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Regional circulatory effects of pancreatic glucagon

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Summary

1. The effects of close intra-arterial injections and infusions of glucagon on blood flow in the femoral, hepatic and superior mesenteric arteries of anaesthetized cats were studied using non-cannulating electromagnetic flow probes.

2. Glucagon dilated the mesenteric vasculature. The threshold dose for rapid injections was less than 1 μg and maximal changes were produced by 10–20 μg. The dilatation began within a few seconds of injection and was unaffected by prior denervation or by pre-treatment with propranolol or atropine.

3. Close intra-arterial glucagon increased the resistance of the hepatic arterial bed but had no effect on femoral vascular resistance. Intravenous glucagon failed to alter renal blood flow.

4. Glucagon is therefore a potent vasoactive substance with its effect wholly or predominantly confined to the splanchnic area.

5. It has been suggested that the cardiac effects of glucagon and isoprenaline resemble each other because both compounds act on the β-adrenoceptor mechanism. The different regional vascular effects of glucagon and isoprenaline suggest that glucagon vasodilatation may not be mediated via β-adrenoceptors.

Introduction

Recent investigations in man and animals have shown that glucagon produces positive chronotropic and inotropic effects on the heart (Glick, Parmley, Wechsler & Sonnenblick, 1968 ; Lucchesi, 1968 ; Williams, Childress, Chip & Border, 1969). Both glucagon and catecholamines stimulate adenyl cyclase in several tissues with the resulting accumulation of cyclic 3′,5′-adenosine monophosphate (cyclic AMP). Robison, Butcher & Sutherland (1967) have summarized evidence that links this increased production of cyclic AMP with the cardiac action of catecholamines and have suggested that adenyl cyclase is in fact the β-adrenoceptor. In addition to its cardiac effects, glucagon also produces a reduction in mean arterial pressure and in total peripheral resistance (Glick *et al.*, 1968 ; Williams *et al.*, 1969) and must therefore be a vasodilator. The purpose of the present study was to determine the regional circulatory effects of glucagon. A similar distribution of glucagon vasodilatation and adrenergic vasodilatation among several vascular beds would provide strong support for the view that vascular β-adrenoceptor responses, like those of the heart, might be mediated by cyclic AMP. On the other hand, any significant differences would suggest that other mechanisms are involved.

Methods

Sixteen cats weighing 3–5 kg were anaesthetized with intraperitoneal pentobarbitone sodium (40 mg/kg). Polyethylene catheters were inserted into an external jugular vein and a common carotid artery for intravenous injections and arterial pressure measurements, respectively. Blood flow was measured in the hepatic artery (ten cats), superior mesenteric artery (nine cats), femoral artery (seven cats), and renal artery (four cats). In several animals multiple flow measurements were made. The abdominal arteries were exposed through a midline laparotomy and separated from enveloping nerve fibres over a length of 1–2 cm. Blood flow was determined with the Biotronex BL-610 electromagnetic flowmeter used in association with non-cannulating probes (Micron Instruments Inc.) and a Beckman S11 Dynograph recorder. Zero flow references were obtained by mechanical occlusion of each artery distal to its probe and were frequently checked during each experiment. Calibration was performed by placing each probe on a convenient artery which was cannulated downstream from the probe. Blood was then withdrawn from the vessel at a known rate using a Harvard infusion-withdrawal pump. The resistance of each vascular bed was calculated by dividing mean systemic arterial pressure (1 mmHg≡1·333 mbar) by mean arterial flow (ml/min). Pilot studies showed that the maximum doses of glucagon used in these experiments produced a rise of only 1–2 mm in portal venous pressure and it was therefore considered justifiable not to measure this variable in the remaining cats because no appreciable error would result from neglecting it in the calculation of superior mesenteric vascular resistance. The drugs used were crystalline glucagon (Eli Lilly Inc.), isoprenaline hydrochloride (Isuprel, Winthrop-Stearns Inc.), atropine sulphate and propranolol hydrochloride (Inderal, Ayerst Laboratories, Inc.). Isoprenaline, propranolol and atropine were dissolved in physiological saline solution. Doses of these agents are expressed in terms of their salts. Glucagon was freshly dissolved in physiological saline solution acidified with HCl to a pH of 2·5. The concentrations of glucagon used were 100 μg/ml, 500 μg/ml and 1 mg/ml. The doses injected ranged from 0·1 to 50 μg. Corresponding volumes of the solvent produced no changes in blood flow. The agents were injected intravenously or directly into the femoral or superior mesenteric arteries using an indwelling 27-gauge needle with the hub removed and the shaft connected to a microsyringe with a polyethylene catheter. Neither mean flow nor the instantaneous flow contour was affected by the presence of the needle. Injections into the hepatic artery were made through a catheter inserted into its gastroduodenal branch.

