The demonstration by Pecile *et al*(4) that rat hypothalamic extracts deplete rat pituitary of growth hormone provided the necessary *in vivo* test for GRF activity (as well as confirmation of the *in vitro* results). Ishida *et al*(5) showed that in rats, pig and beef hypothalamic extracts also deplete the pituitary of growth hormone. They also showed that brain cortex extract, vasopressin, oxytocin, and α-MSH were inactive in this test.

Both by molecular sieving on Sephadex, as well as by biological tests on purified fractions, our studies show that GRF activity is distinct from follicle-stimulating hormone-releasing factor (FSH-RF), luteinizing hormone-releasing factor (LRF), corticotropin-releasing factor (CRF) and thyrotropin-releasing factor (TRF)(14).

Summary. Potent preparations of growth hormone-releasing factor (GRF) were prepared from bovine hypothalamic extracts, by the glacial acetic acid concentration procedure followed by gel filtration on Sephadex. Purified GRF is distinct from vasopressin, oxytocin, α-MSH and other hypothalamic-releasing factors.

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Chronotropic Action of Glucagon on the Sinus Node.* (31261)

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Glucagon, a polypeptide hormone with a molecular weight of 3500, is secreted by the alpha cells of the islets of Langerhans. The liver is the major site of action, where glucagon accelerates hepatic glycogenolysis to produce an increase in peripheral blood sugar. Unger and co-workers have noted falling levels of blood glucose lead to an increased concentration of glucagon in portal and peripheral venous blood(1,2). Other effects of glucagon include inhibition of gastric motility, an antiphlogistic effect, an increase in urinary nitrogen and plasma free fatty acids, and a decrease in plasma amino acids. Regan *et al* have demonstrated an atrioventricular nodal tachycardia following injection of glucagon into the left coronary artery of dogs(3).

Because the results of Regan suggest a positive chronotropic action, we have studied the effect of glucagon by direct perfusion of the canine sinus node through the nutrient artery *in vivo*.

Materials and methods. Mongrel dogs

* This study was supported in part by grants from the Michigan Heart Assn. and U.S. Public Health Service.

weighing 8-12 kg were anesthetized with pentobarbital 30 mg/kg. The chest was opened in the midline, the pericardium incised and pericardial pouch created. The right coronary artery was isolated and the arterial branch to the sinus node cannulated using a technique previously described by James and Nadeau(4). Ligation of the sinus node artery has no significant effect on sinus rhythm because of extensive anastomoses of the artery (5). Pressures were routinely monitored in the right atrium, aorta and in the ligated sinus node artery, and recorded simultaneously with an electrocardiogram and tachogram. Glucagon,† in concentrations of 1×10^{-5} to 1×10^{2} μg/ml dissolved in Ringer's solution, was injected into the cannulated sinus node artery in 2 ml volumes over 5-10 seconds. Control injections utilized Ringer's solution and the diluent present in commercially-available glucagon. All intranodal injections were delivered within 5-10 seconds from a hand syringe. Injections into the sinus node artery produce a transient bradycardia during injection; this is not due to pH, temperature or ion content, has no neurogenic component, occurs with fresh warm autogenous arterial blood, is most likely a local response to physical distention of the sinus node artery (6). For comparative purposes, glucagon also was given into the femoral vein and into the right coronary artery of dogs without a right branch to the sinus node.

Results. Glucagon into the sinus node artery. With serial injections of glucagon at 10-fold increments in 17 dogs, a consistently positive chronotropic effect was demonstrated at 1.0 μg/ml and 10 μg/ml (Fig. 1). With 10 μg/ml of glucagon the mean increment in heart rate was 30 ± 12 (S.D.) beats per minute above control, lasting 17 ± 10 minutes. Occasionally 0.1 μg/ml produced a slightly positive response but no effect was noted at lower concentrations. Concentrations of 100 μg/ml produced variable effects including sinus arrest with an escape AV nodal rhythm. Intravenous glucagon in comparable amounts had no effect on heart rate (Fig. 1). The positive chronotropic effect of

