Br. J. Pharmac. (1971), **43**, 279–286.

Effect of glucagon on ventricular arrhythmias after coronary artery occlusion and on ventricular automaticity in the dog

B. R. MADAN

Department of Pharmacology, S. P. Medical College, Bikaner (Rajasthan), India

Summary

1. In anaesthetized dogs, glucagon (100 μg/kg i.v.), caused a significant increase in heart rate and decrease in mean arterial blood pressure. Ventricular automaticity, as determined by the time to the onset of first vagal escape beat and the number of such indioventricular beats during the 30 s period of vagal stimulation, was not significantly altered.

2. In unanaesthetized dogs with ventricular arrhythmias produced by two-stage ligation of the anterior descending branch of the left coronary artery, glucagon (30 and 100 μg/kg i.v.), restored normal sinus rhythm in a few animals. In the remaining dogs, there was a significant reduction in the ventricular ectopic activity.

3. The significant positive chronotropic response to glucagon elicited in anaesthetized animals was not observed in conscious dogs whose coronary arteries had been ligated.

4. These findings enhance the potential usefulness of glucagon in the treatment of acute myocardial infarction, which may often be associated with disturbances of ventricular rhythm.

5. In the light of observations made by other workers, it is suggested that the antiarrhythmic effect of glucagon may be due to movement of potassium ions into the cardiac cell.

Introduction

Farah & Tuttle (1960) were the first to demonstrate a positive inotropic and chronotropic effect of glucagon, a polypeptide hormone secreted from the alpha cells of the pancreas. This observation has recently been confirmed and extended by numerous workers in several experimental preparations (Lucchesi, 1968 ; Glick, Parmley, Webster & Sonnenblick, 1968 ; Moir & Nayler, 1970 ; Spilker, 1970) and in man (Parmley, Glick & Sonnenblick, 1968 ; Klein, Morch & Mahon, 1968 ; Brogan, Kozonis & Overy, 1969). As a result, use of this hormone has been suggested (Lal & Fletcher, 1969 ; Dolgin, 1970) and actually tried (Eddy, O'Brien & Singh, 1969 ; Murtagh, Binnion, Lal, Hutchison & Fletcher, 1970) in patients with acute myocardial infarction to support the failing myocardium. However, its effect on the ventricular ectopic beats, which often accompany myocardial infarction, is not

known. The work described here was thus undertaken to study its effects on ventricular arrhythmias in dogs after occlusion of the coronary artery.

There are conflicting reports concerning the effect of glucagon on ventricular automaticity. Some investigators reported no effect (Whitsitt & Lucchesi, 1968; Steiner, Wit & Damato, 1969), but others have demonstrated an increase in ventricular automaticity after administration of glucagon (Lipski, Kaminski, Donoso & Friedberg, 1969). In view of this divergence of opinion, the influence of glucagon on ventricular automaticity was also determined, as described here.

Methods

Heart rate, mean arterial blood pressure and ventricular automaticity

Eight adult mongrel dogs of either sex weighing 11–18 kg were anaesthetized with pentobarbitone sodium (30–35 mg/kg i.v.). The trachea was cannulated for artificial respiration under positive pressure. A carotid artery was cannulated for recording mean arterial blood pressure by a mercury manometer (1 mmHg≡1·333 mbar). Bipolar Lead II was recorded with a Galileo electrocardiogram. The right vagus nerve was isolated in the neck and sectioned. Its distal end was stimulated with square wave pulses (duration 1 ms, frequency 50 Hz, 10–14 v) for 30 seconds. The time to the appearance of the first ventricular escape beat and the number of such escape beats (idioventricular rate) occurring during the 30 s period of vagal stimulation were taken as the criteria for ventricular automaticity (Steiner, *et al.*, 1969).

Two to three measurements of ventricular automaticity, which were in good agreement, were taken at 10 min intervals and the results averaged. Glucagon (100 μg/kg), was injected into the cannulated femoral vein, and 5 min later, changes in the time of onset of first escape beat and idioventricular rates were recorded.

