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# Distribution of Receptors for Insulin and Insulin-like Growth Factor I (Somatomedin C) in the Adrenal Gland

Dennis J. Pillion[1,*], Maria Yang[1], and William E. Grizzle[2]

[1]Dept. of Pharmacology
[2]Dept. of Pathology
University of Alabama at Birmingham
Birmingham, AL 35294

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Summary: Rat adrenal glands contain cell surface high-affinity receptors for several peptide hormones. Receptors for IGF-I were abundant in this tissue, but receptors for insulin were relatively scarce. The behavior of adrenal membrane IGF-I receptors in radioligand binding assays was similar to the behavior of IGF-I receptors from other tissues, with a $K_D \approx 6.2 \times 10^{-9}$ M. Covalent cross-linking studies with [$^{125}$I]IGF-I revealed an IGF-I receptor alpha-subunit with Mr ≈ 135,000 on dodecyl sulfate polyacrylamide gel electrophoresis under reducing conditions, as well as a smaller radiolabeled peptide, Mr = 116,000. In contrast, little binding of [$^{125}$I]insulin to adrenal membranes was observed and no labeling occurred in cross-linking studies using [$^{125}$I]insulin. These results contrast with the findings of whole-body autoradiographic studies that indicated substantial binding of [$^{125}$I]insulin to adrenal glands and suggest that IGF-I, rather than insulin, may play a critical role in the growth and development of the adrenal gland.


Receptors for the polypeptide hormones insulin and IGF-I have been isolated and characterized by a variety of techniques, including the recent description of their primary amino acid sequences by the use of cDNA probes (1-3). The receptors for insulin and IGF-I share considerable structural and sequence homology, as well as the capacity to bind their respective ligands with higher affinity than they bind other peptide hormones (4). The tissue distribution of receptors for insulin and IGF-I originally seemed ubiquitous, but more recent findings indicate that a small number of specialized cell

*To whom correspondence and reprint requests should be addressed.

Abbreviations used in this paper: IGF-I, insulin-like growth factor I; IGF-II, insulin-like growth factor II

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surfaces lack these receptors. For example, receptors for insulin are not present on the brush-border membranes of renal proximal tubules or the apical surface of small intestinal epithelial cells (5-7). Conversely, receptors for IGF-I are not found on adult rat liver cell plasma membranes (8). This investigation was designed to evaluate the number of insulin and/or IGF-I receptors on the plasma membrane of adrenal cells.

Previous studies involving whole-body autoradiographic analysis of infused [$^{125}$I]insulin indicated that significant quantities of this hormone were bound to adrenal glands (9). However, these studies failed to exclude the possibility that [$^{125}$I]insulin was being sequestered within the adrenal gland or binding to receptors for IGF-I present therein. Our investigation into the binding of insulin and IGF-I to rat adrenal plasma membranes does not agree with the previously espoused conclusion that the adrenal gland contains high-affinity receptors for insulin. In contrast, we have shown that receptors for IGF-I predominate in this tissue and few, if any, high-affinity receptors for insulin are present on adrenal plasma membranes. The role of IGF-I receptors in the control of adrenal growth, differentiation and function has yet to be elucidated, but the interplay between IGF-I produced endogenously by the adrenal glands and IGF-I secreted by the liver in response to growth hormone may play a critical role in regulating adrenal function and development in both normal and pathophysiological states.

## Materials and Methods

IGF-I was purchased from Amgen, Inc., Thousand Oaks, CA. [$^{125}$I]IGF-I (2000 Ci/mmol) was obtained from Amersham Corp., Arlington Heights, IL. [$^{125}$I]Insulin (porcine, 2,200 Ci/mmol) was purchased from New England Nuclear Co., Boston, MA. Disuccinimidyl suberate was obtained from Pierce Chemical Co., Rockford IL. Other chemicals used were obtained from Sigma Chemical Co., St. Louis, MO.

