Vol. 165, No. 1, 1989 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1989 Pages 204-211

Receptors for Insulin and Insulin-like Growth Factor-I in the Human Adrenal Gland

Dennis J. Pillion[1]*, Patrick Arnold[1], Maria Yang[1], Cecil R. Stockard[2] and William E. Grizzle[2]

[1]Dept. of Pharmacology
[2]Dept. of Pathology
University of Alabama at Birmingham and the Veterans Administration Medical Center
Birmingham, AL 35294

Received October 17, 1989

Summary: Human adrenal glands contain high-affinity receptors for insulin and insulin-like growth factor I (IGF-I). Comparative studies with rat, hamster and human adrenal membranes confirmed that IGF-I receptors are most abundant in rat and hamster adrenals, whereas insulin and IGF-I receptors are present in equivalent numbers in human adrenal glands. Covalent crosslinking studies revealed that the human adrenal gland IGF-I receptor binding subunit migrated on dodecyl sulfate polyacrylamide gels with Mr = 135,000, which is identical to the migration of IGF-I receptor binding subunits isolated from other tissues. Autoradiography of frozen human adrenal slices incubated with [^{125}I]insulin showed prominent, displaceable binding of this radioligand to the zona reticularis, zona glomerulosa, vasculature and medulla; in contrast, [^{125}I]IGF-I binding to human adrenal tissue was most prominent in the zona reticularis and negligible in the medullary region.

The human adrenal gland plays a critical role in metabolic regulation and it represents both an endocrine target, for ACTH, and a hormone-producing tissue, generating catecholamines, cortisol, adrenal androgens, aldosterone and a variety of other adrenocorticoids. Adrenal hormone production appears to be controlled by a combination of endocrine and neural pathways, but its responsiveness to changes in the levels of insulin and IGF-I remain an area of interest. Pillion, Yang and Grizzle (1) recently reported that the rat adrenal gland contains a rich supply of IGF-I receptors, but few insulin receptors. This observation is consistent with results of Dahmer and Perlman (2), who reported that cultured bovine chromaffin cells contain IGF-I receptors and that IGF-I treatment caused an increase in the capacity of these cells to secrete catecholamines in response to high K^+, whereas insulin treatment of these cells had little effect. Brush and Banerjee (3) have also documented the occurrence of large numbers of IGF-I receptors on cultured bovine adrenal endothelial cells, but these cells differ from the chromaffin cells described above in that they also contain a large number of insulin receptors; in this latter respect, adrenal endothelial cells resemble endothelial cells isolated from a variety of other tissues (4-7).

The current study was designed to characterize the binding of insulin and IGF-I to the human adrenal gland. The human adrenal gland differs from the rat and hamster adrenals in its biosynthetic capacities, with cortisol serving as the principal glucocorticoid in humans; hence, hormonal regulation of adrenal growth and metabolism might differ in human vs. rat and hamster adrenal tissue. Previous studies

*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.

0006-291X/89 $1.50

of human adrenal gland insulin and IGF-I receptors have been limited, due in part to the limited availibility of human adrenal tissue, except at autopsy, and the likelihood that autopsy specimens would not provide an accurate representation of the in vivo situation. In this study, we report that human adrenal tissue obtained at autopsy, 2.5-7.0 hours following death, contains fewer membrane receptors for insulin than human adrenal tissue obtained from surgical resections; we also report that human adrenal tissue differs from rat adrenal tissue in that IGF-I receptors are much more abundant than insulin receptors in the latter.

Materials and Methods

IGF-I was purchased from Amgen, Inc., Thousand Oaks, CA. [^{125}I]IGF-I (human, 2,000 Ci/mmol) was purchased from Amersham Corp., Arlington Heights, Il. [^{125}I]Insulin (porcine, 2,200 Ci/mmol) was purchased from New England Nuclear. Disuccinimidyl suberate was purchased from Pierce Chemical Co., Rockford, IL. Other chemicals were obtained from Sigma Chemical Co., St. Louis, MO.

