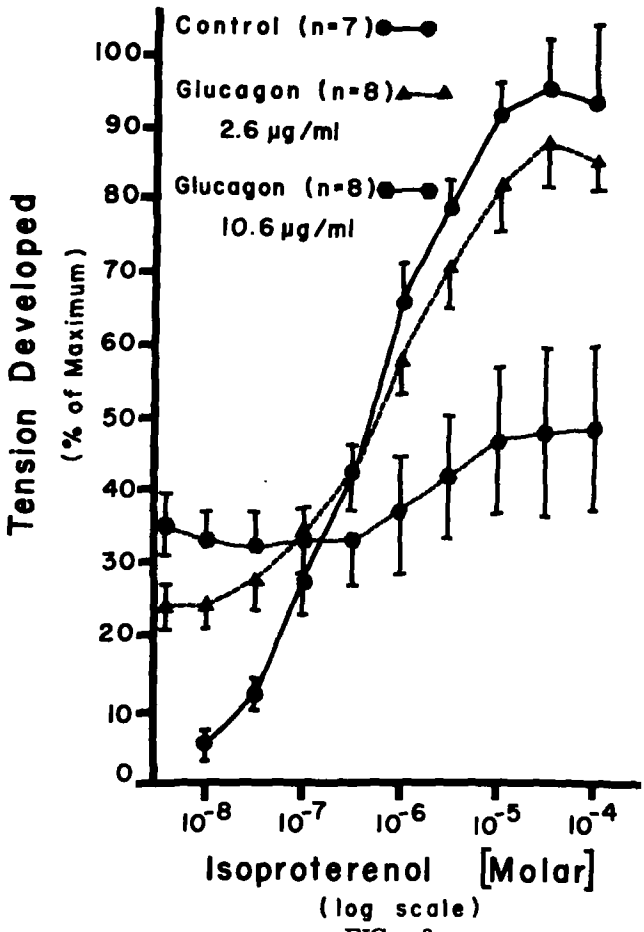

FIG. 3

Effect of Glucagon upon the response of dog isolated papillary muscle to isoproterenol (See FIG. 1 for legend).

increases in the concentration of $CaCl_2$ (640 µg/ml to 1.25 mg/ml; $6x10^{-3}$M to $1.2x10^{-2}$M) produced an increase in isometric tension and a progressive decrease in the response to glucagon. At a concentration of $CaCl_2$ that produced a maximum inotropic response, glucagon ($3.3x10^{-8}$ to $3.5x10^{-6}$M) did not cause a further increase in isometric tension (FIG. 5).

The ability of the tissues to respond to a second paired pulse was not affected by $CaCl_2$ (TABLE 1).

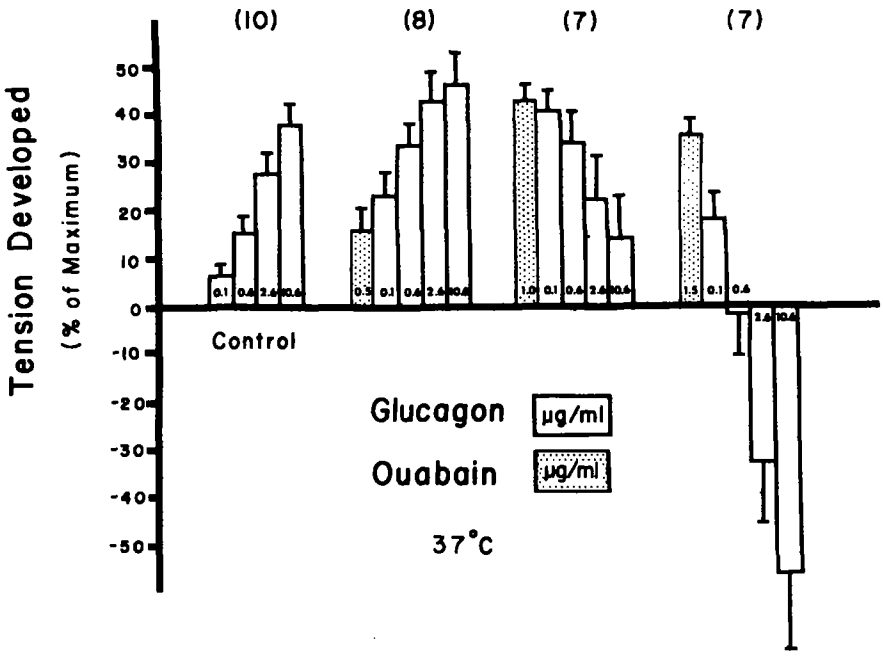

FIG. 4

Effect of ouabain upon the response of dog isolated papillary muscle to glucagon. Dose-response curves for glucagon were obtained after the maximum response to ouabain was obtained. Numbers in parentheses indicate number of muscles used for each group of experiments. Open bars indicate final tension produced by the combination of both drugs. Given are the mean values. Vertical lines represent standard errors of the mean.

