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Research Article

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Evidence for Presence of Insulin Receptors in Rat Islets of Langerhans

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ABSTRACT Binding of insulin to islets of Langerhans was studied. It was found that "specific" binding of [^{125}I]insulin ("specific" binding equals total binding minus nonspecific binding) was saturable with respect to time and insulin concentration and depended on the number of incubated islets. Furthermore, bound insulin was displaced by native insulin in a dose-dependent manner. Bound [^{125}I]insulin was easily dissociated and there was little [^{125}I]insulin degradation both in the incubation medium and during the processes of binding and dissociation. Scatchard analysis of experiments with increasing [^{125}I]insulin concentration and with displacement of insulin binding by native insulin revealed "high affinity" binding sites with a dissociation constant of 0.461±0.08 nM and 3.5×10^6 high affinity binding sites per islet. There also existed "low affinity" binding sites with dissociation constant (K_d) of 43.9 ±11.6 nM and 5.9×10^7 low affinity binding sites. High affinity binding sites of islets from rats pretreated with alloxan decreased by about one half, whereas K_d was unaffected. Because the K_d of specific high affinity binding and mean effective dose (ED_{50}) of the biological effects of insulin on normal pancreatic islets are in the same range (between 0.46 and 1.19 nM), the insulin-receptor interaction may be biologically significant.

INTRODUCTION

Since techniques have been applied that permit measurement of specific binding of insulin to target cells or their subcellular structures, insulin receptors have been demonstrated to be present in ~20 different cell types, as has been reviewed by Kahn and Roth (1). So far, little effort has been made to demonstrate receptor sites for insulin in the pancreatic islets or their isolated cells. Recently insulin has been shown to inhibit insulin secretion of islets of Langerhans (2–5), and to decrease pentosephosphate shunt activity, NADPH/$NADP^+$ ratio (5), and 6-phosphogluconate/glucose-6-phosphate ratio (6). Based on the observation that interaction of insulin with receptors on various cell types represents the first step of its action (1), it seemed worthwhile to investigate whether the above-mentioned effects of insulin on islets of Langerhans are mediated via the interactions of exogenous insulin with insulin receptors in the pancreatic islet. The present study, the results of which were reported in abstract form elsewhere (7, 8), was undertaken to determine whether insulin "specifically" binds to pancreatic islets, whether this binding is related at least in part to B cells of islets, and whether or not such binding correlates with the effect of insulin on insulin release and islet metabolism. Our results suggest insulin receptors are present on pancreatic B cells.

METHODS

Animals. Wistar rats of either sex from a local strain weighing between 250 and 300 g were used. They were kept on a standard pellet diet (Altromin, Lage, West Germany) and tap water ad lib.

Filter. Cellulose acetate Millipore filters from Millipore, Neu-Isenburg, West Germany were used.

Chemicals. Crystalline pork insulin (26 U/mg) was kindly provided by Dr. v. Wasielewski, Hoechst, West Germany. [^{125}I]insulin (sp act, 100–120 μCi/μg with 0.3 atoms of iodine per insulin molecule) was purchased from Amersham Buchler, GmbH & Co., Braunschweig, West Germany. Pilocarpine hydrochloride was supplied by Merck Sharp & Dohme, Darmstadt, West Germany; and alloxan by Merck-Schuchardt & Co., Munich, West Germany. Collagenase of highest purity from Worthington Biochemical Corp., Freehold, N. J., and bovine serum albumin from Behring-Werke AG, Marburg/Lahn, West Germany were used. Insulin radioimmunoassay kits were supplied by Isotopendienst West, GmbH, Dreieich, West Germany. Rat insulin was purchased from Novo Research Institute, Copenhagen, Denmark. A dioxane-based

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scintillator (Rothiszint) was supplied by Carl Roth, Chemikalien, Karlsruhe, West Germany, and a filter-solubilizing scintillator was composed of toluene, 1,520 ml; ethyleneglycolmonomethylether, 545 ml; and PPO (2,5-diphenyloxazole), 8.2 g (Merck Sharp & Dohme). Other chemicals of highest available purity were supplied by Merck Sharp & Dohme.

