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PROTECTIVE EFFECTS OF GLUCAGON DURING THE ANAPHYLACTIC RESPONSE IN GUINEA-PIG ISOLATED HEART

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1 Cardiac anaphylaxis and the effects of glucagon pretreatment were studied in guinea-pig isolated hearts actively sensitized to ovalbumin.

2 Antigen challenge of the sensitized hearts markedly increased creatine phosphokinase (CPK) activity in the coronary venous effluent. Control values of CPK release from the hearts before challenge were 3.56 ± 0.15 mu min^{-1} mg^{-1}. In the first 10 min following challenge, CPK release remained stable at increased levels which ranged between 4.88 ± 0.20 to 5.39 ± 0.38 mu min^{-1} mg^{-1}. There was no correlation between immunologically released histamine and CPK release.

3 Pretreatment of the hearts with glucagon (0.15 μmol l^{-1}) exerted a pronounced anti-arrhythmic activity, reducing the conduction arrhythmias and completely preventing automaticity arrhythmias which normally occurred following ovalbumin challenge.

4 Anaphylactic histamine release was reduced significantly in the presence of glucagon. The percentage inhibition of histamine release from glucagon pretreated hearts, during the first 10 min after challenge, ranged between 58% and 94% of that from hearts similarly challenged in the absence of glucagon.

5 Glucagon significantly elevated sinoatrial nodal automaticity, enhanced atrioventricular conduction, improved coronary flow and reduced contractile force during anaphylaxis. It appears that these effects are caused both by modulating anaphylactic histamine release and by influencing the effects of the released histamine.

6 CPK release from the anaphylactic hearts was significantly inhibited in the presence of glucagon. The average percentage inhibition of CPK activity during the first 10 min after challenge ranged between 42% and 98%.

7 The findings from this study provide experimental evidence for protective effects of glucagon pretreatment during cardiac anaphylaxis.

Introduction

The heart is a primary target organ in systemic anaphylaxis (Capurro & Levi, 1975; Zavecz & Levi, 1976). Cytophylic antibodies bind to cardiac mast cells, and immunological reactions with specific antigens cause mast cell degranulation and release of the mediators of allergic phenomena (Liebig Bernauer & Peskar, 1975). Immunologically released histamine plays a main role in the genesis of most functional changes occurring in the anaphylactic heart (Levi, 1972). The importance of histamine in the cardiac response following mast cell degranulation is apparent from the reduction or abolition of most aspects of cardiac anaphylaxis by histamine H_2-receptor antagonists (Capurro & Levi, 1973; Levi, Allan & Zavecz, 1976; Gristwood, Owen & Smith, 1980).

The possible contributory role of creatine phosphokinase (CPK) in the exocytosis from challenged sensitized mast cells of histamine-containing granules (Magro, 1980), as well as the evidence for increased serum CPK activity during protracted anaphylactic shock (Bernauer, 1976) and in asthma (Burki & Diamond, 1977), led us to investigate both the CPK release during cardiac anaphylaxis and the relationship between histamine and CPK release.

Levi (1971) briefly reported in an abstract on the reduction of the anaphylactic crisis in guinea-pig isolated hearts by theophylline and glucagon. In the present study we have re-examined the effects of glucagon on cardiac anaphylaxis and particularly, the effects of glucagon on immunologically released histamine and on the resultant cardiac arrhythmias. In addition, the relationship between released histamine and various parameters of cardiac function

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were examined. Zlokovic & Andjelkovic (1981) recently demonstrated a reduction by glucagon of CPK release from the heart during ouabain-induced arrhythmias. Since CPK is present both in cardiac muscle (Saks, Lipina, Lyulina, Chesnovsova, Felter, Smirnov & Chazov, 1976) and mast cells (Magro, 1980) we also investigated the effect of glucagon on CPK release during cardiac anaphylaxis.

