Vol. 84, No. 2, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 29, 1978 Pages 293-299

INSULIN RECEPTORS IN ISOLATED MOUSE PANCREATIC ACINI

Murray Korc,* Hariharan Sankaran,† K.Y. Wong,*
John A. Williams† and Ira D. Goldfine*

Metabolism Section, Veterans Administration Hospital and the
Departments of Medicine* and Physiology,† University of California
San Francisco, California 94121

Received July 31,1978

SUMMARY: Specific insulin receptors were measured in isolated mouse pancreatic acini. Scatchard analyses revealed a high affinity binding site with a K_d of 1.67 nM and a lower affinity site with a K_d of 83 nM. Binding of insulin to these receptors was rapid, one-half maximal binding occurring at 2 min and maximal binding at 30 min. Insulin stimulated the uptake of the glucose analogue 2-deoxy-D-glucose; maximum effects were detected at 1.67 μM. Insulin, in contrast, had no direct effects on alpha-aminoisobutyric acid uptake. The finding of high affinity insulin receptors in pancreatic acinar cells supports the hypothesis that insulin may directly regulate specific functions in the exocrine pancreas.

In mammals and birds, islets of endocrine tissue are dispersed throughout the exocrine pancreas. Although the reasons for this unique anatomic arrangement are unknown, it has been speculated that insulin and other islet cell hormones may directly influence pancreatic exocrine function (1,2). Prior studies, employing either intact or perfused pancreas, or pancreatic fragments, have suggested that insulin may regulate glucose oxidation (3), and amylase secretion and synthesis (4,5). These preparations, however, contain heterogeneous cell populations including duct cells, islet cells, and connective tissue. Since many cells, including fibroblasts, have insulin receptors and are insulin-sensitive (6,7), it has been difficult with these preparations both to document a direct effect of insulin on acinar function and to study early steps in insulin action.

0006-291X/78/0842-0293$01.00/0

We have recently described a preparation of isolated mouse pancreatic acini consisting of 20-30 acinar cells that have maintained their original polarity around an intact lumen (8). These acini, which are nearly devoid of duct cells, islet cells, and connective tissue, are exquisitely sensitive to regulation by physiologic pancreatic secretagogues. We now report that insulin binds to specific receptors, and then stimulates 2-deoxy-D-glucose (2DG) uptake in these acini.

MATERIALS AND METHODS

Preparation of Acini. Isolated mouse pancreatic acini were prepared by limited enzymatic digestion employing a mixture of hyaluronidase, collagenase, and chymotrypsin (8). For all studies acini (0.6-1.1 mg protein/ml) were incubated at 37°C in HEPES-buffered Ringer solution pH 7.35 (HR), equilibrated with 100% oxygen (8,9). Acini were always preincubated 60 min and then resuspended in fresh medium prior to measuring binding or transport. Bovine serum albumin (0.5%), which was added to the incubation medium, had low insulin-like activity (7). For (^{3}H)2-deoxy-D-glucose (^{3}H(2DG)) uptake studies, the glucose in the media (5.6 mM) was omitted and replaced by sodium pyruvate (2 mM).

Insulin Binding Studies. Biologically active porcine ^{125}I-insulin was prepared by a stoichiometric chloramine-T method (10). To measure insulin binding, 3.5 ng/ml of ^{125}I-insulin were incubated with 5-15 ml of acini in Falcon culture flasks (25-75 cm^2) at 37°C in a shaking water bath at 60 cycles/min. To separate the hormone bound to receptors, one ml aliquots of the incubation mixture were centrifuged at 300 x g for two minutes. The pellet was then washed twice with 0.9% sodium chloride at 4°C. Nonspecific binding was determined by incubating the labeled hormone in the presence of 100 µg/ml of unlabeled insulin. Specific binding was determined by subtracting nonspecific binding from total binding. Degradation of labeled insulin, as monitored by precipitation of labeled insulin with 10% trichloroacetic acid (11), did not exceed 15% of total.

Uptake Studies. To measure sugar and amino acid uptake, 0.1 µM (^{3}H)2DG (8.26 Ci/mmole, New England Nuclear), or 0.05 µM (^{3}H)α-aminoisobutyric acid (AIB) (10 Ci/mmole, New England Nuclear) without additional carrier were incubated at 37°C with 3-12 ml of acini in either 25 or 50 ml polypropylene plastic Erlenmyer flasks (Nalgene). At specified times, 0.7 ml of the incubation mixture was added to 10 ml of 0.9% saline at 4°C, and collected by filtration on Nucleopore filters (12). Radioactivity was measured as previously described (12). Protein was assayed by the method of Lowry (13), employing a bovine serum albumin standard.

