

## ANTI-INFLAMMATORY ACTION OF GLUCAGON IN RATS

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- 1 Subcutaneous administration of glucagon (1 and 0.5 mg/kg) 30 min before the injection of carrageenin or dextran into the rat's paw reduced oedema and the local exudation of Evans blue previously given intravenously.
- 2 The effect persisted after removal of the adrenal medulla but not after adrenalectomy.
- 3 When glucagon (1 mg/kg, s.c.) was given daily after a local reaction to Freund's adjuvant injected into the paw had developed, a decrease in the reaction was observed up to 12 days. Blood sugar levels remained within the normal range.
- 4 Glucagon may exert an anti-inflammatory effect through the release of adrenal corticosteroids and thus help modulate inflammatory reactions.

### Introduction

An interplay of hormones seems to influence the development of inflammatory reactions (Garcia Leme & Schapoval, 1975). Insulin may act as a pro-inflammatory agent (Garcia Leme, Hamamura, Migliorini & Leite, 1973b; Garcia Leme, Böhm, Migliorini & Souza, 1974). Most of the actions of glucagon oppose those of insulin. The glucagon-producing alpha cells and the insulin-producing beta cells of pancreatic islets are regarded as a single functional unit performing opposing functions (Unger, 1970).

The present experiments were therefore undertaken to investigate whether glucagon affects inflammatory reactions.

### Methods

Male Sprague Dawley (Holtzman) rats weighing between 200-250 g were used.

#### *Production of oedema in rat paws*

Carrageenin (0.5 mg/ml) or dextran (1.0 mg/ml) was dissolved in distilled water and 0.1 ml injected into the subplantar area of one of the hind paws; the other was injected with 0.1 ml of 0.9% w/v NaCl solution (saline). The volumes of the paws up to the tibio-tarsal articulation were determined by plethysmography at various time intervals with a modification of the apparatus described by Winder, Wax & Been (1957). Results are expressed as percentage increase in relation to the initial volume of the paw. Control values (saline-injected paws) were subtracted from values obtained in carrageenin or dextran-injected paws. In these

groups the animals were anaesthetized with pentobarbitone sodium (20-40 mg/kg, i.p.). Complete Freund's adjuvant was used as commercially available; 0.1 ml of the suspension was injected into the subplantar area of one of the hind paws of unanaesthetized rats. The volumes of the paws were determined every three days. The percentage increase in relation to the initial volume, at 3 days after the injection of adjuvant, was designated as 100. This was done to minimize individual variations and because drugs were applied from the 3rd to 15th day after adjuvant injections.

#### *Double coaxial perfusion of the rat paws*

The subcutaneous space of both hind paws was perfused (Garcia Leme, Hamamura & Rocha e Silva, 1970) through polyethylene tubing (3 mm external and 2 mm internal diameter) introduced into the subcutaneous space of the distal part of the paws through a small incision high in the limbs. The perfusing saline solution (37°C) reached the subcutaneous space through a narrower inner tube and was collected through the outer one, the flow being adjusted to 1 ml/10 minutes. The perfusions were carried out for 30 minutes. Evans blue (40 mg/kg; 2% aqueous solution) was injected intravenously 5 min before the perfusions and the amount of dye present in the perfusates estimated by spectrophotometry at 600 nm. Control values (dye in perfusates from saline-injected paws) were subtracted from test values (dye in perfusates from carrageenin or dextran-injected paws).

### Removal of adrenal glands of medullae

The adrenal glands were removed under ether anaesthesia, by the dorsal approach. After adrenalectomy, rats were supplied with saline and water *ad lib.*, and were tested 72-80 h later. No functioning accessory adrenocortical tissue develops within 72-80 hours. Sham-operated animals were also tested at 72-80 h post-operatively.

The medulla was expressed through a small incision in the capsule. Operated and sham-operated rats were tested 72-80 h post-operatively.

### Blood sugar levels

These were estimated by the method of King & Garner (1947) in 0.05 ml whole blood from a tail vein.

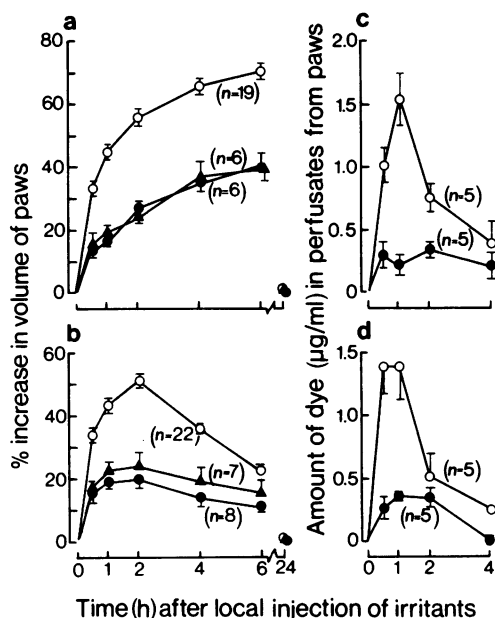
### Drugs

The following drugs were used: dextran, mol. wt. 60,000-90,000 (Nutritional Biochemical Corp.), carrageenin, mol. wt. 60,000-100,000 (Merck, Sharp & Dohme), complete Freund's bacto-adjuvant (Difco Labs.), crystalline glucagon (Lilly), Evans blue (E. Merck AG.), pentobarbitone sodium (Nembutal, Abbott), and indomethacin (Merck, Sharp & Dohme).