Results

	Control values		No. of cats
	Mean	Range	
Mean arterial pressure (mmHg)	115	80–150	16
Blood flow (ml/min per kg cat) in			
Superior mesenteric artery	12·7	7– 22·2	10
Hepatic artery	10·8	8·2– 21·4	10
Femoral artery	2·8	1·2– 6·3	6
Renal artery	7·7	6·8– 8·1	4

Effects of glucagon on superior mesenteric arterial flow (Figs. 1–4)

Rapid injections of glucagon 0·2 to 20 μg (total dose per cat) into the superior mesenteric artery increased flow in this vessel and reduced mesenteric vascular resistance in each of eight cats. The threshold dose varied from 0·1 to 0·8 μg and maximal changes were produced by 10–20 μg. The flow increase began 3–5 s after injection and persisted for 2 to 10 min, depending on the dose. The responses of three cats in which all visible nerve fibres accompanying the artery were deliberately cut appeared to be identical to those with intact innervation. Doses larger than 1 μg reduced systemic pressure. These doses also increased portal pressure in all cats although never by more than 2 mmHg. The pressure and resistance changes produced by 2, 10 and 20 μg are shown in Table 1.

Glucagon infusions at rates of 0·5 to 10·0 μg/min for 5 min into the superior mesenteric artery increased mesenteric arterial flow throughout the period of infusion in each of four cats. These responses were unaltered by pre-treatment with intravenous atropine 0·5 mg/kg or by intravenous propranolol in doses (0·25–0·5 mg/kg) which blocked similar blood flow increases induced by isoprenaline (Fig. 1).

TABLE 1. *Effects of rapid close intra-arterial injections of glucagon on the resistance of the mesenteric and hepatic arterial vascular beds and on systemic arterial pressure*

Glucagon dose (μg)	Fall in arterial pressure (mmHg) (n=12)	Change in vascular resistance (%)	
		Mesenteric artery (n=8)	Hepatic artery (n=9)
2	− 5±1	−34±5	+16±10
10	−13±2	−53±4	+64±7
20	−18±1	−58±5	+153±63

Values presented are means ± standard error.
n, Number of cats.

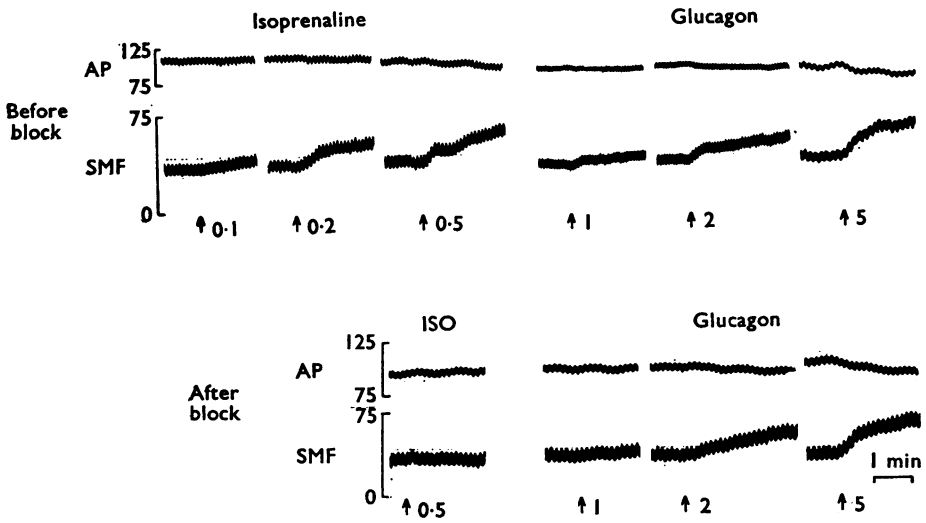

FIG. 1. Effects of isoprenaline and glucagon infused into the superior mesenteric artery on flow in this vessel before and after β-adrenoceptor blockade produced by intravenous propranolol (0·5 mg/kg). The arrows indicate the beginning of the infusion and only that portion of the record up to the maximum response is shown. The doses shown are μg/min. AP, Mean systemic arterial pressure ; SMF, superior mesenteric arterial flow.

