ACTIONS OF GLUCAGON ON THE PERFUSED VESSELS OF THE ISOLATED RABBIT EAR

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Summary: Actions of glucagon on the perfused vessels of the isolated rabbit ear were investigated. The two main actions of glucagon on the perfused ear vessels of the rabbit are: (i) release of noradrenaline which accounts for the constrictor response in low tone preparations. The response depends on the level of 3, 5 AMP. If the level rises as a result of noradrenaline action, constriction sets in. (ii) Glucagon may stimulate the adenylcyclase. In the high tone preparation 3, 5 AMP levels are probably high. Release of noradrenaline by glucagon would have little additional effect.

Key words: glucagon, rabbit ear vessel, adrenaline, 3,5, AMP, noradrenaline release

INTRODUCTION

The external ear of the rabbit comprises cartilagenous skeleton, muscles, sensory nerves and a richly innervated system of vessels supplying the overlying skin. Perfused ear is a convenient model to study the effect of drugs on predominantly cutaneous vascular bed. This preparation maintains a stable resistance over long periods of time (1). The preparation is sensitive to vasoactive substances and gives reproducible results.

Glucagon has potent cardiac actions and reduces the mean arterial pressure and peripheral vascular resistance (6, 16). Ross (12) has reported dilatation of mesenteric bed, increase in resistance of hepatic artery and no effect on the renal and femoral musculature.

Tibblin *et al.*(15) have shown that changes in blood sugar concentration are not essential for the flow responses following glucagon to occur. Therefore blood sugar concentration *per se* cannot be responsible for enhanced blood flow in the splanchnic bed of dogs.

There is a lack of detailed studies of the effects of glucagon on the blood vessels of the skin. The experiments described in the present investigation, were designed to investigate this aspect of glucagon action.

MATERIALS AND METHODS

Adult albino rabbits of either sex weighing 1-1.5 *kg* were used. The technique of perfusion has been described by Kapoor *et al.* (9). The effect of drugs is expressed in terms of reactivity of tissue by measuring the changes in resistance manifested as alteration in the outflow by counting the drops of the effluent in the unit time with an electronic drop counter (C.F. Palmer, Ltd.).

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The perfusion fluid was Ringer Locke's solution, aerated at 37°C. Perfusion for 2 hours preceeded experimentation. This produces a constant low resistance (an atonic preparation). In some preparations noradrenaline was added to the reservoir to produce a concentration of 0.1 $\mu g/ml$. This produces a constant high resistance (tonic preparation). In some preparation vasopressin (2 *U/L*) was substituted for noradrenaline.

A gravity feed apparatus was used to perfuse the vessels at a constant pressure of 85 cms of water. In majority of cases an approximately constant inflow rate of 3 *ml/min* was maintained.

Glucagon was administered by injection in 0.1 *ml* volume of saline into the tubing near the arterial cannula. The time interval between injections was 15 min.

The drugs used were glucagon, reserpine, phenoxybenzamine, vasopressin, noradrenaline, oestrogens, propranolol, atropine. Dilutions were made shortly before experimentation and the solutions in 0.9% sodium chloride.

Six rabbits were injected 2 *mg/kg* of reserpine subcutaneously daily for 3 days before perfusion of ears. Rabbits of either sex were injected intramuscularly stilboestrol 0.5 *mg* followed by 0.1 *mg* daily for 3 days.

RESULTS

In the atonic preparation injection of glucagon caused vasoconstriction. In ten preparation tested glucagon in a dose of 100 *ng* produced a marked vasoconstriction within 40 seconds and the effect persisted for 5 minutes ($p < .001$). In four preparations a dose of 50 *ng* produced a brief and marginally detectable constriction. The vasoconstrictor response was abolished by pretreatment of animals with injection of reserpine and phenoxybenzamine.

In tonic preparations (noradrenaline or vasopressin added to the reservoir) the injection of glucagon produced a dose dependent dilation (Table I). The range of effective concentrations were 10 *ng* to 100 *ng*. Atropine, propranolol, reserpine did not radically affect this dilatation of the tonic vessels by glucagon. Pretreatment of animals with stilboestrol sensitized the vessels to the constrictor action of glucagon.

DISCUSSION

The constrictor effect of glucagon on the vessels perfused with Ringer Locke's solution was abolished by pretreatment with reserpine or phenoxybenzamine indicating that the vasoconstriction may be due to release of noradrenaline from adrenergic nerves. Such a conclusion is in agreement with the findings of Scian *et al.* (13) that glucagon causes release of catecholamines.

The dilator effect of glucagon on the vessels perfused with noradrenaline or vasopressin indicates that it antagonises the pressor effect of noradrenaline and vasopressin in a similar fashion

as PGE_1 does (4). PGE_1 also stimulates the release of noradrenaline (7,8). The rabbit ear vascular bed probably contains beta-adrenoreceptors. Besides, propranolol has been reported to enhance the constrictor response to infused insoprenaline in the noradrenaline perfused ear (2). Nevertheless, propranolol does not affect the dilatation caused by glucagon in the noradrenaline perfused ear. The mechanism must differ from that of the beta-adrenoreceptors. The finding is in agreement with those of Ross (12) who has concluded that glucagon and isoprenaline have different effects on certain vascular beds suggesting that glucagon induced vasodilatation may not be mediated via beta-adrenoreceptors since propranolol failed to block the vasodilatation in mesenteric blood vessels.

TABLE I: Effect of glucagon on the perfused vessels of the isolated rabbit ear.

Pretreatment/perfusion fluids	*Dose of glucagon*			
	50 *ng*	100 *ng*	200 *ng*	400 *ng*
	Mean response represented as percentage difference from control			
Ringer Locke's Solution	—22% S	—30% S	—40% S	—50% S
Ringer Locke's Solution + Noradrenaline	+10%	+15% S	+20% S	+30% S
Ringer Locke's Solution + Vasopressin	+8%	+12% S	+26% S	+40% S
Stilboestrol	—18% S	—26% S	—30% S	—30% S
Reserpine	—1% NS	—2% NS	—2.5% NS	—3% NS

—Constriction +Dilatation NS — $P > .05$ S — $P < .001$

The most interesting feature of the present study is the reversal of the constrictor response to glucagon shown by low tone preparations when noradrenaline or vasopressin is added to the perfusion fluid. Noradrenaline has been shown to stimulate adenylcyclase activity in a number of systems and to antagonize it in others (3). Vasopressin is also thought to stimulate it (11). Stilboestrol raises the level of 3, 5 AMP (14). The high tone of the vessels in the presence of noradrenaline or vasopressin and the loss of dilator response to glucagon when 3, 5 AMP is further increased implies that the high levels of 3,5 AMP are correlated with vasoconstriction.

Flack *et al.* (5) showed that oestrogen causes an increase in the catecholamine content of uterine muscle and progesterone reduces it and they concluded that noradrenaline and 3, 5 AMP are synergists in causing contraction of the myometrium. The same conclusion would seem to apply to vessels of the rabbit ear.

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