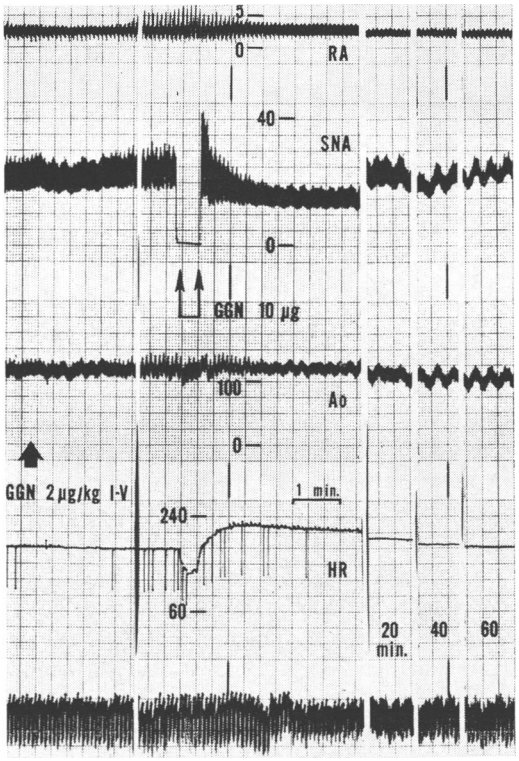

FIG. 1. In this 10 kg dog there is a positive chronotropic effect with glucagon (GGN) 2 ml of 10 μg/ml into the sinus node artery, but no effect from glucagon in the same amount (2 mg/kg) is achieved when given intravenously.

tyramine hydrochloride, 10 μg/ml, was compared to glucagon in 5 dogs (Fig. 2). The maximal acceleration was similar for glucagon and tyramine but the duration following tyramine was briefer. The commercial diluent for glucagon was dissolved in Ringer's solution in concentrations comparable to those obtained in preparing glucagon. Injected into the sinus node artery, the diluent produced no significant tachycardia. 1.0 mg/kg of atropine sulfate intravenously did not significantly alter the positive chronotropic action of intranodal glucagon. Analyses of blood glucose were made during the early experiments. We were satisfied that the positive chronotropic effect of glucagon occurred without any significant change in peripheral blood glucose.

Effect of beta-adrenergic blockade. In 6 dogs the effect of naphthylisoproterenol

† Glucagon was generously supplied by Dr. William Kirtley, Lilly Laboratories, Indianapolis, Ind.

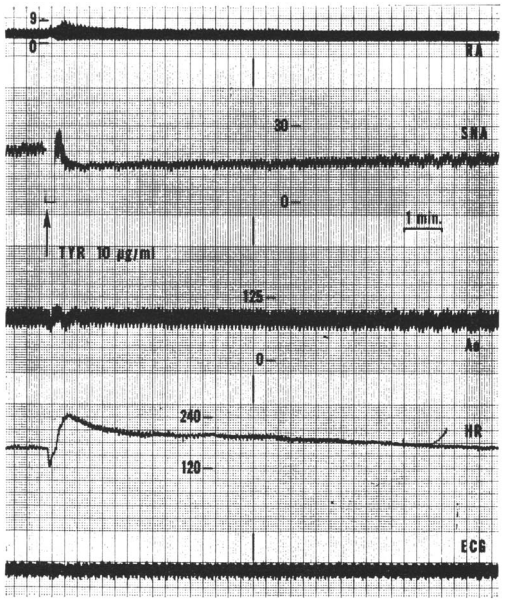

FIG. 2. Intranodal tyramine (TYR) produces a sinus tachycardia similar to, but briefer than that from glucagon.

(pronethalol)‡ on the tachycardia from glucagon was examined. Concentrations of 1 to 10 µg/ml reversed the sinus tachycardia from glucagon and diminished its subsequent effect (Fig. 3). Complete reversal and blockade of the sinus tachycardia from glucagon could be produced consistently only with relatively high concentrations of naphthylisoproterenol (10 µg/ml and greater); however, a direct negative chronotropic action as well as beta-adrenergic blockade occurs at such levels.

Effect of reserpine. Nine dogs were pretreated with reserpine (0.5 mg/kg i.m. for 2 days) and identical experiments with glucagon were carried out. The positive chronotropic action of glucagon was diminished in intensity and in duration, but not abolished (Fig. 4).

Discussion. We have demonstrated a positive chronotropic effect when glucagon is injected through its nutrient artery into the canine sinus node *in vivo*. Such an action might be explained in 3 ways: 1. a direct effect by glucagon on the sinus node; 2. a vagolytic action; 3. an indirect effect through release of local stores of catecholamine. Failure of atropinization to alter the sinus tachycardia leaves only the first and third of these possible explanations tenable.

