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TAURINE BINDING TO THE PURIFIED INSULIN RECEPTOR

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Abstract—Taurine (2-aminoethanesulfonic acid) was shown to bind specifically and reversibly to the purified human insulin receptor. While insulin binding to the purified insulin receptor exhibited characteristic negative cooperativity and an apparent dissociation constant (K_d) of 1.2×10^{-9} M, taurine binding was shown to exhibit positive cooperativity and had a lower affinity for the insulin receptor. The apparent K_d for taurine binding to the purified insulin receptor was calculated to be 130×10^{-9} M and the maximum number of binding sites (B_{max}) was 1.6 nmol/mg receptor protein. Chromatographic data demonstrated that taurine binds to the 138,000 molecular weight subunit of the insulin receptor. Taurine binding to the receptor protein was displaced by either taurine or insulin. Anti-human insulin receptor sera prevented insulin or taurine from binding to the receptor. Taurine binding to the protein was pH dependent, and sulfur-containing taurine analogues were able to displace taurine from the insulin receptor. These data supported our previous *in vivo* and *in vitro* observations that the hypoglycemic properties of taurine appear to be mediated through an interaction of taurine with the insulin receptor.

Taurine (2-aminoethanesulfonic acid) is a predominant amino acid in mammalian tissue [1]. The highest concentrations of this beta-amino acid are found in those tissues that maintain high levels of cellular metabolism, such as heart, skeletal muscle, and brain. Evidence continues to accumulate that taurine may alter cellular metabolism. Ackerman and Heinsen [2] were the first to report a hypoglycemic effect of taurine. These results were confirmed by Macallum and Sivertz [3], who found taurine to be a more potent hypoglycemic agent than the sulfonamides. It was not until nearly 20 years later that Shimzu *et al.* [4] reported that taurine produces an antidiabetic effect in alloxan-induced hyperglycemic animals. This report was confirmed in rats and rabbits made diabetic with either alloxan or ditisione [5] or in mice made diabetic following streptozotocin injections [6].

Until recently, the specific mechanism(s) for the actions of taurine has not been investigated extensively. Donadio and Fromageot [7] were the first to demonstrate that taurine increases glucose utilization in the rat diaphragm. Their results suggested that this pharmacological action of taurine occurred at the level of the sarcolemma. In preliminary studies, Dokshina *et al.* [8] provided evidence that taurine-stimulated glucose uptake is mediated through 3′,5′-cyclic adenosine monophosphate (cAMP). It was observed by Lampson *et al.* [9] that perfusion of isolated rat hearts with taurine is able to potentiate the effects of insulin on glucose uptake. However, *in vivo* evidence from our laboratories has shown that, following the administration of a bolus injection of glucose into WKY rats, taurine, independent of insulin, is able to attenuate the rise in serum glucose levels and increase skeletal muscle glucose levels [10]. These data suggested that taurine may interact with the insulin receptor to mediate the same physiological effects observed for insulin. In this paper, we provide *in vitro* evidence that taurine binds to the purified human insulin receptor. The binding characteristics of this interaction are described.

MATERIALS AND METHODS

Full-term human placenta was used for isolation and purification of the insulin receptor. The crude membrane fraction was isolated by differential centrifugation [11]. The human placenta was homogenized in 50 mM Tris–HCl, pH 7.4, containing 250 mM sucrose, 2 mM EDTA, 2 mM ethyleneglycolbis(amino-ethylether)tetra-acetate (EGTA), 1 mM phenylmethanesulfonyl fluoride, 25 mM benzamidine HCl, 10 mM leupeptin, 0.05 trypsin inhibitor units/ml protinin, 1 mM 1,10-phenathrolinic acid, and 1 mM pepstatin A. The insulin receptor was solubilized from the membrane preparation by extraction with 50 mM Tris–HCl, pH 7.5, containing 1.0% (v/v) Triton X-100 for 1 hr at 24°. The suspension was centrifuged at 200,000 *g* for 90 min at 4°. The clear supernatant fraction was used for purification of the insulin receptor.