Human adrenal tissue was obtained from autopsies through the UAB Human Tissue Procurement Facility. Adrenal tissue was snap frozen 2.5-7.0 hours post mortem and stored in liquid nitrogen vapor phase until analyzed. Fresh human adrenal surgical specimens were also obtained from the UAB Human Tissue Procurement Facility. Surgical samples were flash frozen in liquid nitrogen soon after resection. All adrenal glands were removed during the resection of the adjacent kidney renal cell carcinoma. None of the adrenal glands were involved grossly or microscopically by tumor. Rat adrenal tissue was obtained from adult male Fisher rats as described previously (1). Hamster adrenal tissue was obtained from Syrian (Golden) hamsters obtained by Veteran's Administration, National Cancer Institute cooperative agreement from the Frederick Cancer Center, Frederick, MD. Adrenal cortex and medulla were not separated from each other; sections of whole adrenal tissue were thawed and homogenized with a Tekmar Tissue Mizer (10 seconds, setting 70, two times) in 10 ml of ice-cold buffer A (10 mM Tris-HCl, pH 7.45, 250 mM sucrose). The homogenate was centrifuged at 5,000 g for 15 min to remove cellular debris and nuclei; the 5,000 g supernatant was centrifuged at 33,000 g for 30 min to pellet plasma membranes.

Binding studies were conducted as previously described for rat adrenal membranes solubilized in buffer containing 1% Triton X-100 (1). Radioligands were added to achieve approximately 50,000 cpm/sample and the protein content of binding samples ranged from 35-50 μg protein. Data from different human, hamster and rat adrenal samples were normalized to account for differences in protein content. All binding samples were run in duplicate and the average of the two determinations was used to calculate binding. Crosslinking studies were conducted with adrenal membranes prepared by differential centrifugation as described above and conditions for crosslinking, polyacrylamide gel electrophoresis and autoradiography have been described previously (1,5).

Autoradiography was performed on 10 μ thick frozen sections of human adrenal tissue obtained during surgical resections. Slices were applied to poly-L-lysine coated glass microscope slides and covered with 0.05 ml of buffer containing 0.1% bovine serum albumin in phosphate-buffered saline, pH 6.5, 7.4 or 8.0. After an initial incubation of 3 min to block nonspecific binding, the buffer was drained and replaced with 0.05 ml of the same buffer, containing 50,000-80,000 cpm [^{125}I]insulin or [^{125}I]IGF-I . Paired samples also received unlabelled insulin or IGF-I, 1 μg/ml, to block specific hormone binding sites. The tissues were covered with 25 mm^2 pieces of parafilm to minimize evaporation and incubated at 22°C for 90 min. The incubation was terminated by draining and washing the tissue three times with ice-cold buffer. The slices were air dried overnight and exposed to LKB Ultrafilm for 10-20 days.

Results

Previous work in our laboratory has shown that detergent-solubilized adult rat adrenal membranes contain significant numbers of high-affinity receptors for IGF-I, whereas the number of high-affinity insulin receptors in this tissue is minimal (1). The capacity of human adrenal tissue to bind insulin and IGF-I had yet to be determined and was therefore measured in eight samples of human adrenal tissue obtained during surgical resections. The results presented in Table I indicate that every sample of human adrenal tissue tested bound [^{125}I]insulin in a specific manner, with values for specific binding ranging from 1.10 to 2.90% bound/40 μg protein. In comparison, specific binding of [^{125}I]IGF-I to human adrenal tissue

Table I. Binding of insulin and IGF-I to surgical samples of human adrenal tissue

		% [^{125}I]Insulin Bound / 40 µg protein			% [^{125}I]IGF-I Bound / 40 µg protein		
Patient	Age(yr)	0 µg/ml insulin	10 µg/ml insulin	Δ	0 µg/ml IGF-I	1 µg/ml IGF-I	Δ
1	35	3.19	1.22	1.97	1.83	0.54	1.29
2	39	2.91	0.93	1.98	1.32	0.78	0.54
3	48	3.16	1.25	1.91	2.13	0.94	1.19
4	60	2.45	1.31	1.14	5.51	1.79	3.72
5	63	2.21	0.97	1.24	4.81	1.28	3.53
6	76	1.90	0.80	1.10	3.51	1.25	2.26
7	76	3.01	1.31	1.70	6.09	1.86	4.23
8	78	3.77	0.87	2.90	1.55	0.78	0.77
	Mean±SD	2.88±0.22	1.05±0.07	1.83±0.21	3.03±0.65	1.06±0.16	1.97±0.50