Pretreatment with reserpine caused a diminution in the positive chronotropic action but did not abolish it. Naphthylisoproterenol, a beta-adrenergic blocking agent, reversed and partially blocked the tachycardia. These findings suggest that glucagon releases norepinephrine locally. However, the persisting tachycardia following glucagon in reserpinized dogs as well as the requirement of relatively high concentrations of a beta-adrenergic blocking agent to reverse the tachycardia suggest that a direct action of glucagon on the

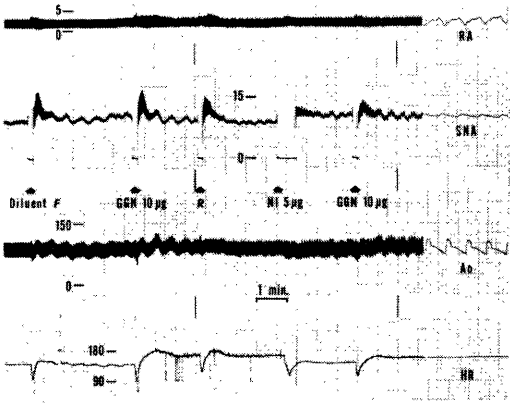

FIG. 3. Effect of intranodal naphthylisoproterenol (NI) on tachycardia produced by glucagon, and its effect on a subsequent injection of glucagon. An injection of Ringer's solution (R) preceded the NI. Some of the sharp tachogram deflections represent premature beats but others are artifacts.

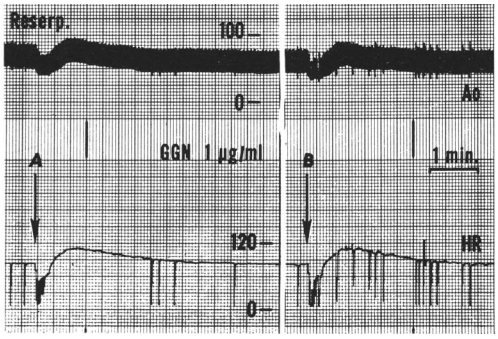

FIG. 4. Effects of intranodal glucagon in a reserpinized dog. Slow heart rate and low central aortic pressure are due to reserpinization. Diminished effect and shortened duration of tachycardia following 2 injections of glucagon (A and B) are depicted.

‡ Dr. Alex Sahagian-Edwards, Ayerst Laboratories, New York, furnished us with pronethalol.

sinus node also may exist. This likelihood is further supported by the fact that tachycardia from glucagon lasted much longer than that from tyramine. The accelerating effects with intranodal glucagon could not be reproduced with comparable amounts of glucagon administered intravenously, indicating that the positive chronotropic action occurred within the sinus node and was not secondary to any effect from recirculated glucagon.

Does glucagon under normal or pathologic conditions have any cardiovascular effect in man? It is known that levels of glucagon in human plasma are increased by hypoglycemia. Many of the symptoms of hypoglycemia are related to elevated circulating catecholamines. Measured levels of glucagon in the fasting state in dogs range from 0-1300 micromicrograms Eq. per ml of plasma(2). During severe hypoglycemia, this concentration may exceed 2000 micromicrograms Eq. per ml or .002 μg Eq. per ml. The positive chronotropic effect in our dogs occurred with concentrations of glucagon which were far greater than those recorded in man. However, secretion of glucagon following hypoglycemia may persist for several hours, thus permitting chronic stimulation of the sinus node. It is possible, therefore, that subliminal levels of glucagon bathing the sinus node over an extended period of time result in a tachycardia, and that some of the cardiovascular symptoms observed during or after hypoglycemic episodes in man may be related to elevated circulating glucagon.

Summary. Glucagon injected into the cannulated sinus node artery of dogs produces a positive chronotropic effect. This effect appears due in part to local release of nodal norepinephrine and in part to a direct action by glucagon on the sinus node.

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Experience with Intrafemoral Transplant of Breast Tumor in Rats.* (31262)

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A technique whereby tumor cells can be introduced into the medullary cavity of the rat femur has been reported(1). This report describes the successful growth of mammary tumor in the same site.

Method and material. The experiments fell into two phases.

Phase I. Sprague-Dawley female rats were divided into 2 groups of 20. The first group received an homologous suspension of DMBA-induced breast cancer cells. Following the inoculation the tumor failed to grow, was at no time radiologically demonstrable and no viable tumor cells remained when the femora were histologically examined.

The second group received an autologous suspension of DMBA-induced breast cancer cells. All the animals in this group developed lytic lesions in the femur, well shown radiologically and confirmed by histology. The difficulty, however, of establishing a uniform group of animals where inoculation of the femur could be performed in a fixed number,

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