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CHOLECYSTOKININ AND INSULIN REGULATE INSULIN-LIKE GROWTH FACTOR II BINDING TO PANCREATIC RECEPTORS; EVIDENCE OF A ROLE FOR INTRACELLULAR CALCIUM

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The binding of ^{125}I-labeled insulin-like growth factor II (^{125}I-IGF II) to mouse pancreatic acini was stimulated (45%) by insulin and inhibited (30%) by cholecystokinin octapeptide (CCK_8). When CCK_8 and insulin were added together, the effect on IGF II binding was similar to that seen when CCK_8 was added alone. Two lines of evidence suggest that this effect of cholecystokinin on basal and insulin-stimulated ^{125}I-IGF II binding was mediated via a change in intracellular calcium: (1) the cholinergic agent carbachol inhibited IGF II binding to its receptors; (2) addition of the Ca^{2+} ionophore A23187 mimicked the effects of CCK_8 and carbachol. In contrast to its effects on IGF II binding to acini, CCK_8 had only small effects on IGF I binding and no effects on insulin binding.

Pancreatic secretagogues such as cholecystokinin (CCK), acetylcholine, secretin, and VIP have numerous effects on the secretion and metabolism of pancreatic acinar cells (1-3). However, in addition to secretagogues, other polypeptide hormones regulate the acinar pancreas: these include insulin, epidermal growth factor (EGF), and insulin-like growth factors (3-8). In acini, insulin and EGF do not directly stimulate secretion but regulate several biological functions including the sensitivity to CCK (4,6,9). The insulin-like growth factors, IGF I and IGF II, are two very closely related circulating hormones under the control of growth hormone (10,11). They are 7000-7500 molecular weight peptides with sequence and structural homology to proinsulin (12,13). It is believed that these hormones promote the growth of many tissues (13). We have recently reported the presence of specific receptors for IGF I and IGF II on acinar cells of the exocrine pancreas (7). In other target cells, the binding of IGF II is enhanced by insulin (14-17). In the present study we present evidence indicating that the binding of IGF II to

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its receptor on pancreatic acini is regulated by both insulin and CCK, and that Ca^{+2} may have a role in this regulation.

MATERIALS AND METHODS

Pancreatic acini. Male white Swiss Webster mice, 20-30 g, were rendered diabetic via the injection of streptozotocin into the tail vein (18). Five to nine days later, isolated pancreatic acini were prepared from these mice according to the method of Williams (18). Diabetic mice were used since effects of insulin are greater in acini from diabetic as compared to normal animals (18).

Binding studies. Pure IGF I and IGF II and a semipurified mixture of IGF I and II were generous gifts from Dr. R. Humbel, University of Zürich, Switzerland. Unlabeled CCK_8 was a gift from Dr. M. Ondetti, Squibb Institute for Medical Research, Princeton, NJ. Porcine insulin (Elanco Products, Indianapolis, IN) and EGF (receptor grade, Collaborative Research Inc., Waltham, MA) were purchased. Hormones were iodinated by a version of the chloramine-T method (6,19). Pancreatic acini (0.2 - 0.3 mg acinar protein/ml) were suspended in a HEPES buffered Ringer (HR) enriched with minimal Eagles medium amino acid supplement, 0.1 mg/ml soybean trypsin inhibitor, 0.5% bovine serum albumin of low insulin-like activity, and gassed with 100% O_2. ^{125}I-radioligands (IGF I and IGF II 60 pM, insulin 100 pM, EGF 40 pM) plus unlabeled hormones were added to the suspension and incubated in a shaking water bath at 37°C. At specified times triplicate samples of the suspension were removed, centrifuged at 300 x g for 2 min, and the pellets then washed twice; radioactivity associated with the washed acinar pellets was measured in a gamma type scintillation counter. Duplicate aliquots of the total incubation mixture were taken at the end of the incubation period and counted to determine the total radioactivity. This value was used to compute the percent of the label bound. Non-specific binding was determined in the presence of an excess of unlabeled hormones (IGF and EGF, 100 nM; insulin 1 μM). Acinar protein was measured using Bio-Rad(R) reagent and binding was normalized to 1 mg protein/ml. Degradation of labeled hormone in the medium was determined by the precipitation of binding supernatants with trichloroacetic acid added at a final concentration of 10% (20).