Human adrenal surgical specimens were collected and frozen until use in binding experiments. Plasma membranes were isolated from tissue homogenates by differential centrifugation and dissolved in buffer containing 1% Triton X-100. Detergent-soluble membrane material was incubated overnight at 4°C with [^{125}I]insulin or [^{125}I]IGF-I (≈60,000 cpm/sample) in the presence or absence of unlabelled ligand. Radioligand receptor complexes were precipitated by the addition of polyethyleneglycol (12.5%) and human gamma globulins (0.4%). Radioactivity in the entire sample and in the pellet was measured in a gamma counter and the percentage of total radioactivity in the pellet was calculated for each sample. Data was normalized for protein to reflect the percentage of radioactivity bound per 40 µg protein. Each sample was tested in duplicate and the mean is presented; variation between determinations was consistently <0.05. Data represent the average of duplicate determinations.

showed more variation, ranging from 0.54 to 4.23% bound/40 µg protein. These results suggest that the distribution of insulin and IGF-I receptors in human adrenal surgical specimens is considerably different than the distribution which occurs in rat adrenal tissue. This hypothesis was tested directly in comparative studies in which detergent-solubilized membrane material from human, rat or hamster adrenal tissues were used concomitantly in binding studies (Fig. 1). The data presented in Fig. 1 are consistent with the hypothesis that human adrenal tissue contains equivalent numbers of insulin and IGF-I receptors, whereas rat adrenal tissue contains more receptors for IGF-I than for insulin. Hamster adrenal tissue appears to contain more insulin receptors and an intermediate number of IGF-I receptors.

These results prompted us to characterize the specificity of the binding sites for insulin and IGF-I in human adrenal tissues. Fig. 2 displays the results of experiments in which [^{125}I]IGF-I was incubated with detergent-solubilized membrane material from samples of human adrenal tissue obtained from surgical resections; [^{125}I]IGF-I binding to its receptor in the adrenal tissue extract was inhibited in a dose-dependent manner by the inclusion of unlabelled IGF-I, with 50% inhibition of binding observed at a concentration of 40 ng/ml unlabelled IGF-I. As expected for a genuine IGF-I receptor, [^{125}I]IGF-I binding to the human adrenal tissue extract was inhibited to a much lesser extent by the inclusion of unlabelled insulin and 50% inhibition of [^{125}I]IGF-I to its receptor was observed in the presence of 1.5 µg/ml unlabelled insulin. These results are similar to our previously published data obtained with rat adrenal tissue (1) and suggest that the IGF-I bound to the human adrenal tissue is actually bound to a high-affinity IGF-I receptor rather than bound to co-existent insulin receptors.

The existence of distinct receptors for insulin and IGF-I in human adrenal tissue has been confirmed and defined by the use of autoradiography of frozen sections of human adrenal tissue obtained during surgical resections (Fig 3). [^{125}I]Insulin binding was observed primarily in the zona reticularis, zona glomerulosa and medulla. Further details of this pattern could be discerned in parallel samples that were covered with an emulsion (not shown); these samples displayed labelling in the adrenal capsule associated with the vascular endothelium. The addition of unlabelled insulin to the incubation