Isolation of rat pancreatic islets. The isolation procedure of pancreatic islets was the one described by Lacy and Kostianovsky (9) and Kuo et al. (10) with slight modifications: three rats were pretreated with 0.3 ml of 4% pilocarpine hydrochloride i.p. After 3 h, pancreases were isolated and minced as described elsewhere (9), washed twice with 20 ml ice cold Hanks' solution containing 3.7 mM glucose and 0.2% albumin. Pancreatic pieces were soaked and stirred using a magnetic stirrer in a 37°C water bath in the presence of 650 U collagenase/g tissue suspension. After 12–14 min of incubation the tissue suspension was passed through a mesh into 10 ml of ice cold Hanks' solution. Islets were separated by sedimentation and collected as described elsewhere (9). This method yields ~400–640 islets/rat pancreas.

Alloxan pretreatment. Rats were injected with 180 mg/kg alloxan i.p. The legends to the figures indicate how long after injection the pancreases were removed. Each time the isoosmolar (6 g/100 ml) alloxan solution was freshly prepared. Urine glucose measured by Glucotest (C. F. Boehringer and Sons, Mannheim, West Germany) was in the range of 0.5–2%.

Binding experiments. After the islets had been isolated, they were washed three times in ice cold Hanks' solution using centrifugation. About 100 islets were incubated in 0.1 ml Krebs-Ringer-bicarbonate buffer (KRB buffer),[1] pH 7.4 containing 2% (wt/vol) albumin, [^{125}I]insulin, and radioactively unlabeled insulin (native insulin) as indicated in detail in the legends to the table and figures. The insulin concentration in the medium before incubation was checked and found to be <8 μU/ml (50 pM). This is far below the concentrations that would be expected to interfere with binding. The incubations were performed at 37°C for 20 min except in the experiments when time dependence was investigated. Incubation was finished by diluting samples with 0.2 ml of ice cold KRB-0.1% albumin buffer; the incubation medium was removed by filtration through cellulose acetate filters positioned with vacuum, and washed with another 0.3 ml of the same buffer. Dilution, filtration, and washing steps took <10 s. Filter radioactivity was determined in a toluene-based scintillator; filtrate radioactivity was also determined to correct for daily declining radioactivity of [^{125}I]insulin within each series. The scintillation counter Isocap 300 (Nuclear-Chicago Corp., Des Plaines, Ill.) was fitted with an external standard.

Each day islets were incubated in the presence of an excess of native insulin (0.1 mM) to correct for nonspecific binding of insulin. Usually about 0.8% of the total radioactivity employed was adsorbed by biological material (20–30%) and filter (70–80%). Blanks were obtained by incubations without islets in the presence and absence of native insulin (0.1 mM). In our experiments total binding, nonspecific binding, and blanks were measured. Specific binding was calculated in each case by subtracting nonspecific binding from total binding.

Degradation studies

Degradation in the medium. 0 to ~400 islets were incubated in the presence of 2.8 nM [^{125}I]insulin for 20 min and centrifuged. [^{125}I]insulin degradation in the supernate was determined by measuring solubility of ^{125}I activity in trichloroacetic acid (12% g/vol).

Degradation during the process of binding and dissociation. Approximately 100 islets were incubated for 20 min as described above in the presence of 2.8 nM [^{125}I]insulin. After centrifugation the islet pellet was resuspended in 100 μl of [^{125}I]insulin-free buffered medium and every 10-μl islet suspension was incubated with 490 μl of the same medium in the absence or in the presence of 0.1 mM native insulin. After 0–40 min ^{125}I activity of islets as well as of medium was determined and corrected for blanks (same procedure without islets). The ^{125}I activity in the medium was precipitated by trichloroacetic acid to determine degradation of [^{125}I]insulin during binding and dissociation. Maximum binding of each experiment at zero time was 100%.

Scatchard analysis. To establish the receptor affinity (dissociation constant, K_d) and the amount of specific binding sites Scatchard analysis (11) was used in which bound/free insulin (B/F) was plotted against bound (B) insulin. The exact data were computed by a method of maximal likelihood; for statistical evaluation Student's t test was used.

