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HYPOGLYCEMIC PROPERTIES OF TAURINE: NOT MEDIATED BY ENHANCED INSULIN RELEASE

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Abstract—Taurine (2-aminoethanesulfonic acid) has been shown to be a potent hypoglycemic agent in the Wistar Kyoto rat (WKY). Glucose and insulin levels were measured in serum at various times after glucose loading (400 mg/kg, i.p.). Pretreatment with taurine (200 mg/kg, i.p.) attenuated the rise in serum glucose levels at 0.5 hr after glucose administration. In addition, taurine also prevented the rise in serum immunoreactive insulin levels. The taurine analogue hypotaurine produced a similar inhibition in the rise of both serum glucose and insulin levels while β-alanine, the carboxylic acid derivative of taurine, was totally ineffective. The enhanced glucose clearance can be explained by an increase in deoxyglucose accumulation in skeletal muscle and liver. In the liver, a 50% increase in glycogen synthesis was observed. A possible interrelationship between taurine and insulin receptor is discussed.

Taurine (2-aminoethanesulfonic acid) is found in high concentrations in mammalian tissues [1]. This β-amino acid is found predominantly in excitable tissues such as heart, skeletal muscle, and nervous system. In addition, high concentrations of taurine are also found in the liver, where taurine is conjugated to form the bile salt, taurocholic acid. Despite recent intensive investigation of the physiological action of taurine, no other definitive roles for this sulfur-containing amino acid have been found.

However, evidence has been accumulating that taurine may play a role in carbohydrate metabolism. Hypoglycemic properties of taurine were first observed by McCallum and Sivertz in 1942 [2]. They reported that taurine was the most potent hypoglycemic sulfur-containing compound examined. In addition, taurine was shown by Donadio and Fromageot [3] to increase glucose utilization by the rat diaphragm. These results suggested that the hypoglycemic action of taurine on blood glucose levels is due to increased glucose uptake. Dokshina *et al.* [4] also reported a similar insulin-like action for taurine, which they believed to be mediated through 3′,5′-cAMP. In addition, an anti-diabetic effect of taurine was reported in alloxan or ditisone diabetic rats and rabbits [5] and in streptozotocin-induced hyperglycemia in mice [6].

Contrary to these observations, several other investigators have not been able to show an effect on glucose utilization. Ray and Rolek [7] reported that taurine did not alter significantly blood glucose concentrations but it did prevent ouabain-induced hypoglycemia. Furthermore, neither glucose utilization in the cerebral cortex [8] nor hepatic mitochondrial respiration [9] was altered following taurine treatment.

In this paper, we present evidence that taurine has hypoglycemic properties in WKY rats. Even more important is the observation that taurine concomitantly prevented an increase in serum insulin levels in response to glucose. These results suggest that the hypoglycemic action of taurine may be related to a direct action of taurine on both liver and muscle plasma cell membranes at or near the insulin receptor.

MATERIALS AND METHODS

Male Wistar Kyoto rats were bred at the Veterinary Resources Branch, National Institutes of Health, Bethesda, MD. The animals were maintained in laminar flow units on a 12 hr light–dark cycle at 24° and constant humidity. Food and water were available *ad lib*. Prior to the day of experimentation, animals were deprived of food for 18 hr.

Glucose and insulin tolerance tests were performed on fasted WKY rats. Animals were pretreated with taurine (200 mg/kg, i.p.) or vehicle, 30 min before injection of glucose (400 mg/kg, i.p.). At the appropriate times, animals were killed and blood was collected. The serum was obtained following centrifugation and assayed for glucose and immunoreactive insulin. Serum glucose was determined by the glucose oxidase method [10], and insulin immunoreactivity was measured by a double antibody radioimmunoassay by Roche Biomedical and Analytics Laboratories, Columbus, OH.

Glucose distribution in the various tissues was determined by the use of [^{3}H]deoxyglucose. Fasted animals were pretreated with taurine (200 mg/kg, i.p.). Thirty minutes later, [^{3}H]deoxyglucose (800 mg/kg, 0.7 μCi/mmole, i.p.) was administered. Animals were killed 1 hr later, and the appropriate tissues were excised, rinsed in saline, and immediately frozen on dry ice. The tissues were weighed and digested with Protosol at 55°. Scintillation fluid was added to the digests, and samples were counted for radioactivity.