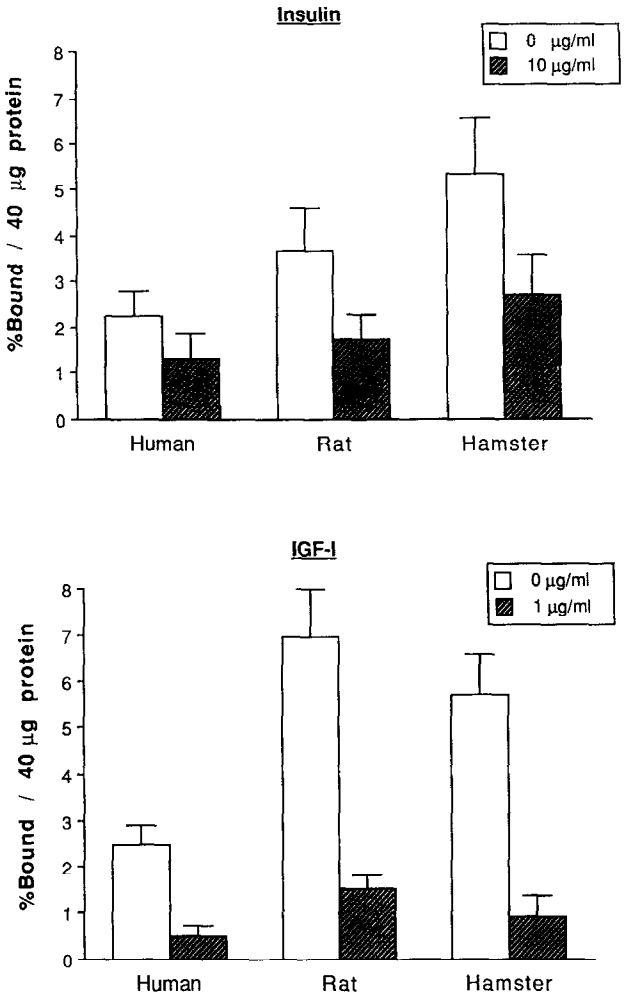

Fig. 1. [^{125}I]Insulin and [^{125}I]IGF-I binding to human, rat and hamster adrenal membranes. Plasma membranes were prepared from tissue homogenates by differential centrifugation and solubilized in Triton X-100. Membrane extracts were incubated overnight at 4°C as described in Table I in the presence of [^{125}I]insulin (upper-figure) or [^{125}I]IGF-I (lower figure) in the presence (stipled bar) and absence (open bar) of the unlabelled ligand. Data represent the mean ± SD from 4 samples of rat and hamster or 8 samples of human adrenal tissue, each assayed in duplicate. Data is normalized to a protein value of 40 µg protein/sample.

medium blocked [^{125}I]insulin binding to the tissue, but the addition of unlabelled IGF-I was less effective. Binding of [^{125}I]IGF-I to companion slides demonstrated prominent labelling of the zona reticularis; the zona glomerulosa showed somewhat less decoration with the ligand and studies with emulsion-coated slides revealed that this was due to diminished labeling of the vascular endothelium with [^{125}I]IGF-I. Binding of [^{125}I]IGF-I to the zona fasiculata or medulla was minimal. The addition of unlabelled insulin to the incubation medium did not block [^{125}I]IGF-I binding, whereas the addition of unlabelled IGF-I completely blocked [^{125}I]IGF-I binding to the human adrenal; these results are consistent with the existence of distinct human adrenal receptors for insulin and IGF-I. The autoradiograms depicted in Fig. 3 were developed from tissues incubated at pH 7.4; samples incubated at pH 8.0 gave similar results, but samples incubated at pH 6.5 displayed no specific radioligand binding. These results are consistent with the pH profile expected for genuine insulin and IGF-I receptors.