Determination of insulin. Insulin released into the medium by islets of alloxan-treated rats was assayed with the radioimmunoassay kit using rat insulin as standard.

RESULTS

Binding of insulin

Time-course of binding. Fig. 1 shows total, nonspecific, and specific binding of [^{125}I]insulin at various incubation periods. Total binding increased with time and was parallel to nonspecific binding after about

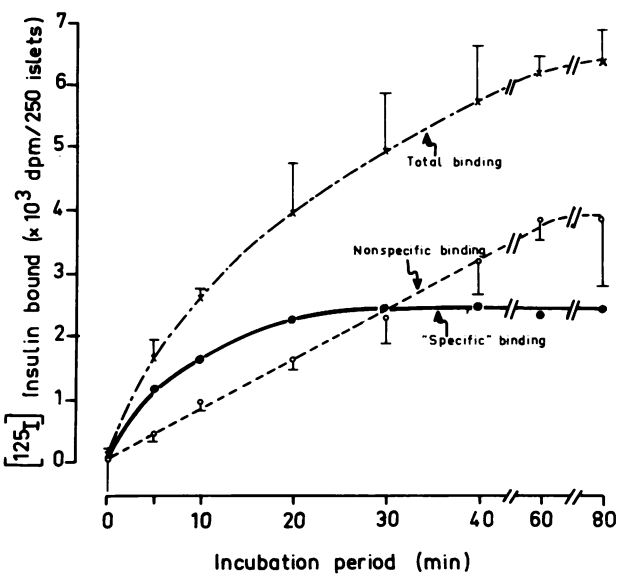

FIGURE 1 Time-course of binding (total, nonspecific, and "specific") of [^{125}I]insulin to isolated rat islets of Langerhans. About 100 islets were incubated in 0.1 ml KRB-albumin (2%) buffer at 37°C for 0–80 min with 2.8 nM insulin in the presence (○) and absence (×) of 0.1 mM native insulin. Results expressed as disintegrations per minute [^{125}I]insulin bound/250 islets: Mean±SEM; four to eight experiments.

[1] *Abbreviations used in this paper*: B/F, bound/free, K_d, dissociation constant, KRB buffer, Krebs-Ringer bicarbonate buffer.

20 min. Nonspecific binding increased linearly from the very beginning for at least 40 min indicating that it is not saturable. On the contrary, specific binding was a saturable process with time, its maximum occurring after 20–30 min.

Further investigations on specific binding (Figs. 2, 3, 4, and 8) therefore, were performed using 20-min incubation periods.

Binding of insulin as a function of the number of islets. When 6–575 islets/0.1 ml were incubated with 2.8 nM [^{125}I]insulin in the absence and presence of 100 μM native insulin (Fig. 2), total binding as well as nonspecific binding were directly proportional to the number of islets with correlation coefficients as indicated in the legend. Subtracting nonspecific binding from total binding yielded specific binding, which was about 60% of the total binding regardless of the number of islets used. Because binding is a linear function of the number of islets, the amount of insulin bound in further experiments can be normalized on the basis of number of islets.

Further results shown in Figs. 3, 4, and 8 are only expressed in terms of specific binding.

Effect of [^{125}I]insulin concentration on specific binding. As shown in Fig. 3, incubation of islets with increasing [^{125}I]insulin concentrations (between 0.2 and 5 nM resulted in a saturable specific binding with respect to insulin concentrations.