RESULTS

Effects of CCK_8 and insulin on IGF II binding to pancreatic acini. When ^{125}I-IGF II (60 pM) was added to pancreatic acini at 37°C, one half maximal binding occurred after 50 minutes of incubation and maximal binding occurred after 120 minutes (fig. 1). At steady state, maximal binding was 26.3 ± 1.1% of total per mg acinar protein (mean ± SE, n=20). When ^{125}I-IGF II was added in the presence of 100 nM unlabeled IGF, binding was reduced to 10% or less of that seen with labeled hormone alone. Addition of either insulin or CCK_8 altered ^{125}I-IGF II binding; insulin increased and CCK_8 decreased this function. The one half maximal effect of insulin was seen at 100 pM and a maximal effect occurred at 10 nM (fig. 2). In five experiments, insulin at 100 nM increased ^{125}I-IGF II binding by 45.1 ± 3.5% (mean ± SE).

useful for probing nucleotide binding sites in numerous proteins [for references cf. (13,14)]. The question of whether the dye interacts with (Na,K)-ATPase and is a suitable tool for the elucidation of the folding pattern of the peptide chains involved in forming the ATP binding site will be tackled in the present paper for the first time.

MATERIALS AND METHODS

ATP from Boehringer (Mannheim) was used as imidazole salt. [^{3}H]ouabain (2.28 Ci/mmole) from Isocommerz (Berlin) was labeled on the butenolide ring by hydrogen-tritium exchange (15). Cibacron Blue F3GA from Ciba-Geigy was a mixture of the meta isomer with the probably active para isomer (16), more than 90 % pure and used without further purification. All other chemicals were of analytical reagent grade. The (Na,K)-ATPase preparations were obtained by established procedures as described by Walter (17) for guinea-pig kidney, by Matsui and Schwartz (18) for guinea-pig heart muscle (omitting here the deoxycholate-treatment) and human heart muscle, and by Samaha (19) for human brain cortex omitting the LiCl-treatment. Protein was determined by the procedure of Bensadoun and Weinstein (20). The assay medium for estimating (Na,K)-ATPase activity contained 2 mM ATP, 4 mM Mg, 80 mM Na, 5 mM K, 80 mM imidazole-HCl (pH 7.4), 0.1 mM EDTA, 0.3 mM phosphoenolpyruvate, 0.2 mM NADH, 9 IU pyruvate kinase and 9 IU lactate dehydrogenase, all in a volume of 2 ml. After addition of aliquots of the enzyme preparations, the decrease of extinction at 334 nm (21) was recorded at 37 °C. Any inhibitory effect of Cb on the auxiliary enzymes did not affect the sufficiency of the optical test. The ATPase activity suppressed by 0.1 mM ouabain was taken as (Na,K)-ATPase activity amounting to 46 (25 °C), 6, 8, and 114 µmole/mg•hr, respectively, in the membranous enzyme preparations from the four sources given in the above-chosen order. The binding of [^{3}H]ouabain to (Na,K)-ATPase from human brain cortex and the dissociation of the complex formed were followed at 37 °C in 40 mM imidazole-HCl buffer solution (pH 7.4) under various conditions specified in the legends to tables and figures. The incubation was terminated by removal of the membrane fragments from the incubation medium by vacuum filtration on glas fibre filters (Whatman GF/C). The filters were washed twice with ice-cold buffer solution, sucked dry and measured for radioactivity in a scintillation spectrometer. The proportion of nonspecific [^{3}H]ouabain binding assessed by omitting ATP, Mg, Na or Mg, P_i from the medium amounted to less than 6 % of saturation binding, and was corrected for so that all data given in the tables were specific bindings. The calculation of the kinetic parameters was carried out with a parameter optimizing program. All experiments were repeated at least twice showing similar results as those presented here.

RESULTS

Inhibitory Effect of Cb on (Na,K)-ATPase Activity.