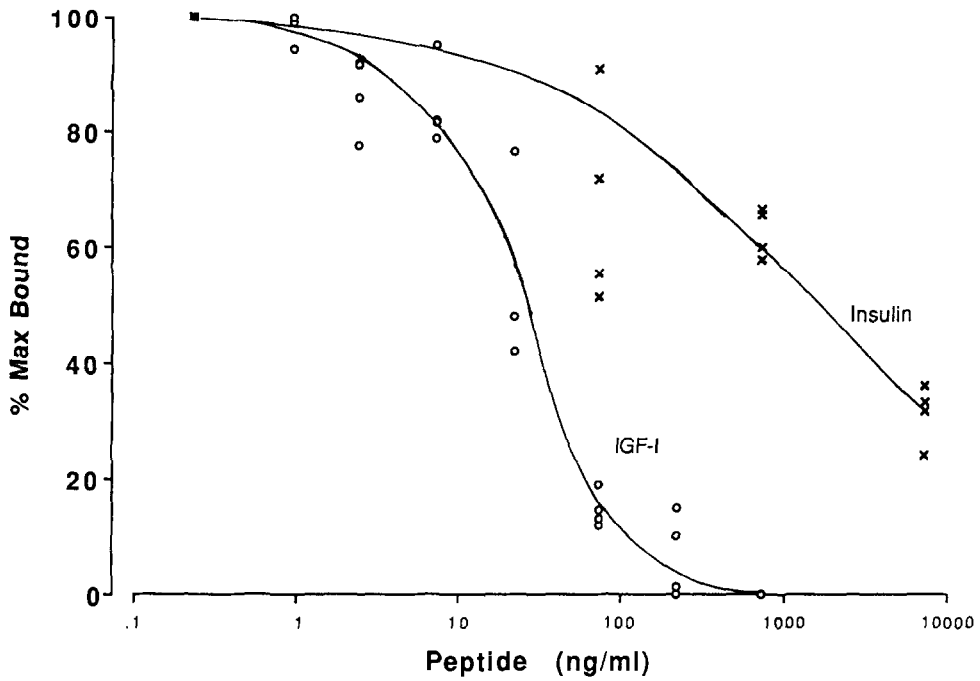

Fig. 2. [125I]IGF-I binding to human adrenal surgical samples. Four samples of human adrenal tissue were obtained at surgical resection and a detergent-solubilized plasma membrane extract was prepared as described in Table I. Binding of [125I]IGF-I to the membrane extract was measured in an overnight incubation at 4°C in samples containing various concentrations of unlabelled IGF-I or insulin. Binding in the absence of unlabelled IGF-1 was assigned a value of 100% after subtraction of non-specific binding activity from all samples, as defined by the residual binding activity that occurs in the presence of 1 µg/ml unlabelled IGF-I. Data is expressed as a percentage of maximal binding for each determination, all of which were run in duplicate.

Further characterization of the human adrenal IGF-I receptor was conducted using covalent crosslinking techniques. Human, rat and hamster adrenal tissues were homogenized and a membrane fraction was isolated. [125I]IGF-I was allowed to bind to its receptor on the adrenal membranes in the presence or absence of 1 µg/ml unlabelled IGF-I and, after washing the membranes, the ligand-receptor

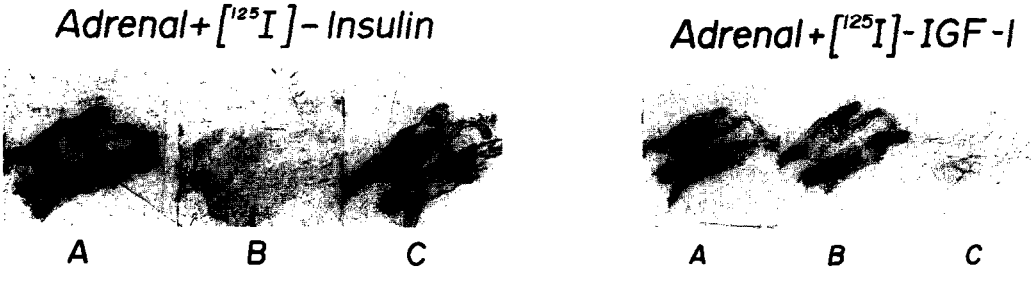

Fig. 3. Localization of insulin and IGF-I binding sites in human adrenal tissue. Frozen sections, 10 µ thick were prepared from human adrenal tissue obtained during surgical resection. Slices were incubated with [125I]insulin (left) or [125I]IGF-I (right) for 90 min at 22°C in the absence (A) or in the presence of 1 µg/ml insulin (B) or 1 µg/ml IGF-I (C). Autoradiography was performed for 15 days after the sample was washed copiously.