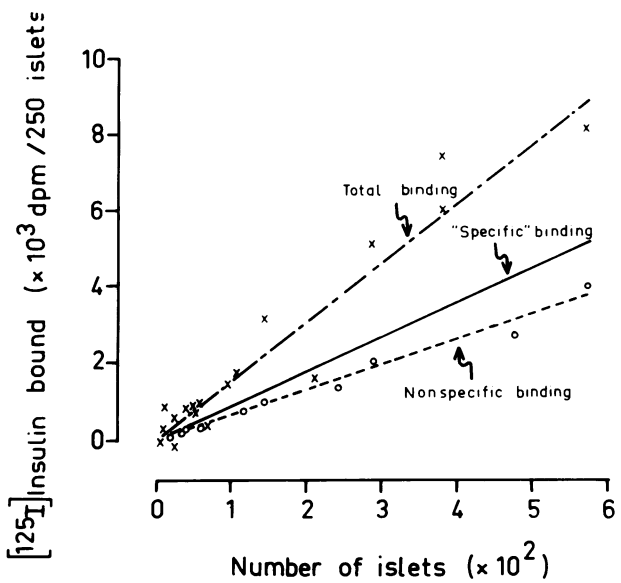

FIGURE 2 Binding (total, nonspecific, and specific) of [^{125}I]-insulin to isolated rat islets of Langerhans as a function of the number of islets in the incubation medium. 6–575 islets were incubated in 0.1 ml KRB-albumin (2%) buffer for 20 min at 37°C with 2.8 nM [^{125}I]insulin in the presence (○) and absence (×) of 0.1 mM native insulin. Correlation coefficients and number of experiments: total binding, r = 0.96 and n = 18; nonspecific binding, r = 0.98 and n = 11.

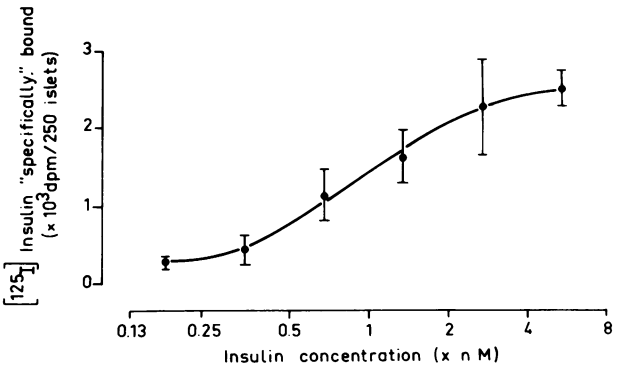

FIGURE 3 Specific binding of [^{125}I]insulin to isolated rat islets of Langerhans as a function of insulin concentration. About 100 islets were incubated in 0.1 ml KRB-albumin (2%) buffer at 37°C for 20 min with 0.2–5 nM [^{125}I]insulin. Results expressed as disintegrations per minute [^{125}I]insulin bound/250 islets: mean±SEM; four to seven experiments.

Displacement of [^{125}I]insulin by native insulin. As shown in Fig. 4, nonlabeled insulin displaced specifically bound [^{125}I]insulin in a dose-dependent manner, the displacement was in the range of 0.1 μM and half-maximal displacement was in the range of 1.2 nM native insulin.

Scatchard analysis. To establish the K_d and the amount of specific binding sites, Scatchard analysis was used (11). When data are derived from displacement studies (Fig. 4), a curvilinear plot is obvious (Fig. 5). Computation of data revealed a K_{d1} of 4.61 $\pm 0.108 \times 10^{-10}$ M. When data were derived from ex-

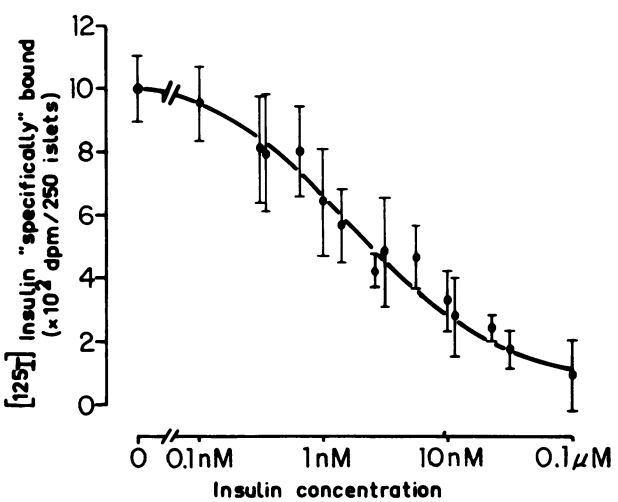

FIGURE 4 Displacement of [^{125}I]insulin from isolated rat islets of Langerhans by native insulin. About 100 islets were incubated in 0.1 ml KRB-albumin (2%) buffer at 37°C for 20 min with 0.7 nM [^{125}I]insulin and increasing amounts of native insulin from 0.1 nM to 0.1 μM. Results expressed as disintegrations per minute [^{125}I]insulin bound/250 islets: mean±SEM; four to seven experiments.

periments using increasing concentrations of [^{125}I]-insulin (Fig. 3), there was no significant difference when compared with the dissociation constants of the above experiments; this indicates that [^{125}I]insulin retained binding properties similar to native insulin.