Methods

Isolated heart preparations

Adult Hartley guinea-pigs of either sex and weighing 250 to 300 g were killed by a single blow to the head. The chest was opened quickly and the heart excised and placed in warm Chenoweth-Koelle's solution at 37.5°C. The heart was perfused according to the Langendorff technique at a flow of 6 to 7 ml min^{-1}. The perfusion solution (Chenoweth & Koelle, 1946) had the following composition (mmol l^{-1}): NaCl 119, KCl 5.6, $CaCl_2 \cdot 2H_2O$ 3.2, $MgCl_2 \cdot 6H_2O$ 2.0, $NaHCO_3$ 25, and dextrose 10, and was continuously gassed with 95% O_2 and 5% CO_2. Before the perfusate entered the aorta it passed through a glass condenser heated by a water jacket maintained at 37° to 38°C. Aortic perfusion pressure was monitored continuously and ranged between 3.9 and 4.9 kPa. A Statham UC 2 strain gauge arch was sutured to the apex of the left ventricle and the transducer adjusted so that a diastolic tension of 1 to 2 g was applied to the heart. Surface electrograms were recorded by means of platinum electrodes, one placed on the right atrium and one on the posterior wall of the left ventricle. Contractile force and the surface electrogram were monitored continuously on a cathode oscilloscope (Beckman, type EO 18) and recorded on an eight-channel Dynograph recorder (Beckman, type R). The coronary venous effluent was collected after flowing over the external surface of the heart. Hearts were perfused for 20 to 30 min before experiments.

Cardiac anaphylaxis

Guinea-pigs were actively sensitized with 4 mg kg^{-1} ovalbumin by means of three intraperitoneal injections 24 h apart. Three weeks after the last injection the hearts were excised and mounted as described above. Sensitization of the animals was confirmed using Schultz-Dale's gut assay (Dale & Hartley, 1916).

Antigenic challenge was accomplished by a slow injection (30 to 45s) of 1 mg ovalbumin into the aortic cannula in a volume of 1 ml saline. When the effects of glucagon were to be studied, perfusion with glucagon solution (0.15 μmol l^{-1} in Chenoweth-Koelle's solution) preceded the antigenic challenge.

Histamine assay

Histamine was determined in the coronary venous effluent by the fluorometric technique as modified by Anton & Sayre (1969). The principle is based on histamine condensation with *o*-phthalaldehyde The fluorescent product was stabilized by making the solution acid, and measured on the fluorometer using a mercury lamp (Beckman, model 772). Results are expressed as nmol histamine $min^{-1} g^{-1}$ heart wet weight. Glucagon at the concentration used did not interfere with the assay.

Creatine phosphokinase (CPK) assay

The determination of CPK activity in the coronary venous effluent was carried out by the conventional kinetic ultraviolet method (Chemnitz, 1979) at 25°C, which is based on the evaluation of the increased optical density (Pye Unicam, UV spectrophotometer) of NADPH per min at 340 nm, resulting from the following reactions:

$$\text{creatine-phosphate} + \text{ADP} \xrightarrow{\text{CPK}} \text{creatine} + \text{ATP} \quad (1)$$

$$\text{glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{glucose-6-P} + \text{ADP} \quad (2)$$

$$\text{glucose-6-P} + \text{NADP} \xrightarrow{\text{G-6-P DH}} \text{gluconate-6-P} + \text{NADPH} + H^+ \quad (3)$$

(HK = hexokinase; G-6-P DH = glucose-6-phosphate dehydrogenase)

CPK release was expressed in mu $min^{-1} mg^{-1}$ heart protein. Protein concentrations were determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

Drugs and chemicals

The following were used: porcine glucagon (Novo Ind., Copenhagen), histamine dihydrochloride (Sigma), *o*-phthalaldehyde (Merck, Darmstadt, lyophilized crystalline ovalbumin (Merck, Darmstadt). CPK activity was measured using a Biochimica test combination (Boehringer, Mannheim). Glucagon was stored at −20°C as a stock solution (100 μmol l^{-1}) in 0.9% w/v NaCl solution (saline) containing 0.1% Tris (hydroxymethyl) aminomethane (Tris buffer) adjusted to pH 8.5; just before each experiment the stock solution was diluted with the perfusion medium. All other chemicals were of reagent grade.

Statistical analysis

Tests of significance for the difference between mean values were performed using Student's *t* test for

unpaired data. Results were considered to be significantly different when the P value by this test was less than 0.05. When correlations between histamine and CPK release were tested, lines of best fit and correlation coefficients (r) were calculated by regression analysis.

Results

Isolated heart anaphylaxis

Antigenic challenge of sensitized hearts resulted in the arrhythmias varying in nature from conduction defects (including first degree of atrioventricular (AV) block and periods of bigeminal rhythm) to disorders and dissociation of automaticity, including single and multifocal ventricular ectopic beats. The incidence, onset time after antigen administration and duration of these anaphylactic arrhythmias are given in Table 1.