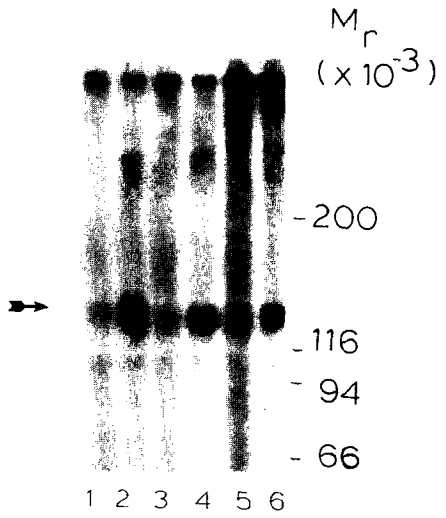

Fig. 4. Crosslinking of [^{125}I]IGF-I to its receptor on rat, hamster and human adrenal tissue. Plasma membranes were isolated from adrenal tissues obtained at sacrifice of rats (Lanes 1 and 2) or hamsters (Lanes 3 and 4) and obtained during surgical kidney-adrenal resections of humans (Lanes 5 and 6). Membranes were incubated for 90 min at 22°C with [^{125}I]IGF-I in the presence (Lanes 1,3,5) or absence (Lanes 2,4,6) of unlabelled IGF-I (1 µg/ml). Membranes were then pelleted by centrifugation, washed and incubated with disuccinimidyl suberate to covalently crosslink [^{125}I]IGF-I to its receptor. Samples were then washed and dissolved in buffer containing sodium dodecyl sulfate and gel electrophoresis was performed on a gel containing 6% polyacrylamide. The autoradiogram of that gel is shown, with the migration pattern of a series of proteins of known molecular weight (Mr - 66,000 - 200,000).

complexes were covalently crosslinked by the addition of disuccinimidyl suberate and separated by polyacrylamide gel electrophoresis. Fig. 4 depicts the autoradiogram of the gel and the results demonstrate that the binding subunit of the IGF-I receptor of human adrenal tissue is similar in size (Mr = 135,000) to that found in rat and hamster tissue.

Finally, we attempted to evaluate the impact of post-mortem changes on human adrenal receptors for insulin and IGF-I. Parallel studies in our laboratory with human kidney tissue have shown that autopsy samples contain little or no insulin and IGF-I binding capacity, whereas human kidney tissue obtained during surgical resection binds considerable amounts of these hormones (Grizzle, Yang and Pillion; manuscript in preparation). The data in Table II represent the results of experiments in which six human adrenal specimens, obtained at autopsy 2.5-7.0 hours following death, were tested for insulin and IGF-I

Table II. Binding of insulin and IGF-I to human adrenal tissue obtained at autopsy

		% [^{125}I]Insulin Bound / 40 µg protein			% [^{125}I]IGF-I Bound / 40 µg protein		
Patient	Post Mortem (hr)	0 µg/ml Insulin	10 µg/ml Insulin	Δ	0 µg/ml IGF-I	1 µg/ml IGF-I	Δ
1	2.5	1.08	0.24	0.84	3.21	0.12	3.09
2	3.5	1.80	0.05	1.75	2.90	0.54	2.36
3	3.5	0.91	0.72	0.19	1.78	0.18	1.60
4	4.5	3.91	3.01	0.90	1.50	1.05	0.45
5	5.0	1.89	0.63	1.26	1.45	0.01	1.44
6	7.0	3.84	3.01	0.74	3.97	1.24	2.73
	Mean±SD	2.24±0.54	1.29±0.57	0.95±0.21	2.47±0.43	0.52±0.40	1.95±0.40

Human adrenal tissue was obtained at autopsy and stored frozen; data was calculated as described in Table I. Each sample was run in duplicate and the average value is presented.

binding capacity. Specific binding of [^{125}I]insulin to human adrenal autopsy tissue was highly variable, with 0.19 to 1.75% bound/40 µg protein. [^{125}I]IGF-I binding to these samples was also variable, with 0.45 to 3.09% bound/40 µg protein.