Male Fisher rats, 350-400 g, were fed ad libitum until sacrifice, which was always between 9:00 and 11:00 a.m. Rats were anesthetized with 1 ml/kg body weight of a mixture containing 50 mg/ml ketamine-HCl and 7.5 mg/ml xylazine-HCl i.m. and the adrenal gland was removed following mid-ventral laporotomy. The adrenal gland was quickly weighed, placed in ice-cold buffer A (10 mM Tris-HCl, pH 7.45, 250 mM sucrose) and homogenized with a Tekmar Tissue Mizer (10 seconds, setting 50, two times). In these experiments, adrenal cortex and medulla were not separated from each other and the tissue homogenate contains both types of adrenal material. A crude plasma membrane fraction was prepared by sequential centrifugation at 5,000 g for 15 min to remove cellular debris and nuclei, followed by centrifugation at 33,000 g for 30 min to pellet plasma membranes. The 33,000 g pellet was resuspended in Krebs-Ringer phosphate buffer, pH 7.6, containing 0.1% bovine serum albumin. A 100,000 g pellet was obtained from the 33,000 g supernatant following a 1 hr centrifugation in a Beckman Ultracentrifuge. In some experiments, crude membrane preparations were dissolved in 1.0 ml of Buffer B (1% Triton X-100, 100 mM KCl, 5 mM Tris-HEPES, pH 7.5, 2.5 mM EDTA, 5 mM N-ethylmaleimide and 100 mM sucrose). Membranes were incubated in Buffer B at 21°C for 60 min with shaking and then centrifuged at 33,000 g or at 100,000 g for 30 min. The supernatant solution was decanted, aliquoted into several tubes and stored at -20°C until used in binding studies.

Binding studies were conducted as described previously (10,11). Frozen aliquots of detergent-solubilized membrane preparations were thawed and their protein content was determined using the method of Bradford (12) with an albumin standard and the appropriate blank correction for the inclusion of Triton X-100 in the tissue extract. Individual adrenal glands failed to yield sufficient quantities of membrane protein to perform binding studies necessitating that 4 adrenal glands be pooled to prepare a single adrenal membrane extract. Unless stated otherwise, only the right adrenal gland from each rat was used. This point becomes critical because left adrenal gland weight is consistently and significantly larger than right adrenal gland weight. [$^{125}$I]Insulin and [$^{125}$I]IGF-I binding experiments were always run simultaneously, using identical tissue extracts, to allow accurate comparisons of the binding of each radioligand to the tissue. Binding experiments were conducted for 16 hours at 4°C unless otherwise stated. Under these conditions, hormone degradation was minimal (< 0.1%) during the binding assay. [$^{125}$I]Hormone bound to receptors was separated from unbound radioligand by precipitation with 12.5% polyethylene glycol and human gamma globulins were included in the assay to serve as co-precipitate. Samples run without tissue extract served as blanks to determine radioligand binding to the tube and trapping in the pellet. Samples run in the presence of 1 µg/ml unlabeled IGF-I or 10 µg/ml porcine insulin were used to determine nonspecific binding to tissue extracts and specific binding was calculated as the difference in radioligand binding in the absence and presence of excess unlabeled ligand.

Covalent crosslinking studies were conducted using disuccinimidyl suberate, as described previously for renal tissues (10,11). Molecular weight marker proteins from BioRad, Inc. were included on dodecyl sulfate polyacrylamide gels and electrophoresis was conducted as described by Laemmli (13).

## Results

Adrenal glands were isolated from adult rats and a crude plasma membrane fraction was prepared by differential centrifugation. Receptors for IGF-I and insulin were solubilized in Triton X-100 and radioligand binding studies were conducted using each hormone (Fig. 1). [$^{125}$I]Insulin binding to adrenal membrane material was consistently low, averaging less than 2% of the total ligand present in the absence of unlabeled insulin and less than 1% in the presence of 10 µg/ml insulin (Fig. 1A). In some experiments, [$^{125}$I]insulin binding to adrenal membranes in the presence of 10 µg/ml insulin was equal to [$^{125}$I]insulin binding in the absence of unlabeled insulin (not shown). In contrast, [$^{125}$I]IGF-I binding to solubilized adrenal plasma membranes averaged over 6% of the total radioligand available in the absence of unlabeled IGF-I and less than 2% in the presence of 1 µg/ml IGF-I (Fig. 1B). It is important to note that the results presented in Fig. 1 were obtained using the same tissue extract and the same amount of adrenal plasma membrane extract for both hormones. Hence, the differences noted in the amount of [$^{125}$I]IGF-I and [$^{125}$I]insulin binding to adrenal membrane material in these studies is not due to differences in tissue preparation or experimental variability, but represent the true relationship of hormone receptor binding capacity in this tissue. It could be argued that detergent-solubilization of adrenal plasma membranes causes inadequate or unequal recovery of receptors for insulin