The solubilized insulin receptor was purified by sequential chromatography on DEAE-cellulose, wheat germ agglutinin, *Ricinus communis*, and agarose-bound insulin columns [12]. The soluble receptor solution was applied to a DEAE-Sephadex column

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(5 × 20 cm) previously equilibrated with 10 mM Tris–HCl, pH 7.5, containing 0.1% Triton X-100 (v/v) and 40 mM NaCl, at a flow rate of 50 ml/hr at 4°. The column was eluted with a 2-liter linear gradient of 40–400 mM NaCl in the Tris–Triton buffer described above. The insulin receptor eluted at a concentration of approximately 100 mM NaCl. The pooled insulin binding fractions were applied to a column containing Concanavalin A-agarose (0.6 × 9 cm) that was equilibrated previously with 10 mM Tris–HCl, pH 7.5, containing 0.1% Triton X-100 (v/v), 1 mM $CaCl_2$, and 0.1 mM $MnCl_2$. The column was washed with buffer, and the insulin receptor was eluted with 25 ml of the buffer containing 0.5 M methyl alpha-D-mannopyranoside. Receptor-containing fractions were dialyzed against buffer to remove the sugar. The receptor-containing solution was applied to a column (0.6 × 9 cm) of *R. communis* 120-agarose. The column was washed with buffer, and the insulin-binding material was eluted with buffer containing 0.5 M D-galactose. The pooled receptor fractions that contained the receptor were chromatographed subsequently on an insulin-succinyldiaminopropylamino Sepharose affinity column (0.6 × 9 cm). The receptor was eluted with 4 M urea buffer, pH 6.3, and immediately diluted one to ten by the addition of 1 M phosphate buffer, pH 7.4, containing 0.1% Triton X-100 and 40% sucrose. After exhaustive dialysis against 50 mM Tris–HCl, pH 7.5, containing 0.05% Triton X-100 for 24 hr at 4°, the insulin binding material was concentrated on an Amicon YM-30 membrane filter. Total purification of the receptor was calculated to be approximately 3000-fold.

The binding assay used during purification of the insulin receptor was the polyethyleneglycol method as described by Cuatrecasas [13]. This procedure also was employed for all studies involving

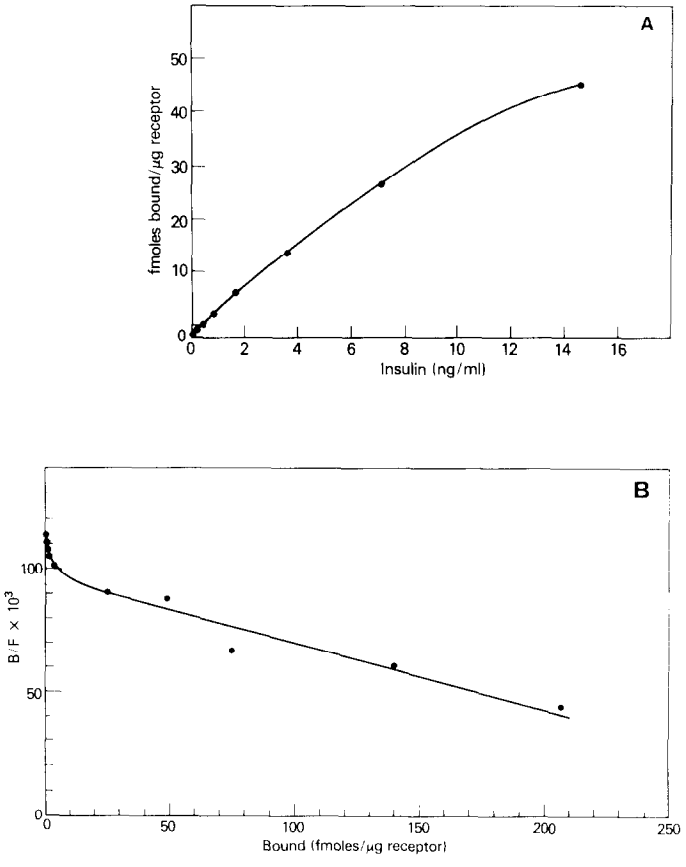

Fig. 1. (A) Insulin binding to the purified insulin receptor. Sepharose-insulin purified insulin receptor (0.5 μg/0.2 ml) was measured for insulin binding as described in Materials and Methods. Following a 10-min preincubation of the insulin receptor in 50 mM Tris–HCl, pH 7.5, that contained 0.03% Triton X-100 and 0.05 M NaCl, [^{125}I]insulin was added. After a 50-min incubation the reaction was terminated by the addition of ice-cold 0.1 M sodium phosphate buffer (pH 7.4) containing 0.1% (w/v) gamma-globulin. The tubes are placed on ice, 0.5 ml of ice-cold 25% polyethyleneglycol was added, and the tubes were vortexed and placed on ice for 10 min. The contents of the tubes were filtered on Millipore filters, and the filters were washed with 3 ml of 8% polyethyleneglycol in 0.1 M Tris–HCl (pH 7.4). The filters were counted for radioactivity. Non-specific binding was determined with unlabeled insulin. (B) Scatchard plot of data from panel A.