Discussion

The role of insulin and IGF-I in the control of adrenal development and adrenal function is not yet understood. This manuscript contains data that strongly suggest that the human adrenal, unlike the rat adrenal, displays a comparable number of receptors for insulin and IGF-I. Comparative studies with autopsy and surgical specimens of human adrenal tissue indicate that autopsy samples generally contain fewer receptors for insulin per mg protein than surgical samples (Table I vs. Table II), whereas the number of IGF-I receptors is not diminished. There is not a consistent inverse relationship between the number of receptors and time after death that the tissue was obtained. This finding does not parallel the results of studies recently conducted in our laboratory with human kidney samples obtained either at autopsy or from surgical resections; kidney autopsy samples display a much more dramatic decline in the number of receptors for both insulin and IGF-I than human adrenal autopsy samples (Grizzle, Yang and Pillion, manuscript in preparation). The reason for this difference may be the presence of renal proteases that more quickly destroy renal hormone receptors following death. The human adrenal gland differs from the rat adrenal gland in that cortisol is the principal glucocorticoid formed in the former, while corticosterone predominates in the latter. Also, surrounding capsular fat is removed easily from the rat and hamster adrenal glands, but is tightly adherent to the human adrenal gland. Nevertheless, we took great care to dissect the fat from the sections of the human adrenal gland used in this study because fat tissue is known to contain large numbers of insulin receptors. The cortex of the human adrenal gland also may contain rare foci of mature fat. Histologic sections from each human adrenal gland used in this study were examined using standard hematoxylin and eosin staining and no such foci were noted in the sections studied. It appears unlikely, therefore, that the occurrence of insulin receptors in human adrenal tissue sections is the result of contamination with adipose tissue insulin receptors. It is not clear at this time if there is a relationship between the population of peptide hormone receptors that occur on the adrenal glands of these different species and the regulation of adrenocortical function or the corticosteroid products secreted from each. However, it has been documented that a complex relationship exists between glucocorticoids and the action of insulin, insulin-like growth factor I and epidermal growth factor, as well as the receptors for these growth factors. Our results indicate that receptors for insulin and IGF-1 are present in distinct regions of the human adrenal gland and these receptors may be involved in the regulation of adrenal growth and function.

Acknowledgments

Supported by grants from NIH to Dennis J. Pillion (HL 01134 and DK 36827) and to William E. Grizzle (CA 13148 and CA 44968) and Veterans Administration Research Funds to William E. Grizzle.

NOTE: While this manuscript was in preparation, Shigematsu et al. (8) reported that fetal and adult human adrenal tissue obtained at autopsy contains high-affinity receptors for IGF-I and localized IGF-I receptors by autoradiography in both the cortical and medulary areas. Our results are consistent with their observations concerning the existence of IGF-I receptors in human adrenal tissue, but our autoradiographic localization of IGF-I receptors, which involved the use of both Ultrafilm and emulsion-coated tissue slices, reveals a greater level of heterogeneity in receptor distribution than their results.

References

1. Pillion, D.J., Yang, M., and Grizzle, W. E. (1988) Biochem. Biophys. Res. Commun. 154, 138-145.
2. Dahmer, M.K. and Perlman, R.L. (1988) J. Neurochem. 51, 321-323.
3. Brush, J.S. and Banerjee, D.K. (1989) Diabetes 38, Suppl. 2, 182A.
4. King, G.L., Buzney, S.M., Kahn, C.R., Hetu, N., Buchwald, S., MacDonald, S.G., and Rand, L.I. (1983) J. Clin. Invest. 71:974-979.
5. Bar, R.S., Peacock, M.L., Spanheimer, R.G., Veenstra, R. and Hook, J.C. (1980) Diabetes 29:992-995.
6. Haskell, J.F., Meezan, E. and Pillion, D.J. (1985) Am. J. Physiol. 248:E115-E125.
7. Im, J.H., Pillion, D.J. and Meezan, E. (1986) Invest. Ophthalmol. Vis. Sci. 27:1681-1690.
8. Shigematsu, K., Niwa, M., Kurihara, M., Yamashita, K., Kawai, K. and Tsuchiyama, H. (1989) Life Sciences 45:383-389.