Glucagon infusions of 0·5 to 10·0 μg/min into the portal vein (two cats) or jugular vein (two cats) also increased mesenteric flow, but no attempt was made to quantitate these changes.

Effects of glucagon on hepatic arterial flow

Rapid injections of glucagon 0·1 to 20 μg into the hepatic artery, portal vein or jugular vein reduced hepatic arterial flow and increased hepatic arterial resistance in nine of ten cats (Table 1). In one animal glucagon injected into the hepatic artery increased hepatic arterial flow and reduced resistance. The changes produced by intrahepatic or intraportal glucagon began within 5 s of injection. Similar responses occurred in three cats in which all visible nerves accompanying the hepatic artery were deliberately cut. Five minute infusions of glucagon at rates of 0·5 to 10 μg/min into the hepatic artery, portal vein or jugular vein also reduced hepatic arterial flow and increased resistance in each of three animals. In contrast, simultaneously recorded superior mesenteric arterial flow increased and mesenteric vascular resistance fell (Fig. 2). The effects of glucagon on hepatic arterial flow were unaffected by the prior administration of intravenous propranolol 0·5 mg/kg (four cats).

Effects of glucagon on renal arterial flow

Close intra-arterial injections could not be given because of the short length of the renal artery. However, rapid intraportal injection of doses up to 20 μg failed to produce any change in renal vascular resistance in four cats whereas simultaneously determined mesenteric resistance declined (Fig. 3).

Effects of glucagon on femoral arterial flow

Intrafemoral arterial injection of glucagon in doses up to 50 μg failed to produce any change in femoral arterial blood flow in six cats, although simultaneously determined mesenteric flow increased. This contrasts strikingly with the effects of intrafemoral isoprenaline, which produced a large increase in femoral arterial flow but only slight changes in mesenteric flow (Fig. 4).

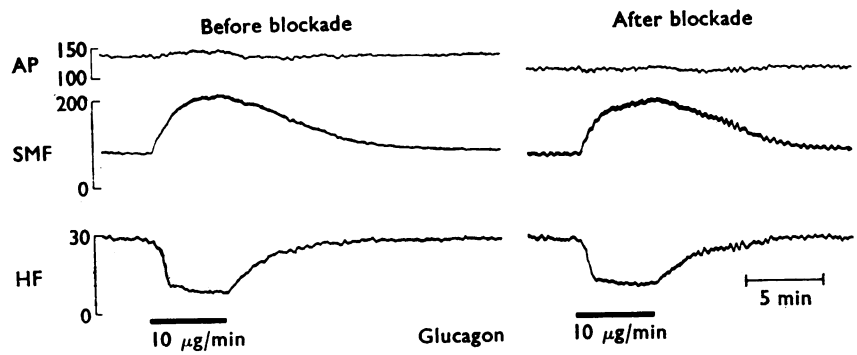

FIG. 2. Effects of intraportal infusions of glucagon (10 μg/min) on blood flow in the superior mesenteric and hepatic arteries before and after β-adrenoceptor blockade produced by intravenous propranolol (0·5 mg/kg). AP, Mean systemic arterial pressure; SMF, superior mesenteric arterial flow; HF, hepatic arterial flow.

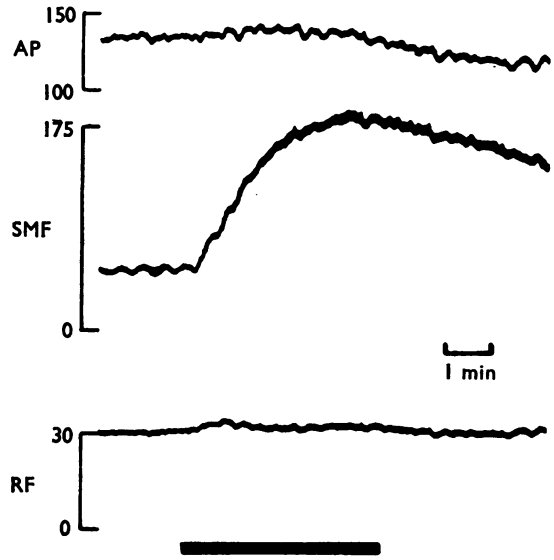

FIG. 3. Effects of intraportal infusion of glucagon 10 μg/min (indicated by the bar) or simultaneously determined mean arterial pressure (AP), superior mesenteric arterial flow (SMF) and renal flow (RF).

