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BRIEF NOTES AND COMMENTS

Cation Requirements for Insulin Secretion in the Isolated Perfused Pancreas

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SUMMARY

The effect of cations on insulin release was studied in the isolated pancreas of the rat perfused with a synthetic perfusate consisting of albumin and buffer. The omission of calcium and magnesium ion completely inhibited insulin release stimulated by glucose. The ionic requirement was specific for calcium ion since perfusates containing calcium but no magnesium permitted normal insulin release by glucose, whereas perfusates containing magnesium but no calcium did not. The presence of only 0.2 mEq. per liter calcium was adequate to permit much, though not all, of the insulin response after glucose to occur. Potassium ion, when raised from 4 to 8 mEq. per liter, directly stimulated insulin release in the complete absence of glucose. DIABETES *15*:910-13, December, 1966.

Although it is well established that glucose directly stimulates insulin secretion in vitro,[1-4] recent studies indicate that a variety of other agents may influence the release of this hormone from the pancreas. Carbohydrates metabolized by the islets such as mannose,[2,3] tolbutamide,[3,5] and glucagon[6,7] can also stimulate insulin secretion in vitro, while epinephrine inhibits the process.[3]

Despite the fact that ions are known to play an important role in the secretion of a variety of proteins from other tissues,[8-10] their direct role at the pancreatic level on the phenomenon of insulin release heretofore has not been established.

The present study utilizes the isolated perfused pancreas of the rat previously described by us[2] which has been modified to employ a synthetic medium consisting of pure albumin in buffer as perfusate instead of diluted whole blood. This modification permits the controlled addition or deletion of known amounts of cations and the study of their effect on insulin release in the absence of glucose.

MATERIALS AND METHODS

The pancreas with the adjacent spleen, stomach, and segment of the duodenum was surgically removed from Long-Evans male rats and placed in the perfusion apparatus previ-

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ously described.[2] Standard perfusate consisted of 25 per cent salt-poor human albumin (Cutter Laboratories) diluted to 4 per cent in Umbreit buffer containing in mEq. per liter: sodium 141, potassium 4, calcium 4.2, magnesium 2.4, phosphate 1.5 and bicarbonate 29. In some experiments either calcium or magnesium ion or both were omitted without replacement; in others, potassium ion was increased to 8 mEq. per liter, accompanied by a corresponding decrease in the content of sodium.

In order to minimize the effect of residual endogenous cation in experiments where calcium or magnesium was to be omitted, the pancreas was preperfused with 50 ml. of the specific cation-depleted albumin-buffer. After this washing procedure, 150 to 160 ml. perfusate was continuously circulated through the pancreas for a ten-minute equilibration period. Following collection of a zero-time sample, glucose (200 mg. per 100 ml.) was added and the circulation continued for one hour.

Oxygen consumption, glucose uptake (in experiments where glucose was added), and valine-C-14 incorporation into protein were monitored as a measure of tissue viability. Oxygen consumption was calculated from the arterial-venous difference in partial pressure of oxygen as measured with an oxygen electrode, and the known flow rates of the perfusate and the oxygen solubility at 37° C. Oxygen tension approximated 450 mm. Hg in the arterial flow and decreased to 200 to 230 mm. Hg in the venous effluent. Oxygen consumption reached a constant and maximal level of about 0.1 ml. per minute per gram dry weight when flow rates were maintained at 10 to 12 ml. per minute.

Glucose in the perfusate was measured by the method of Somogyi and Nelson.[11] Insulin was determined by the immunologic assay of Grodsky and Forsham,[2,12] using pure rat insulin as the reference standard. Methods for the measurement of valine-C-14 into total protein were previously described.[13] Insulin content of rat pancreas was determined immunochemically after precipitation of the proteins with trichloroacetic acid, and extraction of the crude hormone with acid alcohol.[14]

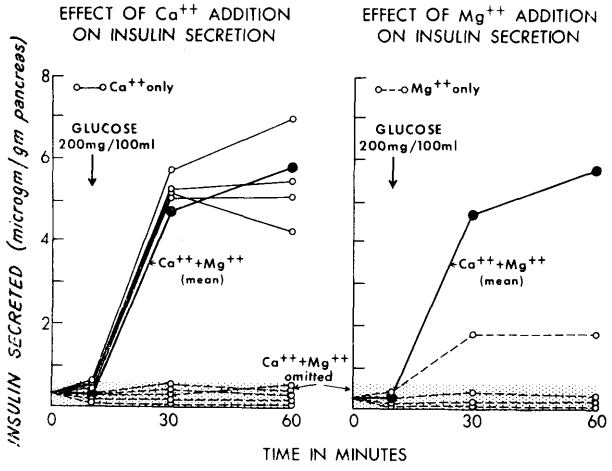

FIG. 1. The effect of calcium and magnesium ions on insulin release stimulated by glucose. The shaded area in each figure shows the mean range of basal insulin levels obtained in fifteen control experiments in which glucose was not added. •——• shows the mean results of twenty-seven perfusions in which glucose was added to perfusate containing normal amounts of calcium and magnesium ions.