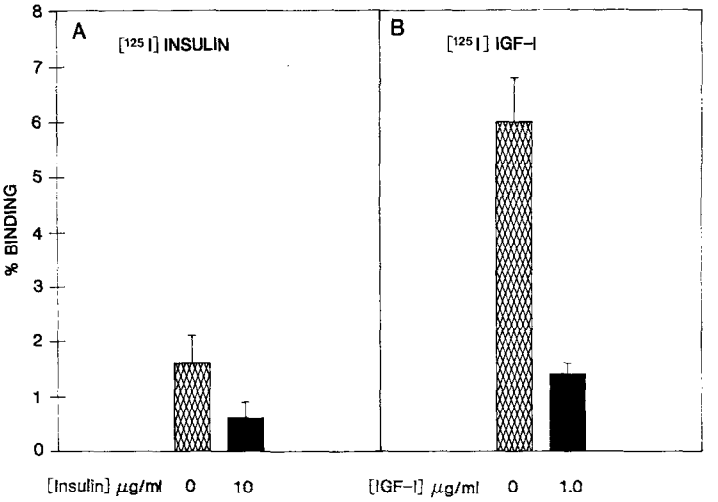


Fig. 1 Insulin and IGF-I binding to adrenal membranes. Rat adrenal plasma membranes were solubilized in 1% Triton X-100 and incubated for 16 hours at 4°C in the presence of [$^{125}$I]insulin (A) or [$^{125}$I]IGF-I (B) with or without the addition of unlabeled peptide. Data represent the percentage of total radioactivity bound to the tissue pellet after precipitation with polyethylene glycol. The mean ± S.E.M. from four separate experiments are presented. Each sample contained 38 μg of solubilized membrane protein.

relative to receptors for IGF-I; this contingency would be reflected in aberrantly low values for insulin binding to the adrenal membrane extract. Parallel hormone binding studies were therefore conducted with aliquots of adrenal plasma membranes not solubilized with detergent. The results of these experiments are comparable to those presented in Fig. 1. Adrenal plasma membranes bound 530 pg [$^{125}$I]IGF-I/mg protein in the absence of unlabeled IGF-I and 143 pg [$^{125}$I]IGF-I/mg protein in the presence of 1 μg/ml IGF-I, for a specific binding of 387 pg [$^{125}$I]IGF-I/mg protein. Paired samples of adrenal membranes bound 104 ng [$^{125}$I]insulin/mg protein in the absence or presence of unlabeled insulin (10 μg/ml).

The nature of insulin and IGF-I binding sites on adrenal plasma membranes was further explored in displacement experiments comparing the binding of each radioligand in the presence of increasing concentrations of the unlabeled hormones (Fig. 2). [$^{125}$I]IGF-I binding to detergent-solubilized adrenal plasma membranes was inhibited more effectively by the addition of unlabeled IGF-I ($IC_{50}$ = 47 ng/ml) than by unlabeled insulin ($IC_{50}$ = 4.4 μg/ml). Scatchard analysis of this data (Fig. 2, insert) reveals that the $K_D$ for IGF-I binding to adrenal membranes is 6.2 x $10^{-9}$ M. The inhibitory effect of unlabeled IGF-I and insulin on [$^{125}$I]IGF-I binding to adrenal plasma membranes is consistent with radioligand binding to a high-affinity Type I IGF receptor rather than binding with lower affinity to an insulin receptor. The reciprocal experiment, measuring [$^{125}$I]insulin binding to adrenal plasma