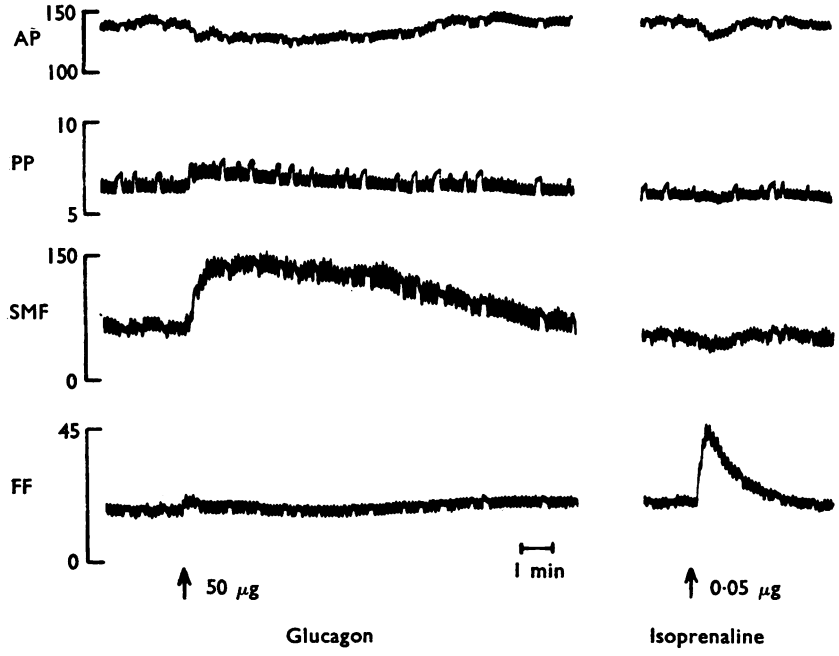

FIG. 4. Comparison of the effects of rapid intrafemoral injections of glucagon 50 μg and isoprenaline 0·05 μg on mean arterial pressure (AP), portal pressure (PP), superior mesenteric arterial flow (SMF) and femoral arterial flow (FF).

Changes in systemic arterial pressure

Regardless of the route of administration, glucagon doses exceeding 1 μg produced a reduction in systemic arterial pressure (Table 1).

Discussion

The present study indicates that, in addition to its well established metabolic actions, glucagon is a potent vasoactive substance. There is, however, considerable variation in the response of the various regional vascular beds. The mesenteric resistance vessels dilate whereas those of the hepatic arterial bed constrict. The femoral and renal vasculature is unresponsive. The action of glucagon on the hepatic and mesenteric vessels is local, for it occurs within a few seconds of close intra-arterial administration and is seen when these vascular beds are denervated. Glucagon may have a direct effect on the vascular smooth muscle of these areas or may possibly induce parenchymal metabolic changes which lead to the observed flow responses. It would obviously be of interest to know the effects of glucagon on isolated arterial preparations, but this information is currently lacking.

The lack of reactivity of the hind limb vessels in our preparation is opposed to the observation of Glick *et al.* (1968), who obtained evidence that glucagon dilates the femoral bed. It is difficult to compare our results with theirs because they used dogs, an isolated leg perfused at constant flow, and much larger doses of glucagon (15–50 μg/kg). Even these large doses produced a mean reduction in femoral vascular resistance of only 10·6%. The reduction in total peripheral resistance observed in man and animals is therefore more likely to be due to mesenteric vasodilatation than to dilatation in skeletal muscle. The increased mesenteric blood flow produced by glucagon is striking. In the experiment illustrated by Fig. 4, glucagon (5 μg/min) produced the same increase in mesenteric flow as isoprenaline (0·5 μg/min). The molecular weights of the two agents are 3,485 and 211 respectively and on a molar basis glucagon is therefore approximately 1·6 times more potent than isoprenaline. The superior mesenteric artery of the cat supplies blood to the whole of the small intestine, parts of the colon and pancreas and the lymph nodes, fat and connective tissues of the mesentery. It is not possible to determine from the present experiments whether all these tissues participate in the blood flow increase, but the small intestinal vessels must be involved for appropriate doses of glucagon more than doubled mesenteric arterial flow and the mass of the other tissues is too small to have accommodated an increased flow of this magnitude. Moreover, dilatation has been demonstrated in denervated isolated canine intestinal loops (Varró & Czernay, 1966).

