Purification and characterization of a feline hepatic insulin receptor

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Objective—To elucidate the functional characteristics of a highly purified soluble liver insulin receptor in cats.

Sample population—Frozen livers from domestic cats were obtained commercially.

Procedures—The feline hepatic insulin receptor was purified from Triton X-100 solubilized plasma membranes by the use of several chromatography matrices, including affinity chromatography on an insulin-Sepharose matrix.

Results—The receptor, although not homogeneous, was purified 3,000-fold. Two silver-stained protein bands were identified following sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with molecular weight of 134,000 and 97,000, which are similar to insulin receptors isolated from other animals. This isolated receptor had steady-state insulin binding by 40 minutes at 24 C. Optimal insulin binding occurred at pH 7.8 and with 150 m*M* NaCl. Under these conditions, a curvilinear Scatchard plot was obtained with the isolated receptor. Using a 2 binding-site model, the feline insulin receptor had a high-affinity low-capacity site with a dissociation constant (K_D; n*M*) of 3 and a low-affinity high-capacity site with a K_D of 1,180. The receptor also had tyrosine kinase activity toward an exogenous substrate that was stimulated by insulin and protamine.

Conclusions and Clinical Relevance—Many of the reported characteristics of the liver insulin receptor in cats are similar to those for the receptor isolated from other animals and tissues, although some differences exist. These similarities suggest that characterization of the feline insulin receptor is important to understanding insulin resistance in cats with diabetes as well as in humans with diabetes. (*Am J Vet Res* 2000;61:1625–1632)

Diabetes mellitus, type-1 or insulin-dependent diabetes mellitus and type-2 or noninsulin-dependent diabetes mellitus, is one of the most commonly encountered endocrine disorders in cats with a reported frequency range of 1 in 800 to 1 in 400 cats.[1-3] Type-1 and -2 diabetes occur in cats,[4-5] with estimates of the relative occurrences of 50 to 70% for type 1 and of 30 to 50% for type 2.[5] Insulin resistance or the requirement for abnormally high concentrations of insulin to achieve normoglycemia,[4] which defines the pathophysiologic characteristics of both types of diabetes,[6] particularly type 2, has been repeatedly associated with reduced insulin receptor function in studies of diabetic humans and rodents[6-19] and with altered function of various components of the insulin signal transduction pathway.[20] It is therefore somewhat surprising that, to our knowledge, no studies exist regarding the structure and function of the insulin receptor in cats, given the importance of insulin receptor function, the apparent direct involvement of altered receptor function in insulin resistance associated with diabetes, the known tissue and species differences in the receptor, and the high prevalence of diabetes in cats. This lack of information has even led to a suggestion in a recent review[21] of diabetes in cats that insulin resistance involves only a postreceptor defect, a conclusion not supported by many studies of insulin resistance seen in rodents and humans.[6-20]

The insulin receptor from human and rat tissues, particularly human placenta and rat liver, have been purified and well characterized.[21-37] The receptor is a heterotetrameric disulfide-linked complex composed of 2 different glycosylated subunits with a stoichiometry of $\alpha_2\beta_2$.[38] The molecular mass of the α- and β-subunits, based on deduced amino acid sequence, is 82 and 70 kd, respectively. The α-subunit is located entirely on the extracellular surface and responsible for binding insulin. The β-subunit is extracellular and intracellular with 1 transmembrane domain and responsible for the tyrosine kinase activity that phosphorylates first the β-subunit and then phosphorylates exogenous proteins (eg, insulin receptor substrate 1). It is this phosphorylation of various proteins that eventually leads to the numerous physiologic actions of insulin.

The similarities in the structural and functional characteristics of the insulin receptor from different species and tissues have promoted the generalized understanding of the molecular mechanisms of the role of the insulin receptor in insulin's action. However, there are numerous known tissue- and species-specific differences in structure and function of insulin receptors,[39] which may affect experimental and clinical approaches to the study and treatment of diabetes mellitus in different species. One of the known differences is the presence of 2 isoforms of the insulin receptor in humans and rats that are generated from tissue-specific alternative messenger RNA splicing sites leading to 2 different α-subunits, 1 that is 12 amino acids longer than the other.[40,41] These isoforms appear to have different functions,[42,43] and their tissue-specific expression may be altered in diabetic humans[44,45;] however, this possibility may not be true

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for partially pancreatectomized diabetic rats[40,46] or other species.