Discussion

Glucagon by itself is a positive inotropic agent (1-5). However, Antonaccio et al (2) have shown that if dog isolated papillary muscles are exposed to a relatively high concentration of ouabain (2×10^{-6} M), glucagon produces only a negative inotropic effect. The present study indicates that this reversal of glucagon's action after exposure to ouabain is dependent upon the concentration of ouabain used. Low concentrations of the glycoside do not interfere with the action of glucagon while high concentrations inhibit the inotropic effect of the hormone (FIG. 4). Other positive inotropic agents were

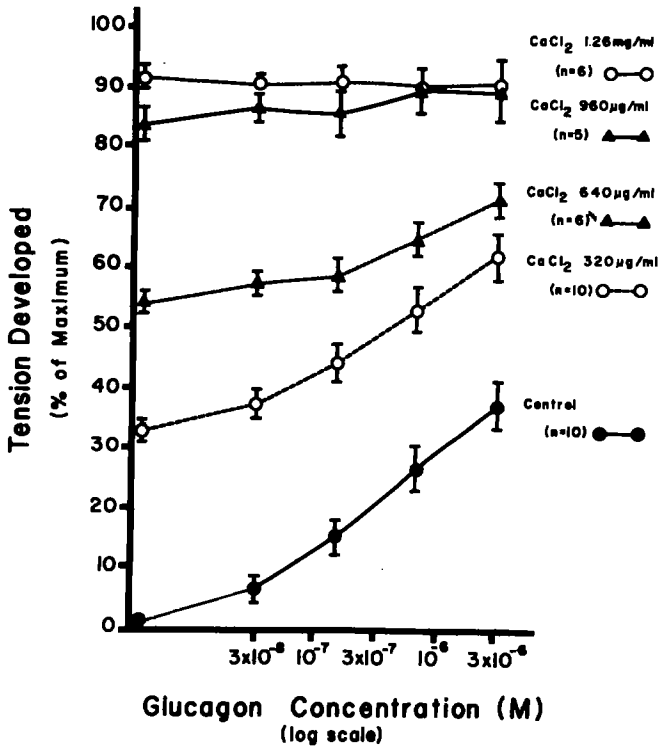

FIG. 5

Effect of $CaCl_2$ upon the response of dog isolated papillary muscle to glucagon (See FIG. 1 for legend).

found to decrease the action of glucagon but only in high concentrations. The response of the tissues to glucagon in combination with low concentrations of theophylline, PGE_1, ouabain, and $CaCl_2$ was not altered; there was, in fact, an additive response (FIG. 1-5). In addition, the combination of glucagon and these drugs in low concentrations did not alter the ability of the muscles to respond maximally to a second paired pulse (TABLE 1). However, relatively high concentrations of theophylline, and ouabain inhibited the response of dog papillary muscles to glucagon (FIG. 1, and 4) and a high concentration of glucagon inhibited the response to isoproterenol (FIG. 3). Concomitantly, there was a decrease in the ability of the tissues to respond to a paired pulse

(TABLE 1). In contrast, the previous exposure of the muscles to PGE_1 or $CaCl_2$ did not decrease the response to glucagon (FIG. 2 and 5) and also did not effect the ability of the muscles to respond to a second paired pulse (TABLE 1). Thus, the positive inotropic effect of glucagon on dog isolated papillary muscles can be inhibited or reversed by relatively high concentrations of other inotropic agents. However, this inhibition occurs only if the combination of drugs antagonizes the ability of the tissue to respond. It is suggested, therefore, that the interaction of glucagon and high concentrations of theophylline, isoproterenol, and ouabain adversely affects the ability of dog isolated papillary muscles to respond maximally and that this results in an apparent inhibition that is non-specific.

References

1. LUCCHESI, B. R., Circulation Res. 22, 777 (1968).

2. ANTONACCIO, M. J., SMITH, C. B. and LUCCHESI, B. R., Submitted for publication.

3. FARAH, A. and TUTTLE, R., J. Pharmacol. Exp. Ther. 129, 49 (1960).

4. GLICK, G., PARMLEY, W. W., WECHSLER, A. S. and SONNENBLICK, E. H., Circulation Res. 22, 789 (1968).

5. PARMLEY, W. W., GLICK, G. and SONNENBLICK, E. H., New Engl. J. Med. 279, 12 (1968).

6. FISHER, R. A., The Design of Experiments, 11th Ed., Oliver and Boyd, Edinburgh (1950).