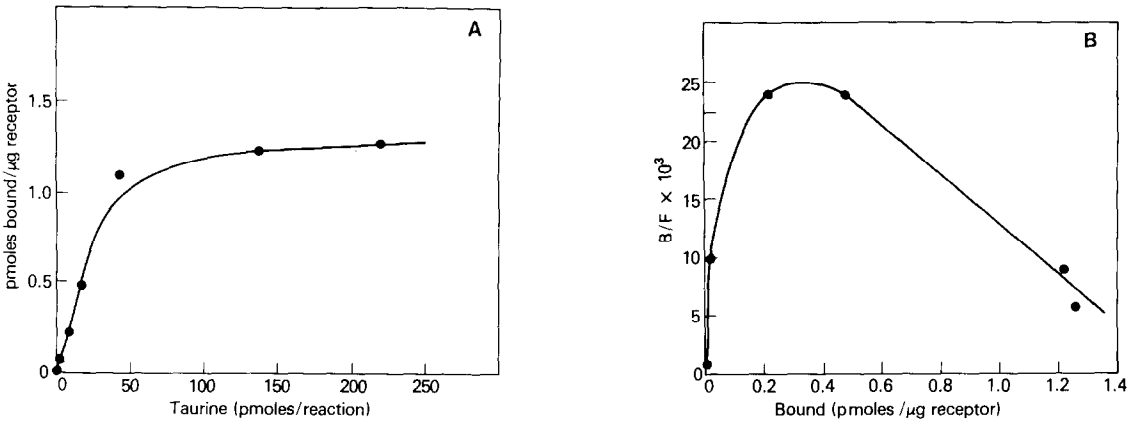

Fig. 2. (A) Taurine binding to purified insulin receptor. Sepharose-insulin receptor (0.5 μg/0.2 ml) was measured for taurine binding. Following a 10-min preincubation of the insulin receptor with 50 mM Tris–HCl, pH 7.5, that contained 0.03% Triton X-100 and 0.5 M NaCl, [^{3}H]taurine was added. After a 50-min incubation, the reaction was terminated by the addition of ice-cold 0.1 M sodium phosphate buffer (pH 7.4) containing 0.1% (w/v) gamma-globulin. The tubes were placed on ice, and 0.5 M ice-cold 25% polyethyleneglycol was added. The tubes were vortexed and placed on ice for 10 min. The contents of the tubes were filtered on Millipore filters, and the filters were washed with 3 ml of 8% polyethyleneglycol in 0.1 M Tris–HCl (pH 7.4). The filters were placed in fluor and counted for radioactivity. Non-specific binding was determined with 50 μg taurine and found to be 10% of total binding. (B) Scatchard plot of data from panel A.

[^{125}I]insulin or [^{3}H]taurine. The final reaction volume for all assays was 0.2 ml. Non-specific insulin or taurine binding was determined in the presence of 50 μg unlabeled insulin or taurine respectively. The final concentration of the unlabeled insulin was 42 μM; and the final concentration of the unlabeled taurine was 2 mM.

Gel exclusion chromatography of the insulin receptor was performed on a column (1.5 × 90 cm) of Sepharose 6B previously equilibrated with Krebs–Ringer bicarbonate buffer, pH 7.5, containing 1.5% Triton X-100. The column was calibrated with the following proteins of known Stokes radii: thyroglobulin, apoferritin, gamma globulin and bovine serum albumin. The receptor was exposed to either [^{125}I]insulin or [^{3}H]taurine prior to application on the column. Fractions (2 ml) were eluted from the column and assayed for radioactivity by crystal scintillation counting for [^{125}I]insulin or liquid scintillation counting for [^{3}H]taurine.