The type and degree of glycosylation varies among insulin receptors in different tissues and species. The α-subunit possesses N-linked glycosylations, and the β-subunit has N-linked and O-linked-glycosylations.[47,48] The large degree of glycosylation increases the apparent molecular mass of the α- and β-subunits on **sodium dodecyl sulfate polyacrylamide electrophoresis (SDS-PAGE)** to 120,000 – 135,000 and 90,000 – 95,000, respectively, from the deduced molecular mass of 82,000 and 70,000.[48] The type of glycosylation on insulin receptors can vary among tissues within the same species[49] and among species[50] and affects the function of the receptor.[47,48] These glycosylation differences concomitant with the differences in primary structure likely play important roles in the numerous tissue- and species-specific differences in insulin-receptor function.[51-53]

As in humans, cats can develop diabetes spontaneously. Therefore, the purpose of the study reported here was to provide information on the purification and characterization of insulin receptors in cats. We report on the apparent molecular mass of the subunits, optimum pH and NaCl concentration for insulin binding, the dissociation constants for insulin binding, and the insulin and protamine stimulation of phosphorylation of an exogenous substrate. To our knowledge, this report is the first to characterize part of the insulin signal transduction pathway in cats. Given the known importance of the impaired insulin receptor function in diabetic humans and rodents,[6-20] this knowledge should contribute to the understanding of the role of altered insulin receptor function in diabetic cats.

Materials and Methods

Purification—Cat livers were purchased from a biological supply house[a] and stored at –70 C. Euthanasia of the random source cats had been performed with phenobarbital administration. Plasma membranes from the livers were prepared by modifications of previous methods.[22-24] The frozen livers were homogenized in a blender[b] with a 1:5 (wt/vol) solution of an ice-cold buffer of 0.25*M* sucrose, 25 m*M* potassium phosphate (pH 7.5, 20 C), 2% (vol/vol) bovine serum, 5 m*M* EDTA, and a mixture of protease inhibitors (50 µg of trypsin inhibitor/ml, 5 µg of bacitracin/ml, 2 µ*M* bestatin, 5 µ*M* E-64, 1 µ*M* pepstatin A, 25 µ*M* tosyl-lysine chloromethyl ketone, 10 µ*M* leupeptin, 5 µg of aprotinin/ml, 5 µ*M* elastatinol, 2 µ*M* antipain, and 10 µ*M* chymostatin). The homogenate was made to 1 m*M* phenylmethylsulfonyl fluoride and again homogenized with a motorized tissue grinder[c] set at #5 for 60 seconds. All subsequent steps were conducted at 4 to 6 C. This homogenate was centrifuged[d] for 30 minutes at 10,800 × g. The supernatant, after passage through 4-layers of cheesecloth, was adjusted to pH 7.5 with 2*M*-Tris buffer and made to 0.1 *M* NaCl and 1 m*M* $MgCl_2$ with 3*M* and 1*M* stock solutions, respectively. This mixture was centrifuged[d] for 45 minutes at 208,500 × g. The pellet was collected using 3-passes of a motorized Potter-Eljeheim homogenizer in 1 ml/g original tissue weight in 50 m*M* sodium acetate, pH 6.3 at 20 C, 5% (vol/vol) Triton X-100, and the protease inhibitors already described in this report. This mixture was stirred for 30 minutes on ice and centrifuged[d] for 30 minutes at 229,000 × g. The supernatant, containing a crude preparation of detergent solubilized membrane proteins, was frozen at –70 C.

The detergent solubilized membrane proteins were applied to a 6.5 × 100 cm column of diethylaminoethyl-sephacel equilibrated with 50 m*M* sodium acetate (pH 6.3, 20 C) and 0.5% (vol/vol) Triton X-100. After the sample was applied, the column was washed with 0.5 L of equilibration buffer and eluted with a linear gradient (2.5 L) of ammonium acetate (0.04 to 1 *M*), pH 6.3, at 20 C, containing 0.5% (vol/vol) Triton X-100. The fractions containing insulin receptors, assayed by specific insulin binding, were pooled. The proteins were concentrated by making the solution 12% (wt/vol) in **polyethylene glycol** (**PEG**, molecular weight of 6,000) by slowly adding ice-cold 50% (wt/vol) PEG. The mixture was allowed to slowly stir for 30 minutes on ice and centrifuged[d] for 20 minutes at 21,000 × g. The pellet was extracted with a small volume of ice-cold 50 m*M* Tris-chloride buffer (pH 7.5, 20 C), 0.1% (vol/vol) Triton X-100, and protease inhibitors (already described). This sample was applied to a 3 × 50 cm column of polylysine-agarose equilibrated in the same buffer but without protease inhibitors. The column was washed with 0.2 L of this buffer and eluted with a linear gradient (1 L) of NaCl (0 to 500 m*M*) in the same buffer. The fractions containing insulin-binding activity were pooled and the proteins again concentrated with PEG. The pellet was extracted with 50 m*M* HEPES-potassium buffered solution (pH 7.5, 20 C), 150 m*M* NaCl, 10 m*M* $MgCl_2$, and 0.1 % (vol/vol) Triton X-100 and applied to a 3 × 12 cm column of wheat germ agglutinin agarose[e] equilibrated in the same buffer. After recycling the sample (flow rate, 0.5 ml/min) at least 8 times, the matrix was washed with 0.2 L of the equilibration buffer. The insulin-binding activity was eluted with 0.3 *M* N-acetylglucosamine in the same buffer. Fractions containing the insulin-binding activity were pooled, concentrated by PEG, extracted with 50 m*M* HEPES-potassium buffered solution (pH 7.5, 20 C), 1 *M* NaCl, and 0.1 % (vol/vol) Triton X-100, and applied to an insulin-agarose[e] column (3 × 12 cm) equilibrated in the same buffer. The sample was recycled (0.5 ml/min) through the column 50 times before the column was washed with 0.5 L of the same buffer. The insulin receptor was eluted with 50 m*M* sodium acetate (pH 5.0, 20 C), 1 *M* NaCl, and 0.1% (vol/vol) Triton X-100. Each fraction (2.5 ml) was collected in tubes containing 0.1 ml of 2*M* Tris-chloride buffer (pH 7.6, 20 C), to increase the pH of the eluted sample, and protease inhibitors. The fractions containing the insulin receptors were pooled and concentrated to 1 ml by putting the sample in a dialysis bag and surrounding the bag with solid PEG (molecular weight of 20,000) for 24 hours at 4 C. The concentrated sample was dialyzed overnight against 0.5 L of 25 m*M* HEPES-potassium buffered solution (pH 7.5, 20 C), 0.1% (vol/vol) Triton X-100, 1 m*M* EDTA, and 20% (vol/vol) glycerol. The dialyzed sample was stored in 0.1 ml portions at –70 C.