The increased mesenteric blood flow produced by glucagon was not blocked by a dose of propranolol which blocked equivalent flow increases induced by isoprenaline. This behaviour is similar to that of the myocardium where glucagon elicits inotropic and chronotropic responses resembling those of isoprenaline but which are unaffected by β-adrenoceptor blockade (Glick *et al.*, 1968 ; Lucchesi, 1968).

The responsiveness of the mesenteric vessels to glucagon, which has also been observed in dogs (Merrill, Chvojka, Berkowitz & Texter, 1962), inevitably leads one to speculate on its physiological importance. The threshold infusion rate for mesenteric vasodilatation is close to 1 μg/min (Fig. 4). This would produce a

mesenteric arterial blood concentration in the cat of approximately 20 ng/ml which is many times higher than the 0·3 to 2·0 ng/ml plasma which represents the fasting range in most species (Unger & Eisentraut, 1967). Extracts of the small bowel of several mammals, however, contain glucagon-like immunological reactivity (Unger, Eisentraut, Sims, McCall & Madison, 1961) and also activate adenyl cyclase (Makman & Sutherland, 1964). The immunological assay indicates levels of 0·15 to 0·38 μg/g and the enzyme method 1 to 3 μg/g glucagon-like activity in small intestinal mucosa. Attempts to purify "gut glucagon" have not yet been completely successful (Unger, 1968), but if it should prove to have the same vasodilator activity as pancreatic glucagon it appears to be present in more than adequate amounts to exert a local regulatory action on intestinal blood flow. It is also conceivable, although no direct evidence is available, that pancreatic glucagon could influence pancreatic blood flow.

The responsiveness of the hepatic arterial resistance vessels to glucagon appears to be similar to those of the mesenteric bed, but the former constrict whereas the latter dilate. The magnitude of the hepatic arterial vasoconstriction produced by the rapid injection of 2 μg glucagon is very similar to that produced by 0·2 μg noradrenaline in a similar cat preparation (Ross & Kurrasch, 1969). On a molar basis glucagon is therefore approximately twice as potent as noradrenaline in constricting the hepatic arterial bed. Nevertheless glucagon almost certainly increases total liver blood flow because of the increased portal flow secondary to mesenteric dilatation. Indeed an increase in total liver flow (BSP method) has been reported in dogs (Shoemaker, Van Itallie & Walker, 1959) and in man (Kibler, Taylor & Myers, 1964) following intravenous glucagon.

It is of interest to compare the regional vascular responses of glucagon with those of isoprenaline:

	Glucagon	Isoprenaline
Femoral	No effect	Dilates
Mesenteric	Dilates	Dilates
Hepatic	Constricts	Dilates (Ross & Kurrasch, 1969)
Renal	No effect	No effect (Green & Kepchar, 1959)

It has been suggested, on the basis of studies mainly on rat heart, that all β-adrenoceptor responses result from stimulation of adenyl cyclase with the consequent accumulation of cyclic AMP which then induces the effector response (Robison *et al.*, 1967). The fact that glucagon increases cyclic AMP in a number of tissues and has a cardiostimulatory action which resembles that of catecholamines supports the view that cardiac β-adrenoceptor responses are related to accumulation of cyclic AMP. Glucagon and isoprenaline have, however, been shown in the present study to have different effects on certain vascular beds which indicates that the basic molecular mechanisms underlying these effects probably also differ. Moreover recent studies with adrenoceptor blocking agents indicate that there are differences between cardiac and vascular β-adrenoceptors (Dunlop & Shanks, 1968; Levy, 1966).

The ingenious hypothesis that all β-adrenoceptor responses may have a single underlying mechanism has proved most fruitful in stimulating further research. Nevertheless currently available evidence is difficult to reconcile with a unitary

theory for all cardiovascular beta-adrenoceptors. This difficulty is compounded by the fact that there is as yet no direct evidence that either glucagon or catecholamines increase cyclic AMP in vascular smooth muscle.

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