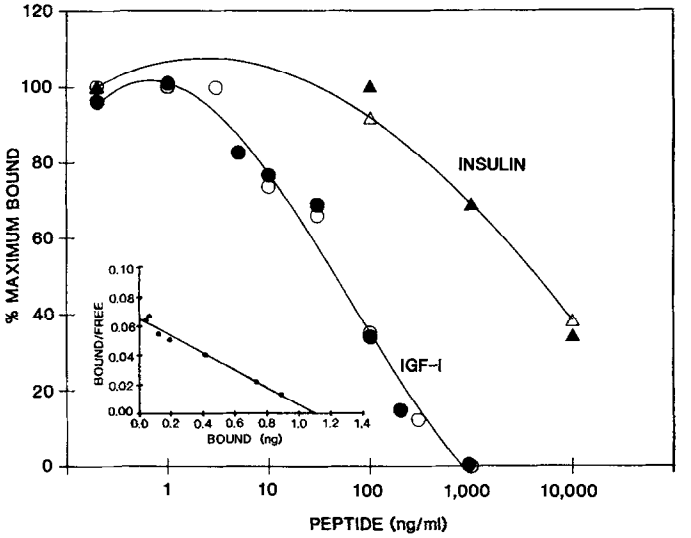


Fig. 2 Binding of [$^{125}$I]IGF-I to adrenal membranes in the presence of unlabeled IGF-I and insulin. Adrenal membranes were solubilized in 1% Triton X-100 and incubated with [$^{125}$I]IGF-I for 16 hours at 4°C in the presence of various concentrations of unlabeled IGF-I or insulin. [$^{125}$I]IGF-I binding in the absence of unlabeled peptide was assigned a value of 100%. Data represent the percentage of maximum binding observed in two separate experiments. Each sample contained 38 µg of solubilized membrane protein. Insert: binding data for [$^{125}$I]IGF-I in the presence of various concentrations of unlabeled IGF-I is converted to expression in a Scatchard plot.

membranes in the presence of increasing concentrations of unlabeled insulin and IGF, was also performed (not shown). The binding of [$^{125}$I]insulin to adrenal tissue was very low in the absence of any unlabeled peptide and no statistically significant dose-dependent inhibition of radioligand binding could be confirmed with the addition of either hormone. This finding is compatable with either of the following two hypotheses: a) adrenal plasma membranes contain only a small number of high-affinity insulin receptors and a large number of high-affinity IGF-I receptors; b) adrenal plasma membranes contain no insulin receptors and [$^{125}$I]insulin bound to this tissue is actually associated with high-affinity IGF-I receptors. These two possibilities are difficult to distinguish on the basis of radioligand binding experiments.

The size of the alpha-subunits of IGF-I and insulin receptors have been characterized in numerous other tissues by covalent cross-linking studies. Adrenal plasma membranes were incubated with [$^{125}$I]insulin or [$^{125}$I]IGF-I in the presence and absence of 10 µg/ml porcine insulin or 1 µg/ml IGF-I, respectively, and the radioligand was covalently cross-linked to its receptor by the addition of dissuccinimidyl suberate as described previously (10,11). Membranes from another tissue, human placenta, were included for comparison.

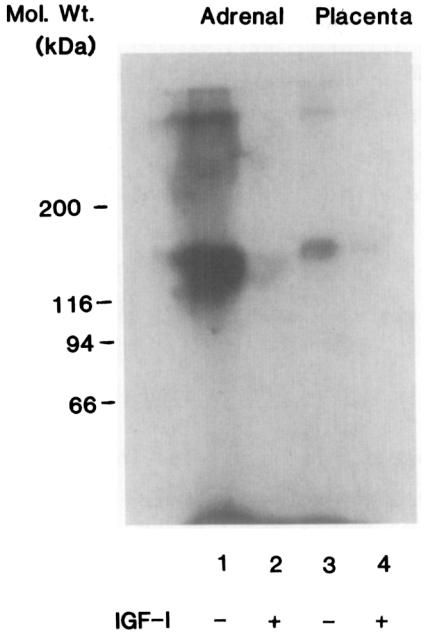


Fig. 3 Crosslinking of [$^{125}$I]IGF-I to plasma membranes from rat adrenal gland and human placenta. Adrenal and placental membranes were incubated with 0.5 μCi [$^{125}$I]IGF-I plus or minus 1.0 μg unlabeled IGF-I for 90 min at 22°C. The samples were washed and crosslinking took place for 15 min at 4°C following the addition of disuccinimidyl suberate. Samples were solubilized in buffer containing 2% sodium dodecylsulfate and 40 mM dithiothreitol and gel electrophoresis was performed with a 7.5% polyacrylamide gel. The gel was dried and autoradiography was performed. Molecular weight markers were included to allow determination of the Mr for the labeled bands. Each sample contained 577 μg of membrane protein.