Insulin binding activity—Determination of insulin binding activity was done essentially as described.[22] Briefly, the receptor preparation was incubated for 1 hour at 24 C with 20,000 cpm (40 p*M*) of receptor grade [^{125}I]-Tyr^{A14} porcine insulin[f] in a total volume of 0.1 ml containing 50 m*M* HEPES potassium-buffered solution (pH 7.8, 20 C), 150 m*M* NaCl, 0.1% (vol/vol) Triton X-100, 10 mg of bovine serum albumin/ml, and either no unlabeled insulin to measure total insulin binding or excess (50 µg/ml) unlabeled porcine insulin[g] to measure nonspecific binding. For Scatchard analysis, various concentrations of unlabeled insulin from 0.1 to 500 ng/ml were added. The receptor- ^{125}I-labelled insulin complex was separated from free insulin by adding 0.1 ml ice-cold 0.2% (wt/vol) bovine γ-globulin[e] and 0.2 ml of 24% (wt/vol) PEG. After incubation for 15 minutes on ice, the mixture, in a 1.5 ml Eppendorf tube, was centrifuged for 5 minutes at

15,600 × g in a refrigerated (4 C) minicentrifuge. The supernatant was aspirated and the pellet washed with 1 ml ice-cold 12% (wt/vol) PEG and treated as described. Finally, the supernatant was discarded and the ^{125}I-contents of the tube determined by an auto-gamma counter.[h] Specific binding is equal to the total binding minus the nonspecific binding. Nonspecific binding was always less than 5% of total binding. Scatchard analysis was by direct fit with a computer program.[i] Characterization of the feline insulin receptor capacity to bind other insulin-like hormones was beyond the scope of this study.

Insulin receptor tyrosine kinase activity—Insulin receptor tyrosine kinase activity was determined by incubating 0.3 µg of purified insulin receptor in a total volume of 15 µl without or with 1 m*M* insulin in 50 m*M* HEPES-potassium-buffered solution (pH 7.8, 20 C), 150 m*M* NaCl, 0.1% (vol/vol) Triton X-100, and 10 mg of bovine serum albumin/ml. The incubation lasted for 30 minutes at 30 C. After incubation, the kinase assay was initiated by addition of 35 µl of a solution that gave a final concentration of 15 m*M* $MgCl_2$, 100 m*M* Na_2VO_4, 200 µ*M* [γ-^{32}P] ATP (1 to 2 Ci/mmol),[f] and 2.5 mg of **polyglutamyltyrosine**[e] (**PolyGT**; 4:1 ratio; mean molecular mass 20,000)/ml. The PolyGT provide an artificial substrate for the insulin receptor's tyrosine kinase activity.[54] After the indicated time, the reaction was stopped by applying 40 µl of the described reaction to a 2 × 2 cm filter paper[j] previously spotted with 0.1 ml of 24% (wt/vol) **trichloroacetate (TCA)**, 2 m*M* sodium pyrophosphate, 0.2 m*M* phosphoric acid, and 1 m*M* ATP. The papers were immediately placed in a stirring solution of 0.5 L of ice-cold 10% (wt/vol) TCA containing 10 m*M* sodium pyrophosphate and 1 m*M* ATP. After 15 minutes, the solution was replaced with another 0.5 L of fresh solution for another 15 minutes. Then the solution was removed and the papers washed 2 times for 15 minutes in ice-cold 0.5 L 5% (wt/vol) TCA. The papers were removed and dried by placing them in 25 ml of ether and drying them at room temperature (20 C) for 20 minutes.[55] The protein-bound ^{32}P on the papers was quantified by liquid scintillation counting. Blank reactions, without exogenous substrate, were counted and subtracted from the amount of phosphate incorporated into PolyGT.