Ventricular arrhythmias after occlusion of the coronary artery

Under anaesthesia with intravenous pentobarbitone sodium (30 mg/kg), two-stage ligation of the anterior descending branch of the left coronary artery was performed in sixteen mongrel dogs of both sexes weighing 9–16 kg (Harris, 1950). A polyethylene catheter was implanted into a femoral vein for injecting test substances. The animals were studied in the unanaesthetized state on the second day after the operation when their electrocardiograms (Lead II) indicated dissociation of atrial and ventricular activity with nearly all beats subatrial in origin.

For studying the antiarrhythmic effects, the animals were divided into three groups. The first group of three dogs served as control and was given 5 ml of isotonic saline intravenously. Groups 2 (five animals) and 3 (eight animals) received respectively, intravenous injections of 30 and 100 μg/kg of glucagon, which was flushed in with 5 ml of isotonic saline. The treatment in the different groups was randomized. The 30 s electrocardiographic records were taken before and at the end of injection and at 5 min intervals thereafter. The total heart and ectopic beats were counted over 30 s and expressed as beats/minute. The lowest incidence of ventricular ectopic beats and the highest total heart rates recorded after the injection were taken as the maximum effect of treatment.

Drugs

A 0·1% solution of crystalline glucagon (Lot No. B 69 of Novo Research Institute, Copenhagen) in sterile physiological saline was prepared on the day of use.

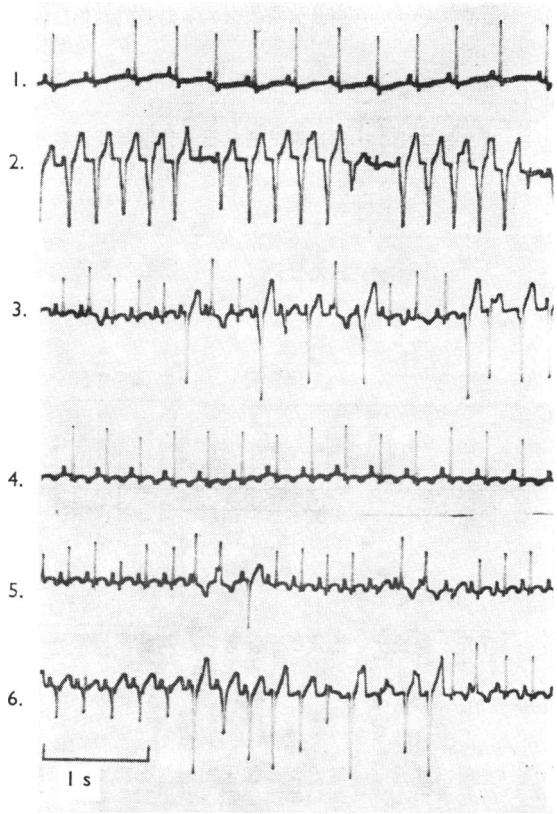

FIG. 1. Effect of glucagon on the electrocardiogram (Lead II) of a dog. (1), Control before ligation of the coronary artery. (2), Ventricular arrhythmia on the second day of operation. (3–6), One, 5, 10 and 15 min after administration of glucagon (100 μg/kg i.v.); normal sinus rhythm was restored after 5 min (4) and ectopic activity reappeared after 10 min (5).

TABLE 1. *Effect of glucagon in anaesthetized normal dogs*

Response studied	No. of dogs	Before glucagon (Mean±S.E.)	After glucagon (Mean±S.E.)	*P* value
Heart rate/min	8	171±9	251±11	<0·01
Blood pressure (mmHg.)	8	128±3	110±1	<0·01
Vagal escape time (s)	7	11·8±3·1	9·2±3·8	>0·05
Vagal escape beats/30 s	7	13·2±3·2	17·8±4·0	>0·05

TABLE 2. *Effect of glucagon in unanaesthetized dogs on the second day after occlusion of coronary arteries*