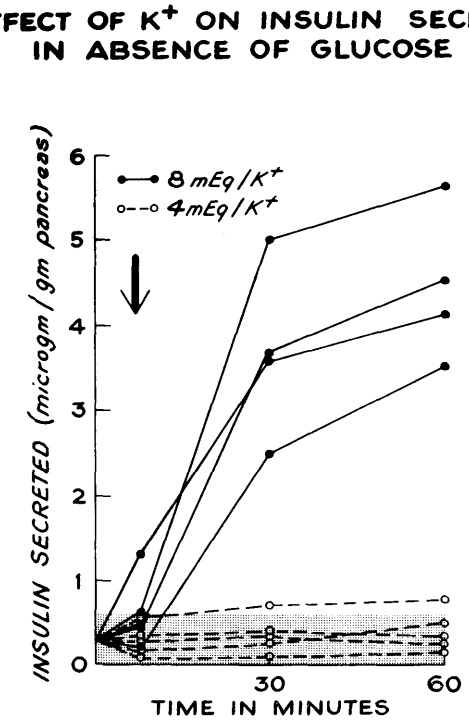

FIG. 2. The effect of potassium ion on insulin secretion in the absence of glucose. The shaded area shows the mean range of basal insulin levels obtained in fifteen control experiments in which glucose was not added.

RESULTS

The mean insulin content of normal rat pancreas assayed immunologically against rat insulin standards was 47 ± 10 μg. per gram pancreas (± × S.E., n = 11). The effect of 200 mg. per 100 ml. glucose recycled through the rat pancreas in perfusate containing normal amounts of calcium and magnesium is shown in figure 1. The glucose elicited a rapid increase in insulin accumulating in the circulating perfusate amounting to 5.8 μg. per gram or about 12 per cent of the pancreatic insulin by one hour. When calcium and magnesium were omitted from the perfusate (figure 1), glucose failed to elicit insulin from the pancreas. When perfusates were used, in which normal concentrations of calcium ion but no magnesium ion were added (figure 1, left), glucose produced a typical stimulation of insulin release. In contrast, when magnesium was present in the perfusate, but calcium ion was still omitted, insulin release after glucose administration remained inhibited (figure 1, right). In all experiments, oxygen consumption (0.1 ml. per minute per gram), valine-C-14 incorporation into protein (15,000 cpm/pancreas) and glucose uptake (10 to 15 mg. uptake) measured at the one-hour period were unaffected by the presence or absence of calcium and magnesium ions. The minimum amount of calcium required to permit normal release of insulin by glucose is being investi-

gated and will be reported in detail elsewhere. In two experiments, the presence of small amounts of calcium ion (0.2 mEq. per liter) resulted in a partial recovery of insulin secretion during glucose perfusion; levels of insulin achieved approximated 2 μg. per gram pancreas or 30 per cent of normal response.

The effect of potassium ion on insulin secretion in the absence of glucose is shown in figure 2. When cations were maintained at normal levels, no insulin release occurred during the sixty-minute perfusion period. The addition of potassium ion to achieve a concentration of 8 mEq. per liter resulted in an immediate and sustained response by the pancreas to secrete insulin. The amount of insulin elicited by potassium ion averaged 8.5 per cent of the pancreatic content or about two thirds of that stimulated by 200 mg. per 100 ml. glucose perfused under identical conditions. Oxygen consumption and amino acid incorporation remained unchanged in these experiments.

DISCUSSION

Previous studies from this laboratory using the above perfusion technic have shown that insulin accumulating in the perfusate arises from the secretion of stored insulin rather than from the de novo synthesis of the hormone.[13] Thus the calcium requirement and potassium stimulatory effects observed in the current study are on the insulin secretory phase. Insulin secretion is known to require the viability of a series of membranes including that surrounding the intracellular granule and the plasma membrane to which the granular membrane ultimately must fuse. Since many membrane systems are known to require potassium and calcium ions for excitation and transport,[15] the potassium-calcium effects on insulin secretion may be at the membrane level. However, a too restrictive interpretation of ion effects solely on the islet membranes cannot be made at this time, particularly in view of the ubiquitous requirements for potassium ion in many phases of intermediary metabolism which may be required for the release of insulin by glucose.[2,3] It is unlikely that an activation of an adenosine triphosphatase similar to that involved in sodium transport is occurring, since this enzyme, though stimulated by potassium, is also activated by magnesium,[16] an ion we find not required for insulin release.