SDS-PAGE—Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed[56] with a 5 to 10 % T: 2.67 % C gradient gel.[k] Proteins were detected by silver staining.[57] Protein concentration was determined by a Coomassie binding assay,[58] using bovine serum albumin as a standard.

Results

Purification—The feline liver insulin receptor was readily purified from Triton X-100 solubilized plasma membranes using chromatography on several matrices (**Fig 1**). The largest degree of purification (**Table 1**) took place with the wheat germ agglutinin agarose, which binds carbohydrate moieties on proteins, and the insulin-agarose, which is a highly specific affinity matrix.[24,25,29,31,33] More than a 3,000-fold purification of insulin receptor resulted in recovery of 1 mg of protein from 2.6 kg of cat liver.

Analysis of the feline liver receptor by SDS-PAGE followed by silver staining revealed that the preparation contained 2 subunits of molecular mass of 134,000 and 94,000. These 2 bands represented 40% of the total 7 bands, assuming equal intensity of different proteins. The relative stoichiometry of the α- to β-subunit was 2:3 (data not shown).

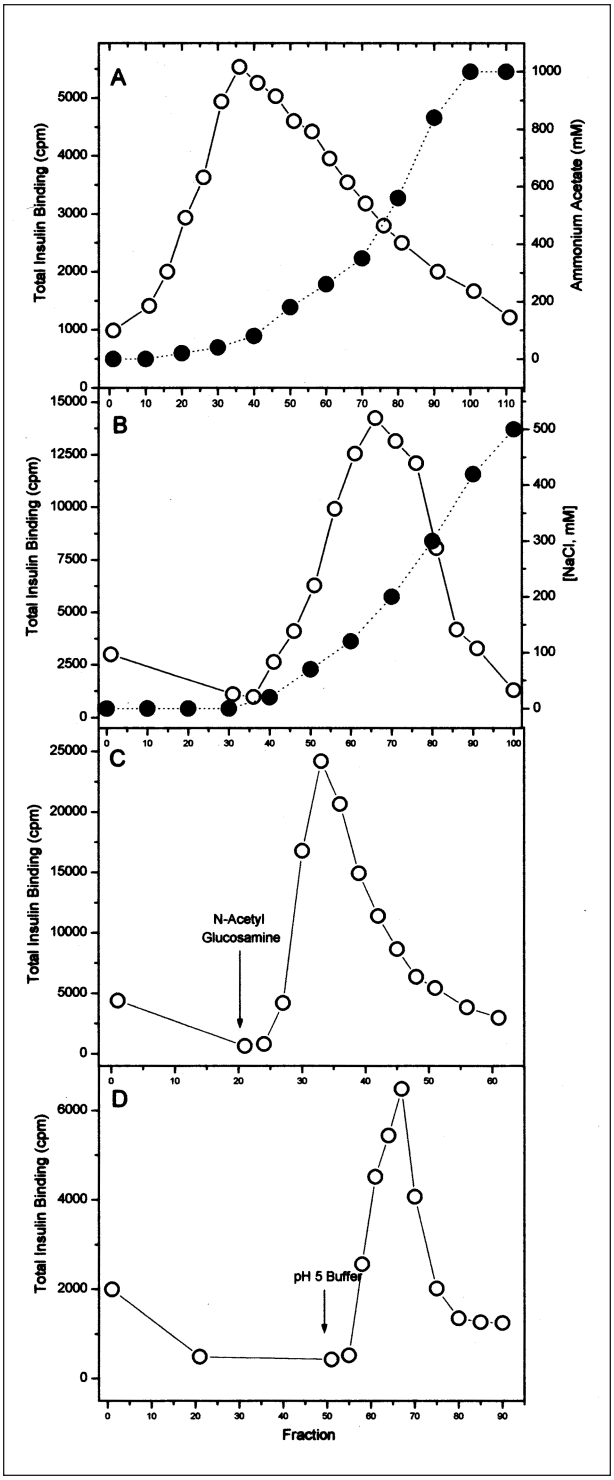

Figure 1—Chromatography profiles for the purification of feline liver insulin receptor. Open circles represent insulin binding (cpm). Panel A: profile of the elution of feline liver insulin receptor from diethylaminoethyl-Sephacel. Closed circles indicate ammonium acetate concentration (m*M*). Panel B: profile of the elution of feline liver insulin receptor from polylysine agarose. Closed circles indicate NaCl concentration (m*M*). Panel C: profile of the elution of feline liver insulin receptor from wheat germ agglutinin agarose. Arrow indicates when elution buffer was used containing 0.3 *M* N-acetyl glucosamine. Panel D: profile of the elution of the feline liver insulin receptor from insulin agarose. Arrow indicates when elution buffer pH was changed to 5.0.