Drug and dose	Dog No.	Ectopic rhythm: Beats/min Before	Ectopic rhythm: Beats/min After*	Ectopic rhythm: % Decrease	Ectopic rhythm: Min after injection†	Ectopic rhythm: Duration (min)	Total heart rate: Beats/min Before	Total heart rate: Beats/min After*	Total heart rate: % Increase	Total heart rate: Min after injection†
Saline (5 ml) (group 1)	1	218	190	12·8	10	—	224	236	5·4	10
	2	180	140	22·2	5	—	184	200	8·7	15
	3	166	156	6·0	10	—	184	194	5·4	5
	Mean	188	162	13·7			197	210	6·5	
	±S.E.	±16	±15	±4·7			±13	±13	±1·1	
			P value‡ >0·05				>0·05			
Glucagon (30 μg/kg) (group 2)	1	178	18	89·9	5	10	190	204	7·4	10
	2	226	30	86·7	10	20	234	248	6·0	5
	3	198	18	90·9	30	10	208	224	7·7	10
	4	136	0	100·0	10	20	174	208	19·3	10
	5	214	180	15·9	10	—	214	226	5·6	5
	Mean	190	49	76·6			204	222	9·3	
	±S.E.	±16	±33	±15·3			±10	±8	±2·2	
		P value‡ <0·01					>0·05			
Glucagon (100 μg/kg) (group 3)	1	208	34	83·7	1	4	218	260	19·2	10
	2	166	60	63·9	5	15	182	196	7·7	1
	3	178	0	100·0	5	15	188	220	17·0	10
	4	188	40	78·7	1	29	232	252	8·6	10
	5	224	98	56·3	10	10	228	232	1·8	5
	6	150	0	100·0	10	25	164	176	7·3	10
	7	194	0	100·0	5	10	230	250	8·7	10
	8	204	180	11·8	15	—	204	210	2·9	10
	Mean	189	52	74·3			206	225	9·1	
	±S.E.	±9	±22	±10·7			±9	±10	±2·2	
		P value‡ <0·001					>0·05			

* Each value is the maximum change in ectopic and total heart rates after injection. † When the maximum effect was seen.
‡ *P* value was obtained by using Student's *t* test.

Results

Experiments on anaesthetized normal dogs

Heart rate and blood pressure

Changes in heart rate and mean arterial blood pressure were recorded immediately before determination of the effect on ventricular automaticity, that is, 4 min after the injection of glucagon (100 μg/kg). There was a significant increase in the heart rate and a decrease in the blood pressure (Table 1). No ectopic beat was observed during the period of sinus tachycardia.

Ventricular automaticity

In one animal, the effect on ventricular automaticity could not be studied because of the appearance of beats of supraventricular origin during vagal stimulation. In the remaining animals, the time to the appearance of the first ventricular escape beat was decreased and the number of idioventricular beats during the period of vagal stimulation was increased 5 min after the administration of glucagon (Table 1). Since these changes were slight and statistically not significant, it appears that glucagon does not alter ventricular automaticity. This is in corroboration of the findings of previous workers (Whitsitt & Lucchesi, 1968; Steiner *et al.*, 1969).

Experiments on unanaesthetized dogs with ligated coronary arteries

Effects of ligation

Unifocal and multifocal ventricular arrhythmias were observed 18–24 h after occlusion of the coronary artery. The total heart rate was 164–236 beats/minute. The ventricular ectopic and sinus beats were 76 to 100% and 0–24% respectively.

In the control group, which was given isotonic saline, there was a reduction of only 6–22% (average=13·7%) in the ectopic beats, which was statistically not significant. This indicates the relative stability of the arrhythmia in the dogs with ligated coronary arteries, which has also been observed previously (Harris, 1950; Jacobson, Schiess & Moe, 1962).

Effects of glucagon

In the glucagon treated groups, there was a statistically significant reduction in the ventricular ectopic beats. Complete suppression of the ectopic beats with restoration of normal sinus rhythm occurred in one out of five animals in group 2, and in three out of nine animals in group 3. In almost all the remaining dogs, there was considerable attenuation of ectopic activity. In most of the animals, the maximum antiarrhythmic effect was observed 5–10 min after the injection; but in a few animals it occurred immediately or 30 min after the administration of glucagon. The duration of antiarrhythmic action, as assessed by the return of ectopic beats to more than 50% of preinjection level, was brief and varied from 4 to 29 minutes. The maximum increase in heart rate, which occurred usually 5 or 10 min after injection, was not statistically significant. The results are summarized in Table 2 and a representative experiment is depicted in Fig. 1.