RESULTS

Insulin Binding. Specific binding of insulin to acini was half-maximal at two minutes and maximal at thirty minutes. While specific binding decreased slightly at later time points, nonspecific binding

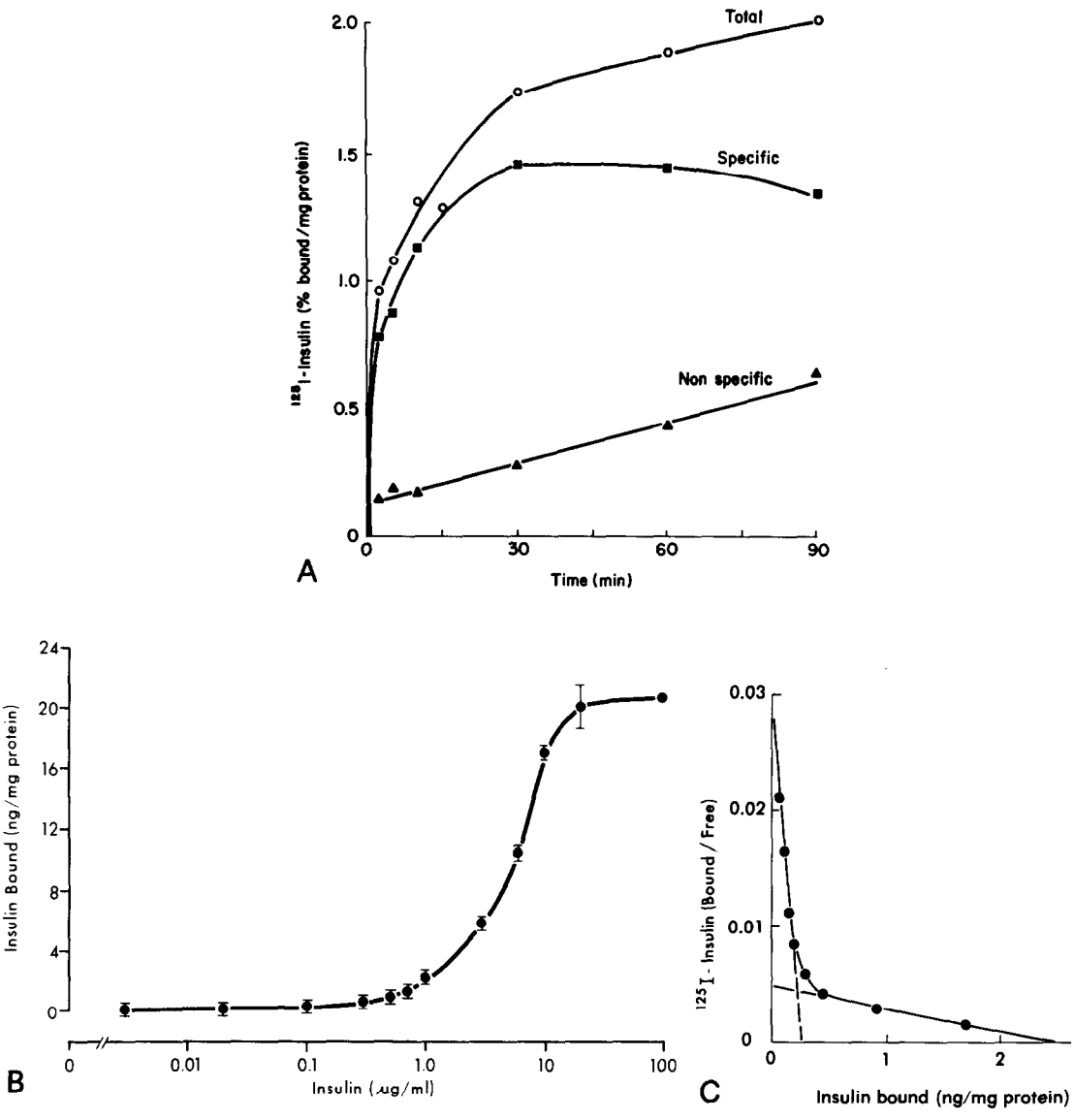

Figure 1. Binding of insulin to isolated acini. A. Time course. Values are the mean of triplicate determinations. B. Dose response; time of incubation, 30 minutes. Values are the mean ± SEM of triplicate determinations. C. Scatchard plot (14) of binding data.