Hearts from sensitized guinea-pigs reacted to antigen by releasing histamine (Table 2). Peak histamine release occurred generally in the second period post-antigen, and reversion to control values had occurred 8 to 10 min after challenge.

Associated with the anaphylaxis were changes in sinus rate, AV conductivity, ventricular contractile force and coronary flow. Maximum increases in sinus rate, PR-interval and contractile force, and the maximum decrease in coronary flow, from the values immediately preceding challenge, are plotted against the amount of histamine released in Figure 1. The sinus tachycardia, increase in PR-interval, increase in contractile force and decrease in coronary flow are related to the magnitude of released histamine.

Antigenic challenge of sensitized hearts greatly increased CPK activity in the coronary venous effluent. From the results given in Table 3 it can be seen that CPK release from the heart remained stable at an increased level during the first 10 min following antigen administration. There is no correlation between histamine and CPK release during cardiac anaphylaxis (Figure 2).

Administration of antigen to non-sensitized hearts released neither histamine nor CPK and there were no changes in cardiac function.

Effects of glucagon on cardiac function

Perfusion of isolated hearts with glucagon (0.15 μmol l^{-1} for 10 min) markedly increased both sinus rate and coronary flow (36%, $P<0.01$ and 19%, $P<0.01$ respectively). There were no changes in contractile force or in AV conduction time. Similarly, glucagon altered neither histamine nor CPK release into the coronary venous effluent.

Table 1 Antiarrhythmic action of glucagon during anaphylaxis in guinea-pig isolated hearts

Glucagon concentration (μmol/l)	n	*Conduction arrhythmias*			*Automaticity arrhythmias*		
		Incidence	*Onset Time* (min)	*Duration* (min)	*Incidence*	*Onset Time* (min)	*Duration* (min)
0.15	8	4/8	1.41 ± 0.22*	0.87 ± 0.26*	0/8	—	—
None	9	8/9	0.42 ± 0.05	5.34 ± 0.48	5/9	2.47 ± 1.01	3.24 ± 0.72

Antigen challenge was 10 min after the start of continuous perfusion with glucagon.
Values are mean ± s.e. mean. *$P<0.01$

Table 2 Effect of glucagon pretreatment (0.15 μmol l^{-1} for 10 min) on the time course of histamine release during anaphylaxis in guinea-pig isolated perfused hearts

	Histamine released (nmol min^{-1} g^{-1})		
	Antigen alone	*Antigen in the presence of glucagon*	*Inhibition* (%)
Control values (before antigen challenge)	1.51 ± 0.09 (5)	1.52 ± 0.08 (5)	
Time after antigen (min)			
0–2	3.12 ± 0.12 (6)	1.78 ± 0.13 (6)*	84
2–4	3.26 ± 0.15 (7)	2.25 ± 0.16 (7)*	58
4–6	2.67 ± 0.10 (5)	1.69 ± 0.11 (5)*	85
6–8	1.95 ± 0.12 (5)	1.55 ± 0.09 (5)*	94
8–10	1.52 ± 0.09 (5)	1.52 ± 0.07 (5)	