Comparison of the effectiveness of glucagon in reducing ventricular ectopic activity in groups 2 and 3 did not show any statistically significant difference ($P>0{\cdot}05$). Thus the antiarrhythmic effect of glucagon does not appear to be dose dependent.

There was no fatality nor any signs of overt toxicity except emesis in one dog treated with 100 μg/kg of glucagon.

Discussion

The depression of cardiac contractility leading to various haemodynamic derangements and occurrence of ventricular ectopic beats ending in fibrillation, constitute two of the most serious complications of acute myocardial infarction (Killip, 1968; Han, 1969). Drugs such as digitalis and catecholamines may strengthen the force of contraction of the failing myocardium, but the use of such agents is restricted because their ability to provoke arrhythmias in the presence of myocardial infarction is increased (Maling & Moran, 1957; Gunnar, Pietras, Stavrakos, Loeb & Tobin, 1967; Han, 1969). Antiarrhythmic drugs cannot be used as a routine for the prevention and suppression of rhythm disturbances accompanying myocardial infarction because they depress cardiac contractility and conductivity (Szekeres & Papp, 1968). The latter action may, under some circumstances, facilitate re-entrant ectopic beats by creating local blocks (Han, 1969). In experimental myocardial infarction in the dog, glucagon augments the contraction of the non-infarcted portion of the left ventricle as indicated by an upward shift of the force-velocity relation (Puri & Bing, 1969) and causes a striking increase in the cardiac output (Maltoff, Parmley, Manchester, Berkovits, Sonnenblick & Harken, 1970) without increase in myocardial oxygen consumption (Kumar, Sharma, Molokhia, Messer, Abelman & Hood, 1970). In corroboration of these experimental findings, the positive inotropic effect of glucagon improves the declining myocardial function in patients with acute myocardial infarction and cardiogenic shock (Eddy, *et al.*, 1969; Murtagh *et al.*, 1970; Vander Ark & Reynolds, 1970).

The results described here indicate that in the dog with a ligated coronary artery glucagon is effective in attenuating ventricular ectopic activity, which is an arrhythmia aetiologically similar to that accompanying acute myocardial infarction in man (Clark & Cummings, 1956; Jacobson, *et al.*, 1962). These findings, when considered together with the observations of other workers, widen the spectrum of potentially useful activity of glucagon in acute myocardial infarction.

While the strong positive chronotropic effect of glucagon reported by numerous workers (Lucchesi, 1968; Glick *et al.*, 1968; Moir & Nayler, 1970) and also seen in the anaesthetized dogs in the present study, may be undesirable in myocardial infarction (Puri & Bing, 1969), it is noteworthy that the hormone failed to cause a significant increase in the heart rate in awake, unanaesthetized dogs with ligated coronary arteries (see Table 2). This lack of significant chronotropic response to glucagon in the conscious dogs may be due to the prevalence of high control heart rates resulting from ventricular ectopic activity as was also seen by Vander Ark & Reynolds (1970) in patients suffering from severe cardiac decompensation with tachycardia. Also relevant to this are the findings that the unanaesthetized dog showed little or no chronotropic response to glucagon compared with the anaesthetized dog (Lucchesi, Stutz & Winfield, 1969).

Although the mechanism of the antiarrhythmic action of glucagon has not been elucidated, it may possibly be related to changes in potassium transfer across the cardiac cell. Development of ventricular arrhythmias after myocardial infarction is attributed to the loss of potassium from the cardiac tissue (Regan, Harman, Lehan, Burke & Oldewurtel, 1967 ; Burke, Asokan, Moschos, Oldewurtel & Regan, 1969). A shift of extracellular potassium to the inside of the cardiac cell may prevent or abolish the arrhythmias, as is achieved by the administration of glucose, insulin and potassium (Sodi-Pallares, Bisteni, Medrano, Testelli & De Micheli, 1963 ; Regan *et al.*, 1967). Glucagon, which increases the release of glucose and potassium from the liver (Sutherland & Rall, 1960 ; Glinsman & Mortimore, 1968) and insulin from the pancreas (Samols, Marri & Marks, 1965) may produce similar shifts of potassium from the extracellular to the intracellular compartments (Bourassa, Eibar & Campeau, 1970) with resultant suppression of arrhythmias.