Values reported are means ± SEM. Significance is listed as $P \leq 0.05$ for three to five measurements per assay point. [^{125}I]Insulin (120 μCi/μg) was prepared according to the method of Cuatrecasas and Hollenberg [14]. [^{3}H]Taurine (20 Ci/mmol; 19 cpm/fmol at 50% efficiency) was obtained from New England Nuclear. Chemical purity was verified by thin-layer chromatography. *Ricinus communis* was purchased from Vector Laboratories. Human polyclonal antibody to the human insulin receptor (Bb) was supplied by Dr. R. Kahn of the Joslin Research Clinic. The remainder of the chemicals and reagents were purchased from Sigma Chemical or Fisher Scientific.

RESULTS

The binding of insulin to the purified human insulin receptor is shown in Fig. 1A. A Scatchard plot of these data demonstrates the characteristic pattern that is indicative of negative cooperativity. The apparent dissociation constant was calculated from these data to be 1.2×10^{-9} M (Fig. 1B). These data are comparable to similar binding isotherms for the interaction of insulin with its receptor [11].

The binding isotherms for [^{3}H]taurine were performed on the same receptor preparations and under identical conditions to those used to determine insulin binding. The taurine binding data (Fig. 2A) were corrected for non-specific binding. These data reveal that taurine binding was specific and saturable. A Scatchard analysis of these data reveals that binding exhibited (1) positive cooperativity, (2) an apparent K_d equal to 130×10^{-9} M, and (3) a B_{max} of 1.6 nmol/mg receptor protein (Fig. 2B).

Under the conditions used to prepare the receptor, exposure of the receptor to insulin leads to subunit dissociation. Preincubation of the receptor with [^{125}I]insulin prior to chromatography on Sepharose 6B resulted in a single insulin binding peak that had a distribution coefficient of 0.53 that corresponded to a Stokes radius of 38 Å (Fig. 3). The second peak represents the unbound [^{125}I]insulin. A similar chromatographic pattern was obtained when the receptor was incubated with [^{3}H]taurine. Both of these radiolabeled ligands could be displaced from the receptor by the addition of unlabeled insulin or taurine prior to adding the sample to the column. These data suggest that taurine binds to the same receptor subunit as insulin.

Since taurine and insulin both interact with the purified insulin receptor, we conducted radiolabeled binding experiments to determine if insulin could displace taurine binding from the purified soluble receptor. The data presented in Table 1 show that radiolabeled taurine can be displaced from the receptor by unlabeled taurine and insulin.

To further establish that taurine binds to the insu-

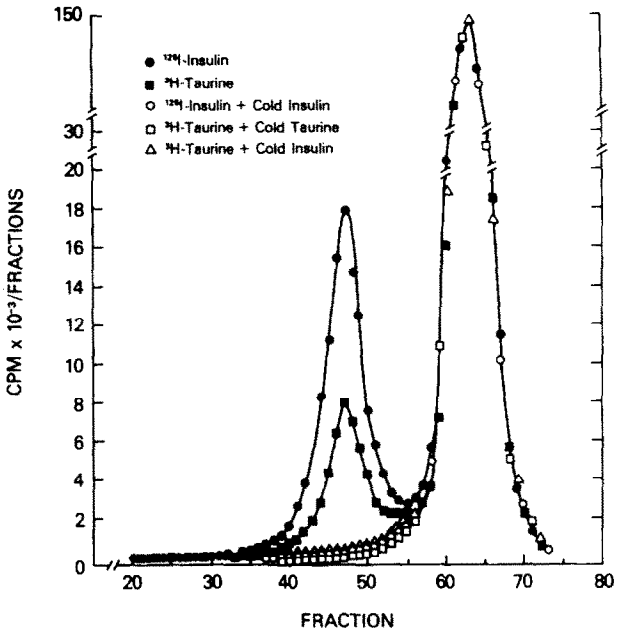

Fig. 3. Chromatogram of taurine and insulin binding to the insulin receptor. Insulin binding material was purified from membranes of fresh term placenta using sequential chromatography on DEAE-cellulose, wheat germ agglutinin, *R. communis*, and insulin-agarose affinity column chromatography. Under these conditions, insulin exposure leads to subunit dissociation of the insulin receptor. Aliquots of the insulin binding material were incubated with 8×10^{-8} M iodinated insulin in the presence and absence of 50 μg native insulin prior to chromatography on a 1.5×85 cm column of Sepharose 6B equilibrated with Krebs–Ringer bicarbonate buffer, pH 7.5, containing 0.1% Triton X-100. Aliquots were also incubated with 9×10^{-10} M [^{3}H]taurine in the presence and absence of 50 μg native taurine or native insulin and chromatographed. Fractions were assessed for radioactivity by solid crystal scintillation for [^{125}I]insulin or liquid scintillation for [^{3}H]taurine.