Table 1—Purification of feline liver insulin receptor*

Step	Volume (ml)	Protein (mg)	Insulin binding† (μg/mg)	Total insulin binding (μg)	Yield (%)	Purification-fold
Solubilized membrane	650	10,730	0.003	32.2	100	1
DEAE-sephacel	78	2,036	0.010	20.4	63	3
Polylysine-agarose	38	488	0.039	19	59	13
WGA-agarose	7	56	0.28	15.7	49	93
Insulin-agarose	1	1	9.1	8.6	27	3,033

*Purification from 2.6 kg of cat liver. †Insulin binding represents specific binding except for the fraction from insulin-agarose column where binding was determined by Scatchard analysis. Nonspecific binding was < 1% of total binding in all fractions except for the solubilized membranes where it was 40% of total binding.
DEAE-Sephacel = Diethylaminoethyl-sephacel. WGA-agarose = Wheat germ agglutinin agarose.

Characterization—The receptor had a linear response between amount of insulin bound and amount of receptor added (up to 2 μg of receptor per assay [**Fig 2**]). Consequently, all subsequent assays of insulin binding was done at ≤ 1 μg/assay to ensure detection of altered insulin binding. Insulin binding was also time-dependent (**Fig 3**). Fifty percent of maximum binding occurred at 14 minutes and reached an apparent maximum by 30 to 40 minutes at 24 C. The effects of various temperatures were not determined. All subsequent binding assays were done after 60 minutes incubation to ensure that steady-state binding had been reached.

Using the described assay conditions, the effect of pH and NaCl concentration on insulin binding was determined. Optimum binding of insulin occurred at a pH of 7.8 (**Fig 4**) and 150 m*M* NaCl (**Fig 5**). Using these conditions, insulin binding by the feline insulin receptor gave a curvilinear Scatchard plot[59] (**Fig 6**). When analyzed with a 2 binding-site model, as in other studies[22,24,25,27-29,31,33,36,42,48,60] of insulin binding to purified insulin receptor, there was a high-affinity low-capacity site and a low-affinity high-capacity site. The mean (± SE) K_D (n*M*) of these sites were 3.0 ± 0.4 and 1,180 ± 44, respectively. Total binding was 9.1 ± 0.1 μg of insulin/mg of receptor or 0.5 mol of insulin/mol of ($\alpha_2\beta_2$) receptor.

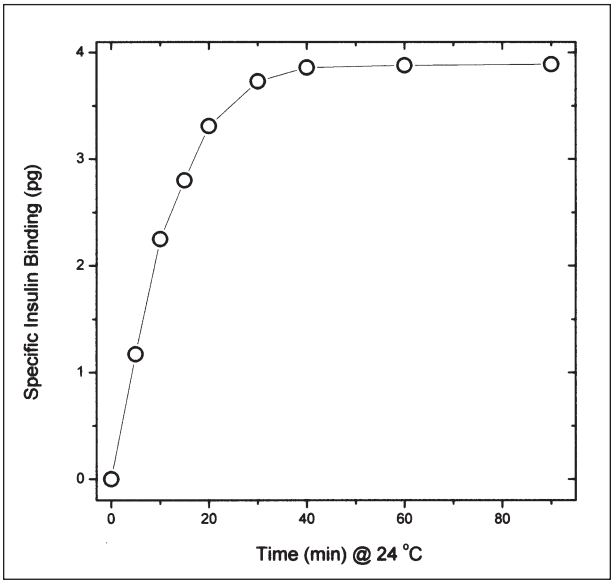

Figure 3—Time course of insulin binding to feline liver insulin receptor (assay conditions: pH 7.8, 1 μg insulin receptor).

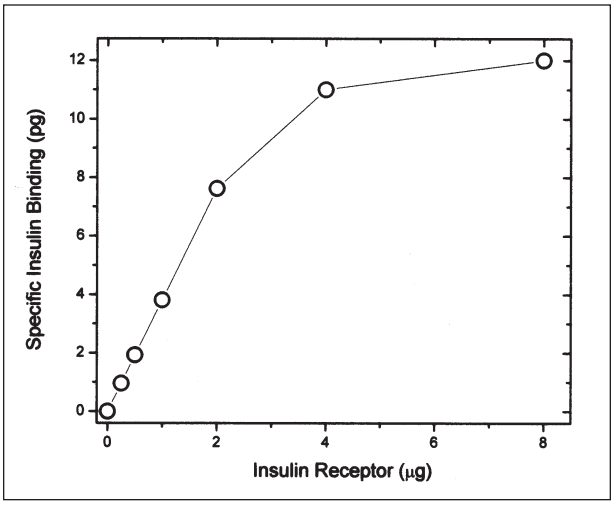

Figure 2—Relationship between amount of feline liver insulin receptor and specific insulin binding of the receptor (assay conditions: 150 m*M* NaCl, pH 7.8, incubation of 60 minutes at 24 C).