I am indebted to Dr. J. Schlichtkrull of the Novo Research Institute, Copenhagen for the generous supply of glucagon and to Shri R. S. Verma for technical assistance.

REFERENCES

BOURASSA, M. G., EIBAR, J. & CAMPEAU, L. (1970). Effects of glucagon on myocardial metabolism in patients with and without coronary artery disease. *Circulation*, **42**, 53–60.

BROGAN, E., KOZONIS, M. C. & OVERY, D. C. (1969). Glucagon therapy in heart-failure. *Lancet*, **1**, 482–484.

BURKE, W. M., ASOKAN, S. K., MOSCHOS, C. B., OLDEWURTEL, H. A. & REGAN, T. J. (1969). Effects of glucose and nonglucose infusions on myocardial potassium ion transfers and arrhythmias during ischemia. *Am. J. Cardiol.*, **24**, 713–722.

CLARK, B. B. & CUMMINGS, J. R. (1956). Arrhythmias following experimental coronary occlusion and their response to drugs. *Ann. N.Y. Acad. Sci.*, **64**, 543–549.

DOLGIN, M. (1970). Treatment of cardiac failure with glucagon. *Am. Heart J.*, **79**, 843–844.

EDDY, J. D., O'BRIEN, E. T. & SINGH, S. P. (1969). Glucagon and haemodynamics of acute myocardial infarction. *Br. med. J.*, **4**, 663–665.

FARAH, A. & TUTTLE, R. (1960). Studies on the pharmacology of glucagon. *J. Pharmac. exp. Ther.*, **129**, 49–55.

GLICK, G., PARMLEY, W. W., WECHSLER, A. S. & SONNENBLICK, E. H. (1968). Glucagon, its enhancement of cardiac performance in the cat and dog and persistence of its inotropic action despite beta-receptor blockade with propranolol. *Circulation Res.*, **22**, 789–799.

GLINSMAN, W. H. & MORTIMORE, G. E. (1968). Influence of glucagon and 3′,5′ AMP on insulin responsiveness of the perfused rat liver. *Am. J. Physiol.*, **215**, 553–559.

GUNNAR, R. M., PIETRAS, R. J., STAVRAKOS, C., LOEB, H. S. & TOBIN, J. R. Jr. (1967). Physiologic basis for treatment of shock associated with myocardial infarction. *Med. Clin. N. Am.*, **51**, 69–81.

HAN, J. (1969). Mechanisms of ventricular arrhythmias associated with myocardial infarction. *Am. J. Cardiol.*, **24**, 800–813.

HARRIS, A. S. (1950). Delayed development of ventricular ectopic rhythms following experimental coronary occlusion. *Circulation*, **1**, 1318–1328.

JACOBSON, E. D., SCHIESS, W. & MOE, G. K. (1962). The effect of hypoxia on experimental ventricular tachycardia. *Am. Heart J.*, **64**, 368–375.

KILLIP, T. (1968). Management of the patient with acute myocardial infarction. *Med. Clin. N. Am.*, **52**, 1061–1075.

KLEIN, S. W., MORCH, J. E. & MAHON, W. A. (1968). Cardiovascular effects of glucagon in man. *Can. med. Assoc. J.*, **98**, 1161–1164.

KUMAR, R., SHARMA, G. V. R. K., MOLOKHIA, F., MESSER, J. V., ABELMAN, W. H. & HOOD, W. B. (1970). Glucagon in experimental acute and healing myocardial infarction: effects on hemodynamics and myocardial oxygen consumption. *Circulation*, **42** (Suppl. III), 176 (Abstr.).