Fig. 5 shows that there may exist a second order of binding sites with lower affinity (K_{d2}) = 43.9±11.6 × 10^{-9} M. The specific binding capacity of "high affinity" binding sites was 5.84 amol/islet whereas that of the low affinity system was 97 amol/islet. This corresponds to about 3.5 × 10^6 high affinity binding sites and 5.9 × 10^7 low affinity binding sites per islet.

Studies with islets of alloxan-treated rats

Insulin secretion. As shown in Fig. 6, pretreatment of rats with alloxan for more than 3 h resulted in a significant decrease of insulin secretion in response to 16.7 mM of glucose.

Scatchard analysis of binding experiments with islets of alloxan-treated rats. To examine a possible decrease in [^{125}I]insulin binding produced by alloxan pretreatment of rats, islets were incubated as previously described. Steady-state insulin binding was measured over a wide range (0 to 0.1 μM unlabeled insulin concentration) and data were plotted in a Scatchard plot (Fig. 5). Fig. 5 shows a leftward shifted curvilinear plot when compared with the Scatchard plot of data obtained from islets without alloxan pretreatment. The high affinity K_d (K_{d1} = 4.35±0.101 × 10^{-10} M is not decreased compared with that of islets without alloxan pretreatment. However, the binding capacity (3.72 amol/islet or 2.25 × 10^6 binding sites/1 islet) is significantly decreased. Low affinity K_{d2} (26.9±6.24 × 10^{-9} M) of islets of alloxan-pretreated rats as well as receptor binding capacity (41.1 amol/islet) are decreased when compared with islets of untreated rats ($P < 0.02$).

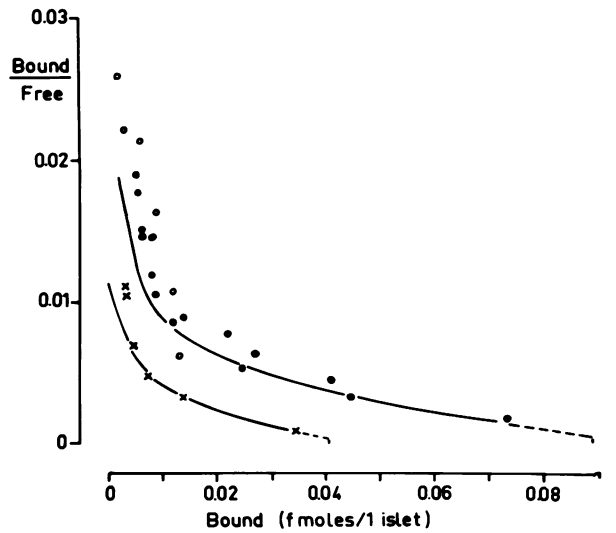

FIGURE 5 Scatchard plot of data shown in Figs. 3 and 4 (experimental conditions described in these figures) and of corresponding data obtained by same experimental conditions except that islets of alloxan-pretreated rats were used (n = 4–16). B/F ratio is plotted against B insulin. ○, increasing concentration of [^{125}I]insulin; ●, displacement of [^{125}I]insulin by native insulin; ×, displacement of [^{125}I]insulin by native insulin using islets of alloxan-pretreated rats.