Values are mean ± s.e.mean (n). *$P<0.01$

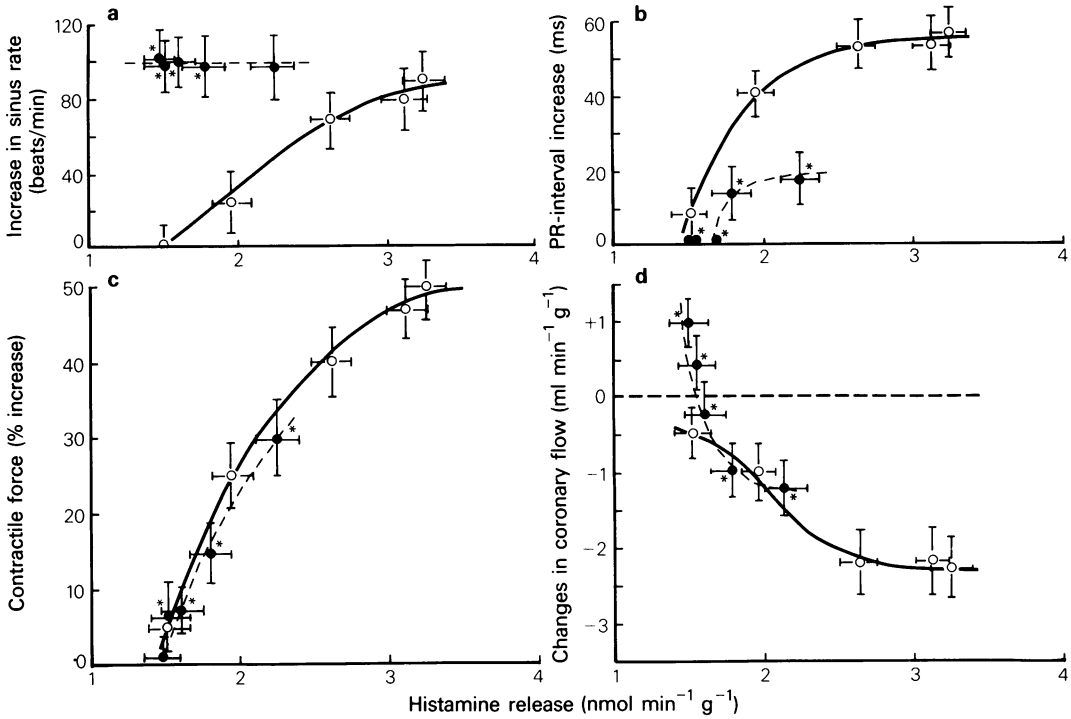

Figure 1 Effects of glucagon on the positive chronotropic (a), negative dromotropic (b), positive inotropic (c) and vasoconstrictor responses (d) to anaphylaxis in guinea-pig isolated hearts. Antigen challenge was 10 min after start of continuous perfusion with glucagon (0.15 μmol l^{-1}). Mean values are shown with s.e.mean; $n = 5-7$ both for anaphylaxis alone (○) and for anaphylaxis in the presence of glucagon (●). In each group, points are recorded during the five 2 min intervals used in Table 2, and for each interval, significance for the difference between values has been calculated: $^*P < 0.05$.

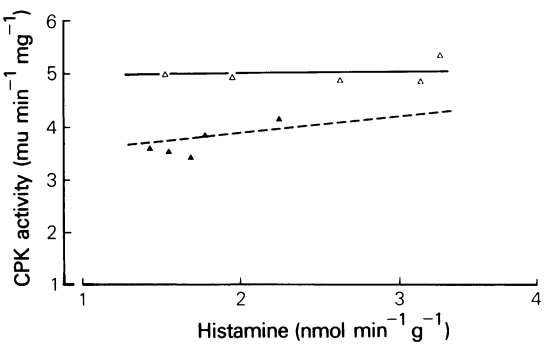

Figure 2 Relationship between histamine and creatine phosphokinase (CPK) release during anaphylaxis in guinea-pig isolated, perfused hearts. Each point represents the mean of 5–7 measurements over the same 2 min intervals in the 10 min following antigen administration. Best lines were calculated by regression analysis. (△) Anaphylaxis alone; (▲) anaphylaxis in the presence of glucagon after challenge, 10 min after start of continuous perfusion with glucagon (0.15 μmol l^{-1}).

Isolated heart anphylaxis in the presence of glucagon

Perfusion of sensitized hearts with glucagon (0.15 μmol l^{-1}) greatly reduced post-antigen conduction arrhythmias, significantly delayed their onset, markedly shortening the duration of conduction disorders and completely prevented abnormal automaticity (Table 1).

Anaphylactic histamine release was significantly reduced in the presence of glucagon whilst time distribution of release was not greatly altered (Table 2). After subtracting control values, the percentage inhibition of histamine release from the challenged glucagon-treated hearts ranged between 58% and 94% of the values given by hearts similarly challenged but in the absence of glucagon. This inhibition is dose-related (Figure 3).

Figure 1 shows the effects of glucagon on heart rate, atrioventricular conductivity, ventricular contraction and coronary flow rate during anaphylaxis. Glucagon itself induced tachycardia in sensitized

Table 3 Effect of glucagon pretreatment (0.15 μmol l^{-1} for 10 min) on creatine phosphokinase (CPK) levels in coronary venous effluent during anaphylaxis in guinea-pig isolated, perfused hearts

	Antigen alone	*Antigen in the presence of glucagon*	*Inhibition (%)*
Control values (before antigen challenge)	3.56 ± 0.15 (5)	3.45 ± 0.25 (5)	
Time after antigen (min)			
0–2	4.88 ± 0.20 (7)	4.22 ± 0.19 (6)**	42
2–4	5.39 ± 0.38 (7)	3.89 ± 0.21 (7)*	76
4–6	4.91 ± 0.17 (6)	3.48 ± 0.27 (6)*	98
6–8	4.99 ± 0.21 (5)	3.57 ± 0.19 (5)*	92
8–10	5.02 ± 0.41 (5)	3.66 ± 0.25 (5)*	86