No covalent crosslinking of [$^{125}$I]insulin to an adrenal receptor could be discerned in autoradiograms of dodecyl sulfate polyacrylamide gels (not shown). Interestingly, [$^{125}$I]IGF-I was covalently coupled not to a single receptor subunit species, but to a broad band that appeared to contain two distinct components, Mr = 135,000 and Mr = 116,000 (Fig. 3, Lane 1). Inclusion of 1 μg/ml unlabeled IGF-I in the incubation mixture preceding the addition of the crosslinking agent caused a marked inhibition of receptor subunit labeling (Fig. 3, Lane 2). In addition to the receptor species mentioned above, a higher molecular weight band (Mr ≃ 300,000) was also specifically labeled with [$^{125}$I]IGF-I in adrenal plasma membranes (Fig. 3, Lanes 1 and 2). Parallel samples containing an equal amount of human placental membranes (Fig. 3, Lanes 3 and 4) contained remarkably less receptor subunit labeling with [$^{125}$I]IGF-I than that observed with adrenal plasma membranes. This placental tissue contained a single, Mr = 135,000, IGF-I receptor subunit rather than the two broad and more intense bands labeled in the adrenal plasma membrane sample. Also of interest is the presence of a

high molecular weight, Mr ≈ 300,000, species labeled by [$^{125}$I]IGF-I in the absence of unlabeled IGF-I (Fig. 3, Lane 3), which corresponds to a similar species found in the adrenal tissue (Fig. 3, Lane 1). This band may represent a subset of IGF-I receptor alpha-subunits not fully dissociated from each other, which are labeled with [$^{125}$I]IGF-I and migrate as an $\alpha_2\beta_2$ complex on the polyacrylamide gel. Alternatively, this band may represent a Type II IGF receptor, which usually migrates at Mr = 260,000 and which is labeled by [$^{125}$I]IGF-I, but which migrates anomalously on this polyacrylamide gel.

## Discussion

The relationship between receptors for insulin and IGF-I has been studied in numerous tissues (see Ref. 4 for review). This report contains data that indicate the presence of high-affinity IGF-I receptors in adrenal membranes. The affinity of the adrenal IGF-I receptor for [$^{125}$I]IGF-I is comparable to the affinity reported using other tissue sources (4,11). Most of the binding studies performed in this investigation were conducted using adrenal membrane material solubilized in 1% Triton X-100. This detergent is widely used to dissolve membrane proteins and has been used successfully to solubilize receptors for both IGF-I and insulin from other tissue membranes. One particular advantage of this approach is that sampling errors due to inhomogeneous solutions of particulate membrane material can be avoided. Of course, care must be taken to ensure that the radioligand binding data obtained with Triton X-100-solubilized membrane material is a genuine reflection of the situation that exists on the intact tissue surface. The data presented in this report does fulfill this criterion, as evidenced by the fact that both binding data and covalent cross-linking data obtained with particulate adrenal membrane material reflects the same relative abundance of receptors for IGF-I and few, if any, receptors for insulin.

Previous data indicating that [$^{125}$I]insulin infused intravenously in rats was found associated with adrenal glands, as well as with numerous other organs, suggested that the adrenal gland was rich in insulin receptors (9,14). Our data do not confirm this hypothesis and we clearly show that adrenal gland membranes contain IGF-I receptors in large numbers and few receptors for insulin (Fig. 1). We believe that the specific binding of [$^{125}$I]insulin to the adrenal gland noted *in vivo* (9,14) represented binding of insulin to these IGF-I receptors. Additional experiments using cDNA probes that recognize rat insulin receptor mRNA and IGF-I receptor mRNA should allow us to unequivocally determine whether or not the adrenal gland produces any insulin receptors.

The adrenal membranes used in the current investigation were comprised of both cortical and medullary tissue. Further studies are warranted to precisely define the localization of these hormone receptors within the

adrenal gland. Similarly, these studies were conducted with adrenal glands from healthy adult, male rats and additional studies with female rats, neonates and rats with various diseases thought to impact on adrenal function should further elucidate the role of IGF-I in adrenal function and development.

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