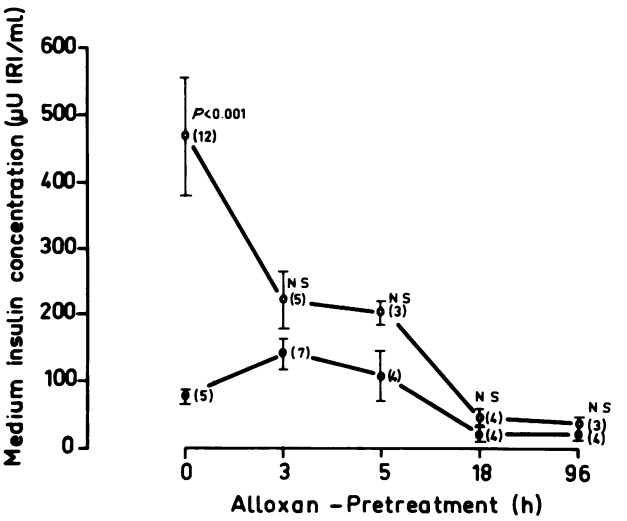

FIGURE 6 Time-dependent effect of alloxan (180 mg/kg) pretreatment of glucose-induced insulin secretion of rat islets of Langerhans. Five islets were incubated in 1 ml/90 min in the presence of 5.6 or 16.7 mM glucose. Mean±SEM; number of experiments in parentheses. ●, 5.6 mM glucose; ○, 16.7 mM glucose. IRI, immunoreactive insulin.

Dissociation and Degradation of [^{125}I]insulin

Insulin degradation in the medium during incubation. Fig. 7 shows that ~5% of [^{125}I]insulin was degraded (soluble in 12% trichloroacetic acid) during a 20-min incubation period at 37°C in buffered medium in the absence of rat islets. This rate increased linearly

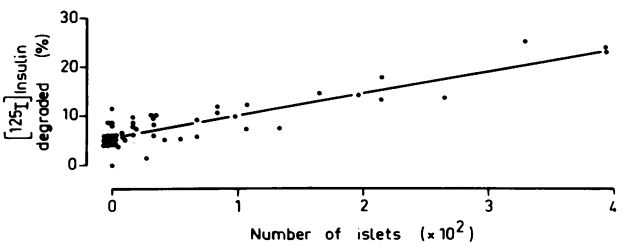

FIGURE 7 Degradation of [^{125}I]insulin in the incubation medium. 0–400 islets were incubated in 0.1 ml KRB-albumin (2%) buffer at 37°C for 20 min with 2.8 nM [^{125}I]insulin. Degradation of [^{125}I]insulin in the medium was determined by measuring solubility in 12% trichloric acid. Results are expressed as percent [^{125}I]insulin degradation of total radioactivity.

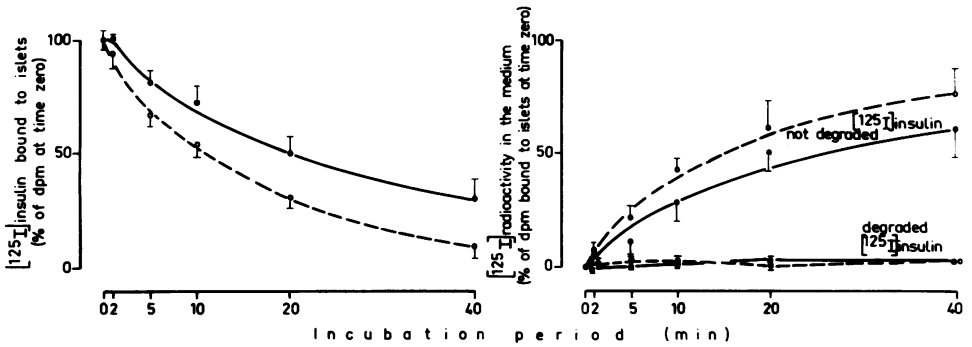

FIGURE 8 Dissociation and degradation of [^{125}I]insulin during receptor interaction. ~100 islets were incubated in 0.1 ml KRB-albumin (2%) buffer at 37°C for 20 min with 2.8 nM [^{125}I]insulin. After centrifugation islet pellet was resuspended in the presence (○) and absence (●) of 0.1 mM native insulin. After 0–40 min the radioactivity of the islets and the washout medium was determined. Using trichloric acid (12% wt/vol) degradation of [^{125}I]insulin in the washout medium was also determined. Maximum binding of each experiment at zero time was 100%. Mean±SEM, number of experiments 7–14.

with the number of islets present in the medium being ~9% (+4%) when ~100 islets (as used in all other experiments) are incubated.