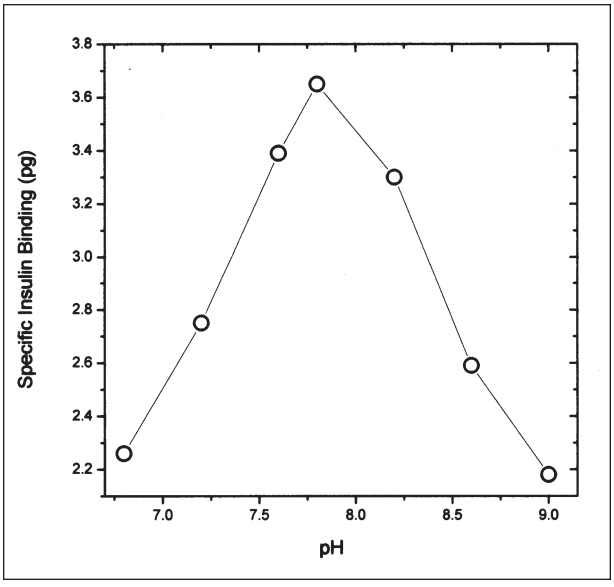

Figure 4—Effect of pH on insulin binding to feline liver insulin receptor (assay conditions: 150 m*M* NaCl, 1 μg insulin receptor, incubation for 60 minutes at 24 C).

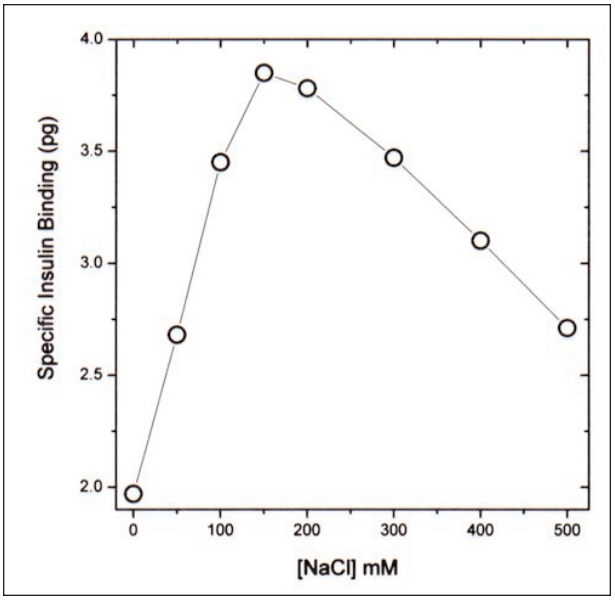

Figure 5—Effect of NaCl concentration on the specific insulin binding to feline liver insulin receptor (assay conditions: pH 7.8, 1 μg insulin receptor, incubation for 60 minutes at 24 C).

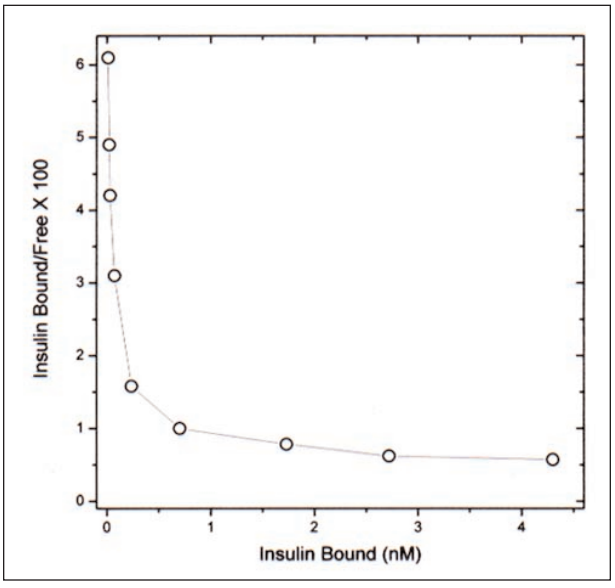

Figure 6—Scatchard plot for the feline insulin receptor indicating the bound insulin (n*M*) versus the ratio of bound/free insulin (assay conditions: 150 m*M* NaCl, pH 7.8, 1 μg insulin receptor, incubation for 60 minutes at 24 C).

The feline liver receptor also had (**Fig 7**) a tyrosine kinase activity with PolyGT, a known artificial substrate for the insulin receptor kinase activity.[54] Phosphorylation was linear with time and stimulated by insulin. Insulin stimulated the receptor's phosphorylation of PolyGT by 2.3-fold (slope of lines, pmol of phosphate incorporated/mg of insulin receptor). Protamine, at 3.6 μ*M*, also stimulated the feline liver insulin receptor kinase activity by 1.8-fold. The combination of protamine and insulin leads to only a modest increase (10%) greater than insulin alone.