The dye showed a rather strong inhibitory effect on enzyme activity (table 1). The inhibition was complete within less than one minute. In contradistinction to the inhibitory effect of Cb on myosin subfragment 1 (22) and on catalytic subunit of protein kinase (23), the interaction of Cb with (Na,K)-ATPase did not result in a slowly reversible inhibition. The activity of the enzyme,

TABLE 2

Inhibition of ^{125}I-IGF II binding by agents which raise intracellular Ca^{2+}

	^{125}I-IGF II bound (% total)
Control	26.3 ± 1.1 (20)
CCK_8 (10 nM)	17.4 ± 1.1 (16)
Carbachol (100 μM)	19.6 ± 1.0 (5)
A23187 (5 μM)	20.7 ± 1.4 (7)

All values are the mean ± SE for the number of experiments shown in parenthesis. Difference between control and various agents was statistically significant ($p<0.01$, Student's t-test).

Effect of agents that act through intracellular Ca^{2+}. Since cholecystokinin carries out all its known biological activities in acini via raising intracellular Ca^{2+} (1,2,21), we studied the effect on ^{125}I-IGF II binding of other agents that raise intracellular Ca^{2+} in acini. Both carbachol, a muscarinic agonist, and ionophore A23187, an agent which increases Ca^{2+} entry into cells, inhibited ^{125}I-IGF II binding (table 2). This effect of CCK was also studied in acini from normal mice, and CCK inhibited ^{125}I-IGF II binding in a manner similar to that observed in acini from diabetic animals (data not shown).

Effect of CCK_8 on binding of other hormones to pancreatic acini. When acini were incubated with CCK_8 there was no effect on the binding of ^{125}I-insulin to its receptors (table 3). In contrast, there was a small effect on ^{125}I-IGF I binding and a marked effect on ^{125}I-EGF binding (6).

TABLE 3

Effect of CCK_8 on the binding of insulin, IGF I and EGF

	^{125}I-ligand bound (% total)	
	Control	CCK_8 (10 nM)
Insulin	3.6 ± 0.3	3.8 ± 0.25
IGF I	14.0 ± 0.4	10.9 ± 0.3
EGF	4.8 ± 0.2	0.6 ± 0.1

Acini were preincubated with CCK_8 for 30 min. Next, ^{125}I-insulin (100 pM), ^{125}I-IGF I (60 pM), and ^{125}I-EGF (40 pM) were added to their respective flasks, and the incubation continued 30 min for insulin and 120 min for IGF I and EGF. Values are the mean ± SD of triplicate samples from a representative of three experiments.

DISCUSSION

In pancreatic acini we have previously reported the existence of separate receptors for IGF I and IGF II (7). In the present study, we find that IGF II binding in pancreatic acini is up regulated by insulin and down regulated by CCK_8. A similar effect of insulin on the IGF II receptor has been reported in rat hepatoma cells (14) and adipocytes (15-17,22). The mechanism of this action of insulin is unknown. One group has suggested that insulin changes the affinity of plasma membrane receptors (14,23) whereas another group has suggested that insulin acts to recruit receptors from the cell interior to the cell surface (24).

We also find that cholecystokinin, in contrast to insulin, lowers the binding of IGF II to its receptor in pancreatic acini. Moreover, when cholecystokinin and insulin are added together the effect of CCK predominates. This observation suggests that CCK acts a step distant to that of insulin. In pancreatic acini cholecystokinin has been shown to exert its numerous cellular effects through a rise in intracellular Ca^{2+} (1-3,21). Accordingly we tested the effects of two other agents that are known to raise intracellular Ca^{2+}, the muscarinic agonist carbachol and the Ca^{2+} ionophore A23187, in acini. These agents, like CCK_8, lowered ^{125}I-IGF II binding. These studies indicated, therefore, that intracellular Ca^{2+} was involved in this regulatory action of CCK on IGF II receptors. Moreover, this effect of CCK is similar to the effect of CCK to inhibit ^{125}I-EGF binding to pancreatic acini (25,26). How Ca^{2+} regulates the IGF II receptor remains speculative. Ca^{2+} could alter either receptor affinity, the kinetics of receptor recycling from the cell interior to the cell surface, or receptor degradation. Further studies will be necessary, therefore, to clarify these issues.

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