Insulin degradation during binding and dissociation. To investigate whether insulin is degraded during the interaction with its receptor (during binding and dissociation) the radioactivity of preloaded islets and of washout medium was determined from 0 to 40 min (Fig. 8). Binding of [^{125}I]insulin to islets decreased in a time-dependent manner. This decrease of binding was accelerated in the presence of 0.1 mM exogenous insulin, being ~90% after 40 min. Most of the bound radioactivity was recovered in the incubation medium (~76% after 40 min). Only ~4% of the ^{125}I radioactivity was soluble in 12% trichloroacetic acid (indicative of insulin degradation).

Biological Significance of specific binding

In Table I the K_d of a high affinity binding site from the displacement study (Fig. 5) is compared with mean effective dose (ED_{50}) of exogenous insulin concerning its inhibition of glucose-induced insulin release and its effect on islet metabolism as calculated from data previously published in detail elsewhere (5, 6). As can be seen, the K_d of specific binding is in the same range as the ED_{50} of the effects of exogenous insulin on insulin secretion, pentosephosphate shunt activity and the NADPH/NADP$^+$ ratio of pancreatic islets. (Although the data of experiments with pentosephosphate shunt activity and NADPH/NADP$^+$ ratio were obtained after 90-min incubations because of technical reasons, we feel that they are comparable with the binding data in the present study because specific binding did not change between 30 and 80 min [see Fig. 1].)

DISCUSSION

Some of the important criteria characterizing insulin receptor (hormone receptor) interactions include specific binding (12) of radioactively labeled insulin to tissues, isolated cells, or membranes depending on the amount of binding; the saturability of this binding with respect to time and ligand concentration; and the displaceability of the radioactive hormone from its binding sites by native hormone.

These criteria hold also for pancreatic rat islets because, as shown in Figs. 1–4, specific binding of insulin depends on the number of incubated islets, is saturable with respect to incubation time and insulin concentration, and is displaceable by native insulin. However, nonspecific binding is not saturable with time, pos-

TABLE I
Comparison of K_d of High Affinity Binding Site of Insulin to Pancreatic Rat Islets

		Reference
	nM	
High affinity binding site	K_d = 0.46	
Glucose (16.7 mM)-induced insulin secretion*	ED_{50} = 1.12	5
Pentosephosphate shunt activity (%)*	ED_{50} = 1.19	5, 6
6-Phosphogluconate/ glucose-6-phosphate*	ED_{50} = 0.91	6
NADPH/NADP*	ED_{50} = 1.05	5

Comparison was made using half maximal inhibitory effects (ED_{50}) of exogenous insulin on glucose-induced insulin secretion, islet pentosephosphate shunt activity, 6-phosphogluconate/glucose-6-phosphate, and NADPH/NADP-ratios.
* Data calculated from experiments published elsewhere (see References).

sibly indicative of some process of internalization and binding of insulin to subcellular fractions. Because specific binding plateaus after ~20 min (Fig. 1), all further studies (Figs. 2–4, 8) were performed using 20-min incubation periods.

However, contrary to other tissues such as fat cells (13), fat cell membranes (14) and liver cell membranes (15), whose K_d was found to be in the range of 5–8 $\times 10^{-11}$ M to 0.08 nM, the affinity of specific binding sites of pancreatic islets was one order of magnitude less, being 46×10^{-11} M for high affinity K_d. At first glance the fact that the K_d of high affinity binding in pancreatic islets is one order magnitude higher than in other tissues might make the physiological importance of islet binding sites seem questionable. It must be considered, however, that the insulin concentration in the intercellular space of islets is assumed to be much higher (16) than insulin concentration in rat serum (17).

From the intercepts of the curves with the abscissa (these curves being derived from displacement experiments [Fig. 4] of the Scatchard plot [Fig. 5]), the specific binding capacity of one islet was calculated to be 5.84 amol/islet of insulin, which corresponds to 3.5×10^6 insulin molecules per one islet. If we assume that one islet consists of about 10,000 cells as suggested elsewhere using islets of obese mice (18), the number of binding sites is ~350/cell. This is somewhat lower than measured in adipose tissue (13) but corresponds to the number found in human peripheral granulocytes (19).