### Results

The subcutaneous administration of 0.5 or 1 mg/kg glucagon attenuated the development of oedema in paws injected with carrageenin or dextran 30 min later. Both doses of glucagon produced similar inhibition of the swelling (Figure 1a,b), possibly because they were absorbed from subcutaneous tissue at similar rates. Measurement of paw volumes made 0.5, 1, 2, 4, 6 and 24 h after the injection of the irritants showed in glucagon-treated rats significantly less oedema than the controls ( $P < 0.01$ , Student's *t* test), with the exception of those obtained at 24 hours. Blood sugar levels, determined 30 min after glucagon (0.5 or 1.0 mg) were 94-142 and 97-147 mg/100 ml blood respectively, and 74-130 mg/100 ml blood in the control group.

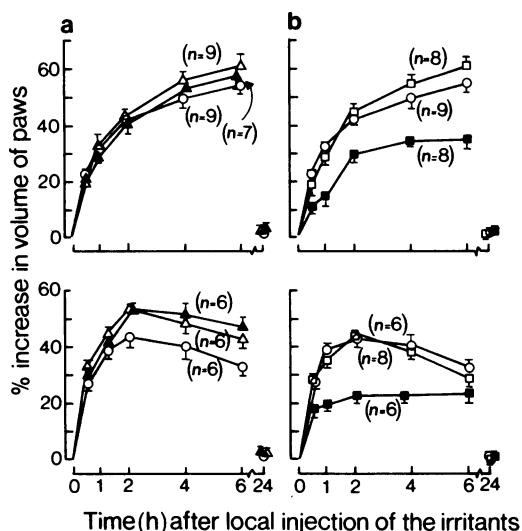
The exudation of Evans blue was also reduced in animals given 1 mg/kg glucagon, subcutaneously, 30 min before the injection of carrageenin or dextran (Figure 1c,d). Significant reduction ( $P < 0.01$ , Student's *t* test) was observed 0.5 and 1 h after the injection of the irritants. The leakage of dye was increased only during the 60 min following the injection of



**Figure 1** Oedema formation in rat paws after the local injection of (a) carrageenin (50 µg) or (b) dextran (100 µg) at 0 hours. (c) Dye (Evans blue, injected, i.v.) concentration in perfusates from rat paws with carrageenin or (d) dextran oedemas. (○) Controls; (▲) rats pretreated with glucagon (0.5 mg/kg, s.c.), 30 min before the injections of the irritants; (●) rats pretreated with glucagon (1.0 mg/kg, s.c.) 30 min before the injections of the irritants. Each point is the mean value; vertical bars show s.e. mean. *n* = number of animals used to obtain each point.

dextran. This confirmed findings with carrageenin (Garcia Leme, Hamamura, Leite & Rocha e Silva, 1973). Therefore the effects of drugs upon increased vascular permeability, as measured by the perfusion technique, should be evaluated during the one hour period following the injection of the irritants. Blood sugar levels were between 84-140 and 78-140 mg/100 ml blood in glucagon-treated and control animals, respectively.

Glucagon (1 mg/kg, s.c.) given to adrenalectomized rats 30 min prior to the injection of carrageenin or dextran did not reduce paw swelling (Figure 2a). No significant differences were found (Student's *t* test) between mean values in glucagon-treated and those in adrenalectomized or mock-adrenalectomized animals. However, glucagon (1 mg/kg, s.c.) decreased oedema produced in adrenal-demedullated animals by carrageenin or dextran for up to 6 or 4 h ( $P < 0.01$ ) respectively (Figure 2b).



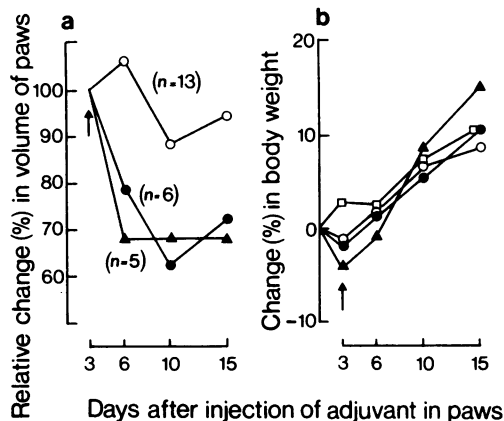
**Figure 2** Oedema formation in rat paws injected with carrageenin (50 µg) (upper graphs) or dextran (100 µg) (lower graphs). (a) Results obtained in adrenalectomized (Δ), sham-adrenalectomized (○) or in adrenalectomized rats pretreated with glucagon (1 mg/kg, s.c.) 30 min before the injection of the irritants (▲); (b) Results obtained in adrenal-demodulated (□), sham-operated (○) or in adrenal-demodulated rats pretreated with glucagon (1 mg/kg, s.c.) 30 min before the injection of the irritants (■). Each point is the mean value; vertical bars indicate s.e. mean. *n* = number of animals used to obtain each point.