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Determination of liver glycogen synthesis was assessed by measuring [^{3}]Hglucose incorporation into glycogen [11]. Thirty minutes after receiving injections of taurine (200 mg/kg) or vehicle, fasted Wistar Kyoto rats were injected with [^{3}H]glucose (800 mg; 21 Ci/mmole). Animals were killed 1 hr later and livers were removed. A sample of the liver was weighed and digested in a steam bath with 30% KOH. The glycogen was precipitated following the addition of sodium sulfate and 95% ethanol. The precipitate was collected by centrifugation and washed twice with ethanol, and the final precipitate was suspended in H_2O. Scintillation fluid was added to the glycogen solution, and the sample was counted for radioactivity.

RESULTS

The effect of taurine on serum glucose levels is shown in Fig. 1. The fasting glucose levels in the WKY rats were approximately 120 mg/100 ml. Intraperitoneal injections of glucose (400 mg/kg, i.p.) produced a significant rise in serum glucose within 0.5 hr. Taurine attenuated the initial rise in serum glucose levels at the 0.5 hr time point. Baseline values were the same for control and taurine-treated animals at all other time points.

Corresponding to the initial rise in serum glucose levels, there was a rise in serum immunoreactive insulin in control animals (Fig. 2). In this experiment there was a five unit increase in immunoreactive insulin 0.5 hr after glucose injection. By contrast, taurine prevented this increase in serum insulin at both 0.5 and 1.0 hr. At all other time points no significant changes were observed between control and taurine-treated animals.

We decided to determine if this increase in glucose uptake without a corresponding rise in insulin levels produced by taurine could be mimicked by equal molar dosages of the taurine analogues, hypotaurine and β-alanine. These results are shown in Table 1. Hypotaurine, the sulfinic acid precursor of taurine, was equally as potent as taurine in inhibiting the rise in both glucose and insulin levels at the 0.5 hr time point. However, β-alanine, the carboxylic acid analogue of taurine, failed to inhibit the rise in glucose and insulin at the same time point.

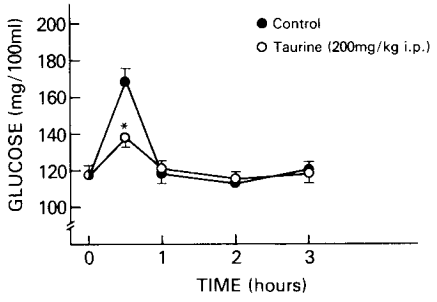

Fig. 1. Effect of taurine on glucose tolerance in fasted WKY rats. One-half hour after injection of rats with taurine (200 mg/kg, i.p.), the animals were injected with glucose (400 mg/kg, i.p.). At the appropriate times the animals were killed, and serum was collected for determination of glucose levels. Values are means ± S.D. of five animals per time point. Significance (*) is reported as $P < 0.05$.

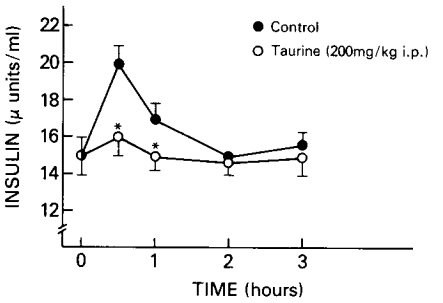

Fig. 2. Effect of taurine on insulin tolerance in fasted WKY rats. One-half hour after injection of rats with taurine (200 mg/kg, i.p.), the animals were injected with glucose (400 mg/kg. i.p.). At the appropriate times the animals were killed, and serum was collected for determination of insulin levels. Values are means ± S.D. of five animals per time point. Significance (*) as determined by Student's *t*-test, is $P < 0.05$.

The increased serum clearance of glucose suggests that there should be an increase in glucose content within certain tissues. To determine which major tissues showed increased accumulation of glucose, we injected [^{3}H]deoxyglucose into control and taurine-pretreated animals. The animals were killed and tissues were removed 1 hr after [^{3}H]deoxyglucose injection. This time point was chosen because serum glucose was at control levels (Fig. 1). Table 2 shows that of the ten tissues examined taurine significantly increased [^{3}H]deoxyglucose accumulation in only two tissues, the liver and skeletal muscle. In the liver we observed almost a 3-fold increase in [^{3}H] deoxyglucose uptake while in skeletal muscle a 40% increase in deoxyglucose accumulation was observed.

To confirm the observation that taurine stimulated glucose accumulation in the liver, we examined the ability of taurine to increase glycogen synthesis in the fasted WKY rat (Table 3). We can see that a single dose of taurine (200 mg/kg, i.p.) was able to stimulate a rise in liver glycogen of approximately 50%.