Values are mean ± s.e.mean (*n*). *$P < 0.01$; **$P < 0.05$.

hearts. After antigen administration the heart rate remained constant and was unaffected by released histamine. Atrioventricular conduction during anaphylaxis was significantly enhanced in the presence of glucagon. However, for the same degree of histamine release, the PR-interval was less than in the absence of glucagon; the curve relating histamine release and the increase in the PR-interval was thus displaced downwards and to the right. Positive inotropic effects occurring in anaphylaxis were significantly reduced in the presence of glucagon and depended only upon the magnitude of histamine release. In the range 1.52 to 2.25 nmol $min^{-1} g^{-1}$ histamine, the inotropic effects appear to be virtually unaffected by glucagon. Coronary flow during anaphylaxis was significantly increased in the presence of glucagon and the curve illustrating a correlation between released histamine and flow rate is displaced upwards and to the left.

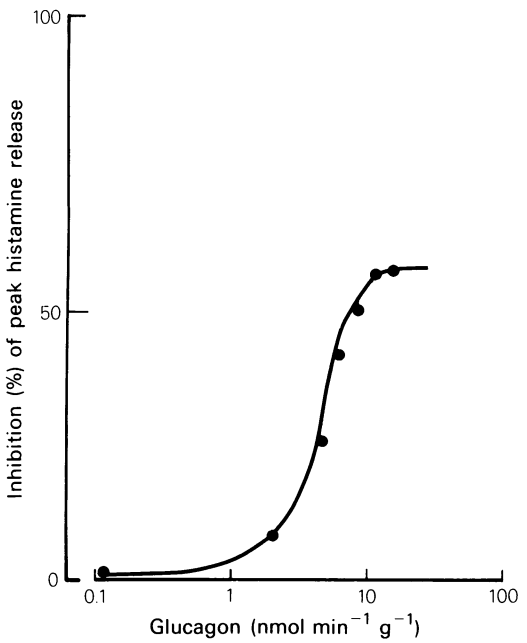

Figure 3 Inhibition (%) of peak histamine release (2–4 min after antigen) from the anaphylactic heart. Antigen challenge was before and 0, 2, 4, 6, 8, and 10 min after the start of continuous perfusion with glucagon (0.15 μmol l^{-1}). The inhibition of histamine release is plotted against the cumulative glucagon concentration in the same period. Mean values are shown ($n = 3$).

CPK release from the anaphylactic heart was significantly inhibited in the presence of glucagon (Table 3). Based on these data the average percentage inhibition of CPK activity in coronary venous effluent after antigen challenge of sensitized hearts in the presence of glucagon ranged between 42% and 98%. There was no correlation between histamine and CPK release from the anaphylactic heart in the presence of glucagon (Figure 2).

Discussion

The results of isolated heart anaphylaxis have confirmed the well-established finding (Levi, 1972; Capurro & Levi, 1975; Gristwood *et al.*, 1980) that histamine released from mast cells in the heart (Giotti, Guidotti, Mannaioni & Zilletti, 1966) greatly alters cardiac function. The time course of this release (Table 2) and the time to onset of the conduction and automaticity arrhythmias (Table 1) show that these occurred during the period of peak histamine release. Likewise when histamine release was spontaneously reversed to control values, no further arrhythmias were observed. Sinus tachycardia, PR-interval prolongation and the increases in contractile

force and coronary flow that occur during anaphylaxis appear to be related to the amount of histamine released (Figure 1). The positive chronotropic and inotropic responses to anaphylaxis are mediated through H_2-receptors (Levi *et al.*, 1976) whereas the negative dromotropic (conductivity) effect appears to be H_1-receptor mediated (Levi, Capurro & Lee, 1975). Since histamine is believed to have no direct vasoconstrictor effect in guinea-pig isolated heart (Broadley, 1975), the apparent correlation between released histamine and the reduction in coronary flow (vasoconstriction) might perhaps be attributed to either a histamine-induced arrhythmia and/or myocardial compression resulting from changes in contractile force and heart rate.