lin receptor, we decided to examine the binding of taurine and insulin to the receptor in the presence or absence of anti-human insulin receptor sera (Table 2). Preincubation of the receptor in the presence of the anti-human insulin receptor sera was able to inhibit insulin binding to the receptor. When compared to a normal IgG control, the anti-sera also inhibited more than 98% of the taurine from binding to the receptor.

In assessing the specificity of taurine for the receptor, we examined the ability of three common taurine analogues to displace taurine binding from the receptor (Fig. 4). In this set of experiments, we incubated equal molar concentrations of hypotaurine, cysteic acid, and beta-alanine with the taurine–receptor complex. In addition to unlabeled taurine being able to displace the taurine binding from the receptor, another sulfonic acid, cysteic acid, that is a precursor of taurine reduced taurine binding by approximately 66% of control and hypotaurine, the sulfinic acid analogue of taurine, produced a similar inhibitory effect. However, beta-alanine, the carboxylic acid derivative of taurine, had only a slight inhibitory effect on taurine binding. Because taurine binds to the receptor, and cysteic acid and hypotaurine were found to displace taurine binding while beta-alanine had little effect on taurine binding, it appears that the sulfur-containing acid group on the alpha-carbon is responsible, at least in part, for the interaction of taurine with the insulin receptor.

Table 1. Displacement of taurine from the purified insulin receptor

	Taurine bound (fmol/0.2 ml)
[^{3}H]Taurine	449 ± 19
+ Unlabeled taurine	135 ± 10*
+ Unlabeled insulin	121 ± 6*

Samples of the purified human placental insulin receptor, which migrated with a K_{av} of 0.53, were assayed for specific binding of the [^{3}H]taurine in the presence or absence of 50 μg unlabeled insulin or taurine. Results are the means of quadruple determinations ± SEM.

* Significance, as determined by Student's *t*-test, is $P < 0.05$.

Figure 5 shows that the binding of taurine to the purified insulin receptor was pH dependent. The pH profile was bell shaped, and maximum binding occurred at pH 7.5. The pH dependency suggests that the hydrogen ion concentration of the buffer affects taurine binding.

DISCUSSION

These studies describing the interaction of taurine with the insulin receptor provide the first evidence at the cellular level that may explain the hypoglycemic

Table 2. Anti-human insulin receptor antibody displacement of binding from the insulin receptor

	N	Normal IgG (fmol bound/0.2 ml)	Anti-human insulin receptor antibody (fmol bound/0.2 ml)
Insulin	4	35.5 ± 7.0	5.16 ± 0.2*
Taurine	4	240.1 ± 11.7	3.4 ± 0.9*

Samples of purified insulin receptor were treated for 16 hr at 4° with a 1:30 dilution of undiluted human anti-insulin receptor sera. Protein A was added to a final concentration of 20%. Precipitates were removed by centrifugation, and the supernatant fractions were assayed for specific insulin and taurine binding as described in Materials and Methods. Controls were performed using normal IgG. Values are means ± SEM.

* Significance, as determined by Student's *t*-test, is $P < 0.05$.

effect of pharmacological concentrations of taurine. The binding of taurine to the insulin receptor that was purified from human placenta was specific, saturable and reversible and exhibited positive cooperativity. The apparent K_d was calculated to be 130×10^{-9} M, and the B_{max} was determined to be 1.6 nmol/mg protein. Because of the ability of taurine to displace insulin binding and vice versa, taurine appears to act as an insulin agonist. In addition, the sulfonic and sulfinic acid analogues of taurine, cysteic acid and hypotaurine, also were able to displace taurine binding from the insulin receptor.