increased continuously throughout the incubation period (Figure 1A). When specific insulin binding was plotted as a function of the insulin concentration in the media, half-maximal binding occurred at 5 μg/ml, and maximal binding occurred at 20 μg/ml (Figure 1B). Scatchard analysis

(15) of this binding data was compatible with two orders of binding sites, a high affinity site with K_d of 0.01 μg/ml (1.67 nM) and a low affinity site with a K_d of 0.5 μg/ml (83 nM) (Figure 1C). Other studies with acini indicated that hormones unrelated to insulin, such as glucagon, growth hormone, or TSH, did not influence the binding of labeled insulin to receptors. In contrast, proinsulin had 2-5% and desoctapeptide insulin had less than 1% the potency of insulin.

Transport Studies. Insulin stimulated the uptake of the glucose analogue (^{3}H)2DG into isolated mouse pancreatic acini. Both control and insulin-stimulated (^{3}H)2DG uptakes were linear up to 45 minutes (Figure 2A). A detectable effect of insulin was seen at 0.01 μg/ml (1.67 nM), half-maximal stimulation occurred at 0.25 μg/ml, and maximal stimulation at 10 μg/ml (1.67 μM) (Figure 2B).

The uptake of (^{3}H)AIB into acini was rapid; maximal uptake was seen after 20 min of incubation. In contrast to the stimulation of glucose uptake, insulin had no effect on (^{3}H)AIB uptake at either earlier time points or at steady state (Table I). This lack of an effect of insulin on amino acid uptake is in concert with previous studies suggesting that insulin does not stimulate amino acid transport into the exocrine pancreas (3,15).

DISCUSSION

Our current study establishes the presence of specific high-affinity insulin receptors in acinar cells from mouse pancreas. Scatchard analyses are consistent with two orders of binding sites having affinities similar to those described for insulin receptors in other tissues (16). A comparison of the dose response curves for insulin binding (Figure 1B) and 2DG uptake (Figure 2B), suggests that a large fraction of the binding sites needs to be occupied before

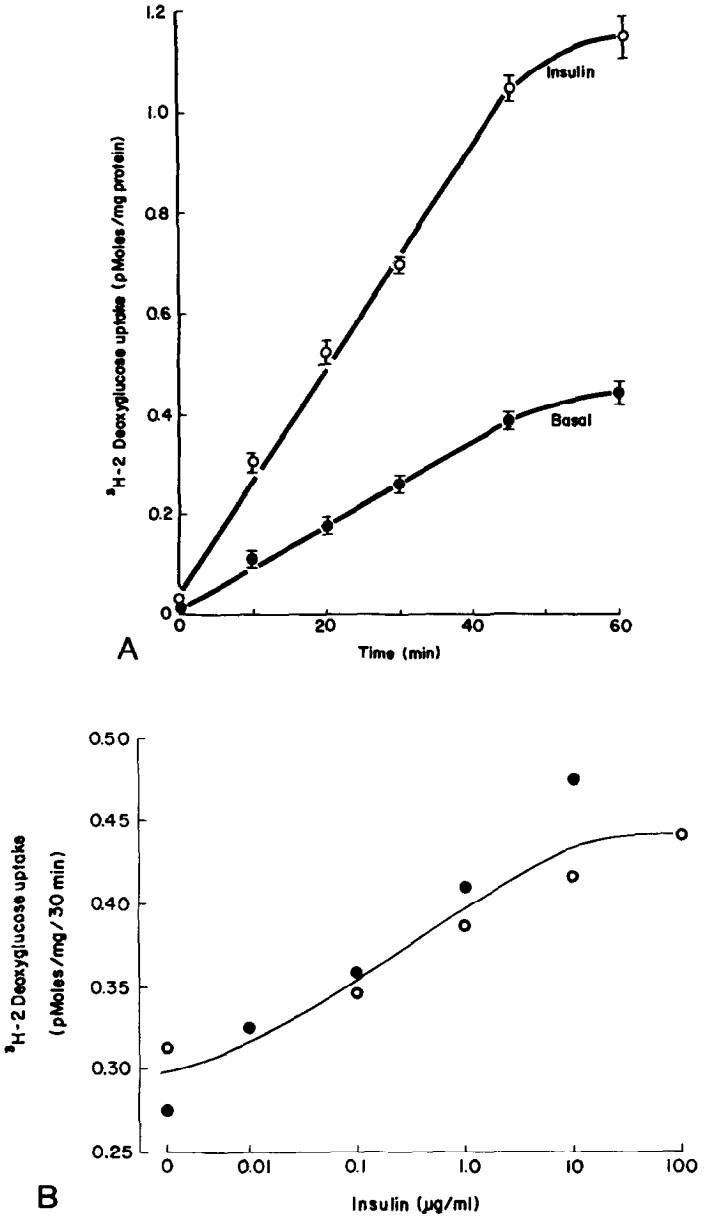

Figure 2. Effect of insulin on (^{3}H)2DG uptake. A. Time course; insulin concentration, 1.0 μg/ml. Values are the mean ± SEM of triplicate determinations. B. Dose response; open and closed symbols represent two different experiments. Time of incubation, 30 minutes.