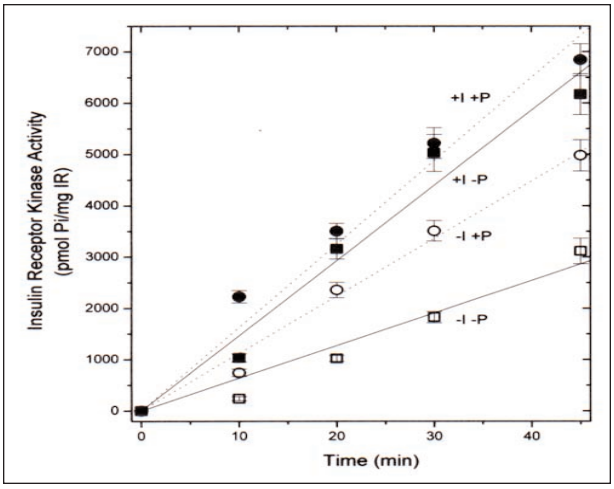

Figure 7—Effects of insulin (I) and protamine sulfate (P) on feline liver insulin receptor kinase activity (pmol of phosphate incorporated/mg of insulin receptor) with polyglutamyltyrosine (polyGT) as substrate. Assay as described in Materials and Methods. Squares and circles indicate absence and presence, respectively, of protamine sulfate (15 μg/ml). Open and closed symbols indicate absence and presence, respectively, of insulin. The SE (n = 3) for each data point is shown (when not seen, the SE was less than the diameter of the symbol). The mean slopes (pmol of phosphate incorporated into polyGT/min/mg of insulin receptor) and SD for the incubations without either insulin or protamine (-I-P), without insulin but with protamine (-I+P), with insulin but without protamine (+I-P), and with insulin and protamine (+I+P) are 63.6 ± 4.6, 112.1 ± 3.7, 146.6 ± 7.7 and 162.6 ± 7.5, respectively.

Discussion

In our study, purification of the feline insulin receptor was similar to purification of insulin receptor isolated from human placenta[24,25,28,31,32,34] and rat liver.[25,33,35] Use of polylysine agarose and PEG precipitation to concentrate the receptors are, however, new modifications used in the purification steps of our study. Purified feline liver insulin receptor had a stoichiometry of binding of 0.5 moles of insulin/mole of $\alpha_2\beta_2$ receptor. This compares with 0.6 to 1.5 moles of insulin/mole of receptor from human placenta,[24,25,28,30,31,32] 0.6 to 1.5 moles insulin/mole of receptor from rat liver,[25,33,35] and 1.7 moles insulin/mole of receptor from rat skeletal muscle.[29] Whereas wide variation in binding stoichiometry may be obtained because of several analytic problems inherent in this determination,[31] the general value of 1 to 1.5 moles of insulin/mole of receptor seems to be generally acceptable.[12,26,60] Given the results of the densitometric analysis (data not shown), with an assumption of linearity in staining intensity with different proteins in silver-stained gels, 40% of the feline insulin receptor preparation was represented by the α- and β-subunits of the insulin receptor. This suggests that a homogeneous feline liver insulin receptor preparation would have a binding stoichiometry of 1.2 moles of insulin/mole of receptor. This is clearly within the range of preparations of purity for apparently homogeneous insulin receptor preparations and within the generally accepted binding stoichiometry. However, there may also be intrinsic differences in binding affinity for the insulin receptor among species and tissues.[39,61] For example, when the receptor was isolated from rat liver and human placenta in the same laboratory,[25] the placental receptor had 15% greater binding capacity for

similarly purified rat liver receptor. Until homogenous preparations of feline liver receptor are obtained, the question of absolute binding stoichiometry of the feline insulin receptor remains unanswered.

The pH optimum for insulin binding for the feline liver insulin receptor of 7.8 was the same as the receptor isolated from human skeletal muscle[36] and human lympocytes[62]; however, it was higher than the receptor isolated from human placenta[28] (pH 7.6) and rat adipose tissue[22] (pH 7.5) and lower than the receptor isolated from rat liver (pH 8.0).[63] The binding of insulin by the receptor is sensitive to the hydrogen ion concentration at physiologic blood pH of the cat[64] of 7.35, suggesting that acidosis or alkalosis may alter insulin binding and thereby insulin action. Furthermore, it would seem that there is a tissue and species difference in the effects of pH on insulin binding. The optimum NaCl concentration for insulin binding of 150 m*M* is about the same as the NaCl concentration found in feline blood.[64]