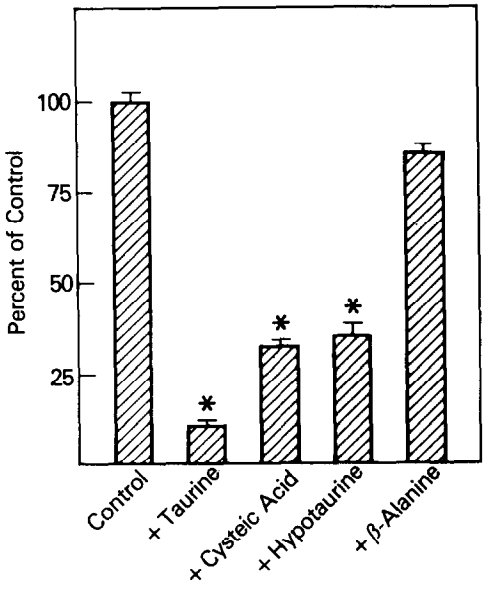

Fig. 4. Displacement of taurine binding from the insulin receptor by taurine analogs. Sepharose-insulin purified insulin receptor (0.1 μg) was incubated with 17.2 pmol/0.2 ml [^{3}H]taurine. After equilibrium, the taurine receptor complex was incubated in the presence of 50 μg unlabeled taurine, hypotaurine, cysteic acid or beta-alanine for an additional 10 min. Taurine binding was determined as described in Materials and Methods. Maximum taurine binding was 34,839 cpm. Values are expressed as mean ± SEM of three samples. Key: (*) significantly different from control ($P \leq 0.05$).

Not only is taurine an apparent insulin agonist for binding to the insulin receptor, but taurine also appears to mediate several of the physiological actions of insulin. In particular, taurine has been shown (1) to reduce blood glucose levels [2–6]; (2) to stimulate glucose uptake into cells [7, 8]: (3) to enhance glycolysis [9, 10] and glycogen production [10]; and (4) to stimulate amino acid uptake (unpublished data from our laboratories). These actions of taurine are consistent with the observed effects of the interaction of insulin with its receptor on cell membranes.

The data presented in this manuscript are the result of using pharmacological concentrations of taurine (86 nmol/L) to determine its binding properties to isolated purified human insulin receptor.

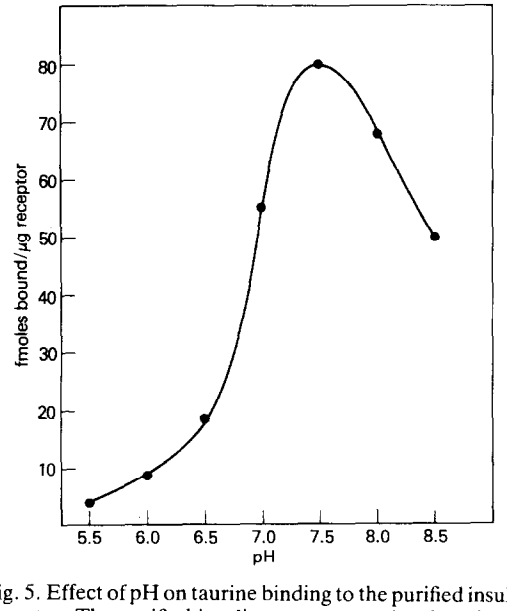

Fig. 5. Effect of pH on taurine binding to the purified insulin receptor. The purified insulin receptor was incubated with [^{3}H]taurine (17.2 pmol/0.2 ml) in 50 mM Tris, 0.03% Triton X-100, 0.5 M NaCl at 24° at the various pH levels listed. Samples were analyzed for taurine binding as described in Materials and Methods.

The concentration of taurine used in these isolated experiments was less than the reported amount of taurine that is present in the circulation. Human plasma or serum has been reported to contain 25–150 μmol/L of taurine. Because, during sample preparation, taurine may be released from leukocytes and platelets, cells that contain high concentrations of taurine, the lower figure may represent a more accurate measurement of taurine in serum or plasma [1]. Furthermore, muscle tissue contains approximately 75% of all taurine in the human body, and muscle is a primary tissue for glucose utilization. The physiological significance of the ability of taurine to modulate blood glucose levels and mimic the cellular actions of insulin is unknown. Research efforts by us and other investigators are attempting to elucidate the mechanism of taurine action on cellular function. Further *in vivo* and *in vitro* research by our laboratories is focusing on a detailed analysis of the interaction of taurine with the insulin receptor. A comprehensive understanding of this interaction may provide additional insight into the cellular mechanism(s) of taurine actions.

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