In the allergic manifestations of protracted anaphylactic shock (Bernauer, 1976) and asthma (Burki & Diamond, 1977), elevated levels of serum CPK have been demonstrated. In this work we have shown that CPK release can also occur during cardiac anaphylaxis *in vitro*. In the light of CPK being present in mast cells, possibly as a contributory component of contractile process during histamine exocytoses (Magro, 1980), the question is raised whether the elevated coronary venous CPK levels are from damaged cardiac tissue or from mast cell granules. Our data suggest CPK release may be from the damaged myocardium because there is no positive correlation between histamine and CPK release (Figure 2). Furthermore, when coronary venous histamine levels have returned to control values, CPK levels remained high. This is in accordance with the findings of the retention of CPK intracellularly during histamine release from challenged sensitized mast cells (Magro, 1980).

In isolated guinea-pig hearts, using experimental conditions which damaged the myocardial cell wall, Tanz & Opie (1978) showed the release of lactate dehydrogenase (LDH). They suggested that this release, which preceded any electrophysiological disturbances, represented a very sensitive test, reflecting the integrity of myocardial cell wall. In the present study we have demonstrated CPK release; we suggest that these increased CPK levels following antigen challenge reflect the presence of both real and potential arrhythmogenic foci.

When guinea-pig isolated hearts were perfused with glucagon (0.15 μmol l^{-1} for 10 min) we obtained increases in both sinus rate and coronary flow, whereas contractile force was unaffected. These results are in accordance with recent results describing responses of rat and guinea-pig hearts to glucagon (Rogers, MacLeod & McNeill, 1981; MacLeod, Rogers & McNeill, 1981).

The glucagon results show that the cardiac anaphylactic crisis was markedly reduced. The data suggest that the antiarrhythmic action of glucagon in cardiac anaphylaxis involves several factors. These include, inhibition of immunological histamine release, vasodilatation of the coronary vessels, increase in sinoatrial nodal automaticity and enhancement of atrioventricular conduction velocity. Inhibition of histamine release and vasodilatation probably play a major role in the mechanism of this antiarrhythmic activity, since the action of released histamine on cardiac conducting tissue, as well as concomitant impairment of coronary flow, are two major factors involved in the genesis of anaphylactic arrhythmias (Levi, 1972; Capurro & Levi, 1975). It has been shown that the antiarrhythmic action of glucagon in ouabain-induced arrhythmias is mediated through a supraventricular action to elevate sinus rate above that of the dominant ventricular focus. This allows a return to dominance by the sinus node (Wilkerson, Partlow Pruett & Patterson, 1977). It might be supposed from the present results that elevated sinoatrial nodal automaticity could be a contributory factor in preventing automaticity arrhythmias during anaphylaxis.

It is clear that in this experimental model, glucagon is involved in inhibiting immunological histamine release (Table 2; Figure 3). Levi (1971) showed that glucagon inhibited the immunological release of histamine in a dose-dependent fashion and suggested that the effect was mediated through activation of the adenylate cyclase system in cardiac cells and that the subsequent increase in intracellular cyclic AMP modulates histamine release. However, Rogers *et al.*, (1981) showed that perfusion of the guinea-pig heart with 10^{-7} M glucagon had no effect upon cyclic AMP levels, at least when measured after 3 min. Other experiments, based on direct anaphylactic histamine release from the pure target cells, have shown that the above mentioned hypothesis of a modulatory effect of cyclic AMP on histamine release, by Levi (1971) is inadequate (Skov, Geisler, Klysner & Norn, 1976). It seems that a more complex mechanism of the effects of glucagon has to be considered. Glucagon, in addition to modulating anaphylactic histamine release, also influences the effects of the released histamine on AV-conduction (Figure 1b), most probably as a result of its direct actions on the AV node (Lipski, Kaminsky, Donoso & Freidberg, 1972) and of its vasodilator action on the guinea-pig coronary vessels (Rogers *et al.*, 1981).

Glucagon markedly reduced anaphylactic CPK release from the heart (Table 3) although this was not related to histamine release (Figure 2). It might be suggested that reduced CPK levels reflect less leaky myocardial cell membranes. However, it is not completely clear whether this effect of glucagon can be solely attributed to inhibition of histamine release and vasodilatation.

Some years ago, glucagon was introduced and

established in clinical medicine as a cardiotonic and antiarrhythmic agent (Parmley & Sonnenblick, 1971; Hurwitz, 1973). Based on the observations from this experimental work, it seems that glucagon might have useful clinical applications in cardiac patients with failure and arrhythmias due to allergic immediate reactions.

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