Daily administration of glucagon (1 mg/kg, s.c.) from the 3rd to 15th day after injection of Freund's adjuvant into the rat's paw, produced a decrease of the already developed oedema ( $P < 0.01$ , Student's *t* test). A similar reduction was produced by daily injections of indomethacin (2 mg/kg, i.p.) (Figure 3a). In the three days following Freund's adjuvant injection rats lost some weight as compared to a control group. However, after this period, treated and untreated animals gained as much weight as the control group (Figure 3b). Blood sugar levels determined in all groups at the beginning of the experiments and at days 6, 10 and 15 were within the normal range.

## Discussion

Our results suggest that glucagon may have an anti-inflammatory action which is likely to depend on release of corticosteroids as it was ineffective in adrenalectomized rats.

Glucagon was effective when given before



**Figure 3** (a) Effect of daily injections of glucagon (1 mg/kg, s.c.) (●), or indomethacin (2 mg/kg, i.p.) (▲) from the 3rd (arrow) to the 15th day on oedema induced in the rat's paw by the local injection of 0.1 ml Freund's adjuvant. (○) Untreated rats injected with adjuvant. Percentage increase in relation to the initial volume of the paws, estimated 3 days after the injection of adjuvant, was arbitrarily taken as 100 and subsequent changes expressed in relation to it. (b) Changes in body weight during the observation period. (○) Six untreated rats injected with 0.1 ml saline in the paw; other symbols as in (a). *n* = number of animals used to obtain each point.

carrageenin or dextran or when given after the initial signs of adjuvant arthritis had developed. In the latter case daily doses of glucagon for 12 days were as effective as daily doses of indomethacin (2 mg/kg). However, indomethacin does not act through the release of corticosteroids (Garcia Leme & Schapoval, 1975). As pointed out by Whitehouse (1973/74), administration of a drug after the onset of inflammation bears more relationship to its potential use as an anti-inflammatory agent than prophylactic administration. The daily administration of glucagon may impair the regulation of blood sugar levels. However, daily doses of glucagon (1 mg/kg, s.c.) did not produce a persistent deviation from the normal range.

Serum concentrations of corticosterone were increased in rats after paw injections of carrageenin (Garcia Leme & Schapoval, 1975). Subcutaneous administration of corticosterone reduced the oedema resulting from a subsequent injection of carrageenin (Garcia Leme & Schapoval, 1975) and attenuated the vascular responses to histamine and 5-hydroxytryptamine given intradermally (Garcia Leme & Wilhelm, 1975). Plasma glucagon levels are elevated in patients with infections (Santeusano, Rocha, Faloona, Muller &

Unger, 1972) and in animals following intravenous injection of bacterial pyrogens (Bloom, 1973). Release of glucagon was also induced by stress (Bloom, Daniel, Johnston, Ogawa & Pratt, 1973).

Catecholamine release from the adrenal glands does not seem to be required for the anti-inflammatory action of glucagon. However, in man, adrenaline stimulates glucagon secretion (Gerich, Karam & Forsham, 1973).

Significant glucagon release during sympathetic nerve stimulation has been seen in the cat (Esterhuizen & Howell, 1970), dog (Renold, 1972) and calf (Bloom, 1973; Bloom, Edwards & Vaughan, 1973). Bloom *et al.* (1973) deduced from their results that any stimulus of sufficient intensity to constitute a 'stress' may cause a non-specific increase in sympathetic activity, which could raise plasma glucagon concentrations.

Similar mechanisms may occur during inflammatory reactions. Catecholamines from the adrenal glands or increased sympathetic activity may enhance glucagon secretion which in turn

would increase blood levels of corticosteroids and thus suppress inflammation.

Stimulation of the hypothalamo-pituitary-adrenal axis with release of corticosteroids was observed during the early phases of acute inflammatory reactions (Garcia Leme & Schapoval, 1975). Furthermore, insulin may act as a pro-inflammatory agent (Garcia Leme *et al.*, 1973b; Garcia Leme *et al.*, 1974). It is interesting that adrenaline inhibits the release of insulin and stimulates the release of glucagon, both *in vitro* and *in vivo* (Malaisse, Malaisse-Lagae, Wright & Ashmore, 1967; Porte, 1967; Leclercq-Meyer, Brisson & Malaisse, 1971). Glucagon may act as an endogenous anti-inflammatory hormone that could be of therapeutic value when administered in chronic inflammation.

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