LAL, S. & FLETCHER, E. (1969). Glucagon therapy in heart failure. *Lancet*, **1**, 731.

LIPSKI, J. I., KAMINSKI, D. M., DONOSO, E. & FRIEDBERG, C. K. (1969). The electro-physiologic properties of glucagon in the normal canine heart. *Am. J. Cardiol.*, **23**, 124–125.

LUCCHESI, B. R. (1968). Cardiac actions of glucagon. *Circulation Res.*, **22**, 777–787.

LUCCHESI, B. R., STUTZ, D. R. & WINFIELD, R. A. (1969). Glucagon: its enhancement of atrioventricular nodal pacemaker activity and failure to increase ventricular automaticity in dogs *Circulation Res.*, **25**, 183–190.

MALING, H. M. & MORAN, N. C. (1957). Ventricular arrhythmias induced by sympathomimetic amines in unanaesthetized dogs following coronary artery occlusion. *Circulation Res.*, **5**, 409–413.

MALTOFF, J. M., PARMLEY, W. W., MANCHESTER, J. H., BERKOVITS, B., SONNENBLICK, E. H. & HARKEN, D. E. (1970). Hemodynamic effects of glucagon and intraaortic balloon counterpulasion in canine myocardial infarction. *Am. J. Cardiol.*, **25**, 675–682.

MOIR, T. W. & NAYLER, W. G. (1970). Coronary vascular effects of glucagon in the isolated dog heart. *Circulation Res.*, **26**, 29–34.

MURTAGH, J. G., BINNION, P. F., LAL, S., HUTCHISON, K. J. & FLETCHER, E. (1970). Haemodynamic effects of glucagon. *Brit. Heart J.*, **32**, 307–315.

PARMLEY, W. W., GLICK, G. & SONNENBLICK, E. H. (1968). Cardiovascular effects of glucagon in man. *New Engl. J. Med.*, **279**, 12–17.

PURI, P. S. & BING, R. J. (1969). Effects of glucagon on myocardial contractility and hemodynamics in acute experimental myocardial infarction. Basis for its possible use in cardiogenic shock. *Am. Heart J.*, **78**, 660–668.

REGAN, T. J., HARMAN, M. A., LEHAN, P. H., BURKE, W. M. & OLDEWURTEL, H. A. (1967). Ventricular arrhythmias and K transfer during myocardial ischemia and intervention with procaine amide, insulin or glucose solution. *J. Clin. Invest.*, **46**, 1657–1668.

SAMOLS, E., MARRI, G. & MARKS, V. (1965). Promotion of insulin secretion by glucagon. *Lancet*, **2**, 415–416.

SPILKER, B. (1970). Comparison of the inotropic response to glucagon, ouabain and noradrenaline. *Br. J. Pharmac.*, **40**, 382–395.

SODI-PALLARES, D., BISTENI, A., MEDRANO, G. A., TESTELLI, M. R. & DEMICHELI, A. (1963). The polarizing treatment of acute myocardial infarction. *Dis. Chest*, **43**, 424–432.

STEINER, C., WIT, A. L. & DAMATO, A. N. (1969). Effects of glucagon on atrioventricular conduction and ventricular automaticity in dogs. *Circulation Res.*, **24**, 167–177.

SUTHERLAND, E. W. & RALL, T. W. (1960). The relation of adenosine-3,5-phosphate and phosphorylase to the action of catecholamines and other hormones. *Pharmac. Rev.*, **12**, 265–299.

SZEKERES, L. & PAPP, Gy. J. (1968). Antiarrhythmic compounds. *Progr. Drug. Res.*, **12**, 292–369.

VANDER ARK, C. R. & REYNOLDS, E. W. Jr. (1970). Clinical evaluation of glucagon by continuous infusion in the treatment of low cardiac output states. *Am. Heart J.*, **79**, 481–487.

WHITSITT, L. S. & LUCCHESI, B. R. (1968). Effects of beta-receptor blockade and glucagon on the atrioventricular transmission system in the dog. *Circulation Res.*, **23**, 585–595

(*Received March* 25, 1971)