Islets of Langerhans contain heterogeneous types of cells, such as A cells, B cells (60–90%), D cells, and connective tissue cells. Therefore, interpretation of our results obtained so far from binding studies is complicated because the question remains as to what type of islet cell binds insulin. Because it is of special interest to determine whether or not B cells specifically bind insulin, an attempt was made to demonstrate the B cells are involved in insulin binding by using islets of alloxan-treated rats. These islets showed no significant insulin release in response to glucose (Fig. 6) after 5 h pretreatment, indicating damage to the B cell. This is in accordance with in vivo results of other authors (20), who found a decrease of serum insulin concentration in rats in spite of sharply increasing serum glucose concentrations. This has been reported to be accompanied by histological changes (21) and a reduction of B cell volume within as little as 15–30 min after alloxan pretreatment.

When B/F insulin is plotted as a function of total hormone concentration F, islets of alloxan-pretreated rats show a decreased B/F over the entire range of insulin concentrations when compared with controls (no alloxan pretreatment) (Fig. 5). Since alloxan pretreatment leads to a loss of ~50% of binding sites and because alloxan is known to selectively destroy B cells of islets, it may be concluded that B cells possess insulin receptors. But although we cannot conclude from this experiment that only B cells of the pancreatic islets are involved in insulin binding, there is no evidence that this might also be the case with A cells and D cells because results of several authors regarding the biological effects of insulin on glucagon secretion are conflicting (22, 23); an influence of insulin on somatostatin secretion could not be established in recent experiments (24). However, the possibility that loss of binding sites by alloxan-pretreatment reflects down-regulation of insulin receptors cannot be fully ruled out by these experiments.

In the Scatchard plot (Fig. 5), in addition to the high affinity K_d, in normal islets (no alloxan pretreatment) there also exists a low affinity K_d that is about 100 times lower than the high affinity K_d. This holds true even for islets of alloxan-pretreated rats (Fig. 5), since their Scatchard curve was nearly parallel but shifted leftward. Whether this low affinity binding site represents a second type of specific binding and whether or not this is of physiological significance in pancreatic islets remains to be evaluated. As far as liver plasma membranes (25) and various tissues (26–28) are concerned, a heterogeneity of binding sites or one class of negatively cooperating binding site for insulin have been demonstrated. Other authors (13–15), however, have reported a single class of high affinity binding sites in adipose tissue (13) or isolated liver cell membranes (15) and fat cell membranes (14). The low affinity binding sites as observed in pancreatic islets may simply represent an adsorptive phenomenon (29) because we used islet tissue, but no isolated cells. Because specifically bound [^{125}I]insulin is easily dissociated, however, and since dissociation is more pronounced in the presence of an excess of native insulin, this may be indicative of a negatively cooperative site-site interaction, as is found to be a characteristic feature of insulin receptors in other tissues (30).

In contrast to other tissues such as liver and granulocytes (31, 32), degradation of [^{125}I]insulin by pancreatic islets is not of major significance, since it occurs neither in the incubation medium nor during the process of binding and dissociation. This is consistent with the data of others (33) who found 95–100% intact exogenous insulin in the perfusate of isolated rat pancreas.

A prerequisite for attributing specific binding of insulin to an insulin receptor is that the insulin exhibits biological effects in the target tissues (34). This appears to be the case for the pancreatic islet (Table I). With regard to fat cells there is some controversy whether (14, 15) or not (35) the K_d of binding is within the physiological range. Nonetheless, in pancreatic islets the K_d of insulin binding is similar to the ED_{50} of the biological effects of insulin, i.e., inhibition of glucose-induced insulin secretion and inhibition of pentose-phosphate shunt as well as decrease of the NADPH/$NADP^+$ ratio.

In conclusion, insulin exhibits specific binding (total binding corrected for nonspecific binding) to pancreatic islets that contain B cells. This binding is a saturable process with respect to time and insulin concentration. The binding of [^{125}I]insulin is reversible and displaceable by native insulin, the number of binding sites being in the range of 10^7 per one islet. Since the K_d of binding and displacement is similar to the ED_{50} of the biological effects of insulin on pancreatic islets, our data suggest that insulin receptors are present in rat pancreatic islets containing B cells.

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