Observations that the secretion of a variety of substances is stimulated by potassium ion, requires calcium ion, and is comparatively independent of magnesium ion,[8-10] suggest that the ionic effects we observed for insulin release are not specific for this hormone but general to all systems where material stored in granules is extruded from the cell.

At this time the role that calcium levels play in insulin release in intact animals is unclear, since the lowest plasma calcium levels compatible with life may still be much higher than that required to maintain the secretory system. The fact that potassium ion stimulates insulin secretion in vitro in the absence of glucose may be of more immediate significance, since the levels required for stimulation are within pathophysiologic ranges. The marked depletion of total body potassium known to occur in severe diabetes[17] where insulin secretion is impaired, or in the temporary diabetic state produced by the thiazide diuretics[18] may in part be related to this direct effect of potassium on pancreatic insulin release. The recent observation of impaired insulin release after glucose in subjects with hypokalemia in man and the improvement of the insulin release mechanism after potassium therapy[19] is consistent with our observation that potassium ion can act independently of glucose to promote insulin release.

ACKNOWLEDGMENT

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EDITORIAL

CHANGE IN EDITORS

The Journal DIABETES salutes Dr. Irving Graef in this final issue published under his Editorship. Dr. Graef became Associate Editor in 1956, and succeeded Dr. William C. Stadie as Editor in 1960. During his tenure as Editor, the Journal DIABETES has enjoyed unusual growth for a periodical devoted to a single disorder and its related metabolic problems.

There have been remarkable changes in DIABETES during this period. The number of manuscripts received annually has been ever increasing thus allowing representation of many interests. Of particular note is the acquisition of papers reporting new work in the basic sciences. Dr. Graef has consistently encouraged new ideas and has striven for literary excellence. He has been alert to new developments, particularly abroad, and has featured them in "Brief Notes and Comments" and in editorials.

The Journal thanks Dr. Graef for his untold hours of work, conscientious correspondence with authors, encouragement of the young writer, and development of DIABETES to its present status. The new Editor, Dr. Harvey C. Knowles, Jr., with Dr. David M. Kipnis and Dr. Henry T. Ricketts as Associate Editors, will endeavor to continue publication at the same levels of enthusiasm and excellence set by Dr. Irving Graef.

ABSTRACTS

Angervall, L.; and Säve-Söderbergh, J. (Patholog. Institute and Sahlgrenska Sjukhuset, Univ. of Göteborg, Sweden): MICROANGIOPATHY IN THE DIGESTIVE TRACT IN SUBJECTS WITH DIABETES OF EARLY ONSET AND LONG DURATION. Diabetologia 2:117-22, 1966.

Verbatim Summary. The digestive tract has been examined by light microscopy in seventeen subjects with diabetes of early onset, long duration and with various causes of death, in six "normal" controls and ten controls with uremia and/or hypertension. In the diabetics grave lesions were demonstrated to a large extent in the capillaries and venules of the oral mucosa, and in small arteries and arterioles in the gastrointestinal tract (from the oesophagus to rectum). The capillary and venular lesions were similar to those earlier described in the skin of diabetics. The arterial lesion was characterized by a hyalin, strongly PAS-positive, picrinophilic (eosinophilic), richly fat-containing (sudanophilic) wall thickening accompanied by heavy reduction of the lumen, endothelial atrophy and medial degeneration. Similar arterial lesions were demonstrated in the kidneys, pancreas, liver, spleen, adrenal glands, testes, prostate and ovaries. The study suggests that hypertension was not of essential importance to the development of the arterial lesions. It is assumed that the grave arterial lesion accentuates terminally in the course of diabetes, where final complications such as ischemia and adrenocortical overactivity are of pathogenetic significance.

Anselmino, K. J.; and Hoffmann, F. (Rheinische Landesfrauenklinik Wuppertal, and Evangelisches Krankenhaus, Essen-Werden, W. Germany): THE PANCREATROPHIC (INSULOTROPHIC) PRINCIPLE OF THE ANTERIOR PITUITARY GLAND. Deutsch. Med. Wschr. 91:1401-05, August 1966.

A review of the authors' early experiments regarding the existence of an insulotropic hormone supposedly elaborated by the anterior pituitary gland. They suggest, and quote some circumstantial evidence from the literature, that the active principle is not identical with growth hormone. O.V.S.

Benjamin, Fred; and Casper, Donald J. (Depts. Obstet. and Gynec., Queens Hosp. Center Affiliation and Long Island Jewish Hosp., Jamaica, N.Y.): ALTERATIONS IN CARBOHYDRATE METABOLISM INDUCED BY PROGESTERONE IN CASES OF ENDOMETRIAL CARCINOMA AND HYPERPLASIA. Amer. J. Obst. Gynec. 94:991-96, April 1, 1966.

The effect of a long-acting progestational compound, Dela-