The feline liver receptor had the 2 known functions of the insulin receptor (ie, insulin binding and insulin-stimulated tyrosine kinase activity). Insulin binding to the feline liver insulin receptor had the same pattern as seen in studies of the insulin receptor from various tissues and animals.[60,61] This pattern of binding reveals heterogeneity of sites, negative cooperativity, or a combination of both. This type of binding suggests[60] that at low physiologic concentration 1 molecule of insulin binds to both α subunits at different binding sites. The second molecule of insulin at higher nonphysiologic concentrations competes for 1 of the binding sites already occupied, possibly leading to negative cooperativity and a lower affinity but higher capacity. The K_D for insulin binding of the high affinity site, 3 n*M*, for the feline liver receptor is similar to that reported for other soluble purified receptors of 0.5 n*M* for the receptor from rat skeletal muscle,[29] of 1 to 2 n*M*[28] and 0.27 n*M*[37] for the receptor from human placenta, and 0.44 n*M* for the receptor from rat liver.[37] These similarities are particularly striking considering the potential methodologic problems in measuring binding affinities with the insulin receptor.[31] A substantial portion of the feline liver receptor would be occupied in vivo when considering the physiologic range of feline blood insulin concentration of 60 to 100 p*M* and 0.8 to 1.65 n*M*.[2,65,66] It is apparent from several in vivo studies that important physiologic effects of insulin can occur at blood insulin concentrations well below the K_D values of insulin binding, and different actions of insulin appear to require different concentrations of insulin suggesting a difference in the signal transduction process for different physiologic effects. For example, in human adipose tissue where 50% of insulin binding would occur at 5 n*M* insulin, 50% stimulation of 3-0-methylglucose transport requires only 50 p*M* insulin, whereas 50% phosphorylation of insulin receptor requires 2 n*M* insulin.[67] Similarly, in human skeletal muscle, blood insulin concentration of 0.7 n*M* accounts for 50% of the maximum glucose disposal, whereas 50% activation of the insulin receptor kinase activity requires 3.8 n*M* insulin.[68] These results indicate that with < 30% occupancy of insulin receptor at physiologic insulin concentration, the glucose disposal rate is 90% of maximum.[69] Results of another study[70] suggest that only 2% of the insulin receptors are occupied to achieve maximum insulin stimulation of glucose transport in adipocytes. Therefore, there may be spare receptors for certain physiologic actions of insulin.[69,70] The relationship between insulin receptor occupancy, insulin concentration, glucose disposal rate, and receptor kinase activity is not known in the cat.

The feline liver insulin receptor kinase activity toward an exogenous substrate, PolyGT, was stimulated 2.3-fold by insulin, which is similar to the receptor isolated from human placenta (3- to 5-fold)[32,71] and human skeletal muscle (3-fold).[72] However, higher values (5- to 19-fold) and differences among the receptor from various tissues (2.3 times greater insulin stimulation of rat liver receptor, compared with human placenta) have been reported.[37] These differences may be possibly inherent to the tissue or species origin of the receptor,[25,51] caused by various experimental differences (eg, ATP concentration, temperature, insulin concentration), or both.

The feline liver insulin receptor kinase activity also had a large stimulation by protamine, which has been reported for the insulin receptor isolated from human placenta[73,74] and rat liver.[75] The stimulation of the feline insulin receptor by 3.6 μ*M* protamine sulfate was similar to that seen with the human placenta with 10 μ*M* protamine.[74] It has been suggested[76] that polybasic effectors might interact at the insulin receptor *C*-terminal acidic domain of the β-subunit, thereby regulating kinase activity of the receptor without the presence of insulin. It is unclear whether this effect is specific or of physiologic relevance, as suggested[77] for regulation of calmodulin phosphorylation by the receptor, or whether some as yet unknown mediator interacts with the receptor at this site and thus regulates the kinase activity.

The structural and functional characteristics of the cat liver insulin receptor appears similar to the same characteristics measured in the receptor from human and rodent tissues. The assay conditions identified with the highly purified cat liver receptor should form the basis of an insulin receptor assay, both insulin binding and kinase activity, for various cat tissues. More detailed studies are needed of the cat insulin receptor to determine, among other things, if the receptor's functions are altered in diabetes mellitus and other pathophysiological conditions with altered insulin resistance as it is in rodents and humans. These studies will set the stage to elucidate the cellular processes of insulin action in the cat and thereby provide a mechanistic approach to understanding, treating and possibly preventing type-2 diabetes in cats.

[a]Pel-Freez Biologicals, Rogers, Ariz.
[b]Waring Products Division, New Hartford, Conn.
[c]Polytron, Brinkman Instruments, Westbury, NY.
[d]Sorvall Division, Kendro Laboratory Products, Newtown, Conn.
[e]Sigma Chemical Co, St Louis, Mo.
[f]NEN Life Science Products, Boston, Ma.
[g]Eli Lilly Co, Indianapolis, Ind.
[h]Packard, Downers Grove, Il.
[i]Enfitter, Biosoft, Cambridge, UK.

[j]3M, Whatman Inc, Clifton, NJ.
[k]Mini-Protein II System, BioRad Laboratories, Hercules, Calif.

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