stimulation of 2DG uptake can become significant. This finding in acini is in contrast to other tissues such as fat cells where the full biologic

TABLE I.

(^{3}H)AIB UPTAKE BY CONTROL AND INSULIN TREATED

ISOLATED MOUSE PANCREATIC ACINI

Minutes of (^{3}H)AIB Uptake	(^{3}H)AIB Uptake (pmoles/mg protein)	
	Control	Insulin (1 μg/ml)
2	0.43	0.41
8	0.84	0.80
20	1.32	1.36
45	1.30	1.36

Values are the mean of two determinations. Insulin was added at time 0.

effect of insulin on glucose transport is elicited by occupancy of only a small number of the insulin receptors (16). Although the reasons for this phenomenon in acini is unknown, it may partly reflect a chronic desensitization of the acinar cell by the high levels of insulin secreted from the neighboring islets.

In our preparation detectable stimulation of (^{3}H)2DG uptake occurs at an insulin concentration of 0.01 μg/ml, and maximal stimulation occurs at 10 μg/ml, levels that are considerably higher than those found in peripheral plasma. However, since the levels of insulin in the pancreatic vein are twenty-fold higher than those in the peripheral circulation (17,18), and since levels within the pancreas itself are undoubtedly even higher, it is possible that insulin plays a role in the regulation of glucose transport in the exocrine pancreas *in vivo*.

While the administration of insulin to animals *in vivo* has been reported to regulate a variety of pancreatic exocrine functions (17,5), is it not clear whether these actions are a direct effect of insulin. The present study is the first to document specific insulin receptors

in isolated mouse pancreatic acini. This in vitro study, therefore, lends support to the hypothesis that insulin could regulate acinar cell functions in vivo.

ACKNOWLEDGMENT

This investigation was supported by the medical research service of the Veterans Administration and by grants AM-19415 and AM-21089 from the National Institutes of Health.

REFERENCES

1. Henderson, J.R. (1969). The Lancet ii, 469-470.
2. Youngs, G. (1972). Gut 13, 154-161.
3. Danielsson, A., and Sehlin, J. (1974). Acta Physiol. Scand. 91, 557-565.
4. Kanno, T., and Saito, A. (1976). J. Physiol. 261, 505-521.
5. Soling, H.D., and Unger, K.O. (1972). Europ. J. Clin. Invest. 2, 199-212
6. Gavin, J.R., III, Roth, J., Jen, P., and Freychet, P. (1972). Proc. Nat. Acad. Sci. USA 69, 747-751.
7. Rechler, M.M., Podskalny, J.M., Goldfine, I.D., and Wells, C.A. (1974). J. Clin. Endocrinol. Metab. 39, 512-521.
8. Williams, J.A., Korc, M., and Dormer, R.L. (in press). Am. J. Physiol.
9. Williams, J.A., Cary, P., and Moffat, B. (1976). Am. J. Physiol. 231, 1562-1567.
10. Goldfine, I.D., and Smith, G.J. (1976). Proc. Nat. Acad. Sci. USA 73, 1427-1431.
11. Freychet, P., Kahn, R., Roth, J., and Neville, D.M., Jr. (1972). J. Biol. Chem. 271, 3953-3961.
12. Korc, M., Williams, J.A., and Goldfine, I.D. (1978). Submitted for publication.
13. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). J. Biol. Chem. 193, 265-275.
14. Scatchard, G. (1949). Ann. N.Y. Acad. Sci. 51, 660-672.
15. Couture, Y., Dunnigan, J., and Morisset, J. (1972). Scand. J. Gastroenterol. 7, 257-263.
16. Goldfine, I.D. (1978). Receptors in Pharmacology, pp. 335-377, Marcel Dekker, Inc., New York.
17. Unger, R.H. (1967). J. Clin. Invest. 46, 630-645.
18. Kanazawa, Y., Kuzuya, T., and Ide, T. (1968). Amer. J. Physiol. 215, 620-626.