Table 1. Changes in plasma glucose and insulin levels from basal levels produced by taurine analogues in WKY rats*

	Dosage (mg/kg, i.p.)	N	Glucose (mg/100 ml)	Insulin (units)
Control		5	83 ± 10	10 ± 1
Taurine	200	5	68 ± 8	1 ± 1
Hypotaurine	175	5	54 ± 13	1 ± 3
β-Alanine	143	5	104 ± 11	12 ± 2

* Thirty minutes prior to injection of glucose (800 mg/kg, i.p.) in fasted WKY rats, taurine or taurine analogues were injected at equal molar dosages. The animals were killed 0.5 hr after glucose injection, and plasma was collected for glucose and insulin determinations as described in Materials and Methods. Values reported are the differences observed from basal levels.

Table 2. Effect of taurine on [^{3}H]deoxyglucose uptake in fasted WKY rats*

Tissue	[^{3}H]Deoxyglucose uptake Control	Taurine-treated
Adrenals	21,250 ± 2,532	19,375 ± 3,727
Brain	74,759 ± 16,487	83,695 ± 9,480
Gastrocnemius	1,542 ± 204	2,164 ± 378†
Heart	613 ± 25	688 ± 33
Kidney	20,167 ± 1,478	17,016 ± 1,122
Liver	771 ± 71	2,097 ± 522†
Lung	633 ± 96	725 ± 130
Pancreas	2,400 ± 100	2,286 ± 195
Spleen	1,805 ± 260	1,325 ± 142
Serum	11,302 ± 3,128	12,247 ± 3,346

* Fasted WKY rats were injected with taurine (200 mg/kg) or vehicle. One-half hour later, the animals were injected with [^{3}H]deoxyglucose (800 mg/kg). The animals were killed 1 hr later, and the various tissues were removed, rinsed in physiological saline, and frozen until assay. The tissues were weighed and digested with Protosol. Scintillation fluid was added to the solubilized tissue, and samples were counted for radioactivity. Data are expressed as cpm/g tissue or cpm/m serum. The values represent the means ± S.E.M. of five animals per group.

† Significance according to Student's *t*-test is $P < 0.05$.

DISCUSSION

The present studies indicate that taurine acts at the level of the cell membrane to produce its hypoglycemic effects in Wistar Kyoto rats. The rise in serum glucose produced by a bolus injection of glucose was attenuated significantly following taurine pretreatment. This enhanced taurine-mediated glucose clearance from the serum appears to be associated with increased glucose accumulation in skeletal muscle and liver. Furthermore, we showed that in the liver the increased glucose uptake can be translated in enhanced glycogen synthesis. These observations are consistent with other investigators, who reported a hypoglycemic action for taurine [2–9]. In addition, the liver and skeletal muscle are the same tissues shown by Silaeva *et al.* [5] and Donadio and Fromageot [3], respectively, to increase glucose uptake. Furthermore, no significant changes were observed in the brain, these results are consistent with the findings of Laborit and Thuret [8] who reported that, in the cerebral cortex, taurine had no effect on glucose utilization, lactate production, or oxygen consumption. However, taurine in the presence of insulin has been reported to stimulate glycolysis, glycogenesis and oxygen utilization in the perfused rat heart [12]. These latter results are consistent with our findings since *in vivo* the liver and skeletal muscle are the primary tissues which have glucose uptake.

Though previous *in vivo* studies in normal animals examined the hypoglycemic effect of taurine, they neglected to examine the effects of taurine on serum insulin levels. In our acute studies, we found that taurine not only inhibited the rise in serum glucose levels but also prevented the rise in serum insulin. Following chronic taurine administration in streptozotocin-induced diabetic mice, taurine was found to suppress the hyperglycemia due to pancreatic β-cells damage without significantly altering serum immunoreactive insulin [6]. These data suggest that the action of taurine is unrelated to a direct stimulation of pancreatic insulin release.

Since taurine enhances glucose uptake without increasing serum insulin, it appears that taurine may have a direct action on the cell membrane to enhance glucose transport. Dokshina *et al.* [4] have suggested that the insulin-like action of taurine is mediated through cAMP. However, the mechanism of action for insulin does not appear to be mediated through cyclic nucleotides [13]. Alternatively, several lines of evidence suggest that taurine may act directly on the insulin receptor to facilitate glucose uptake. First, Kulakowski *et al.* [14] have identified a low affinity taurine binding protein, which, like the insulin receptor, is a glycoprotein and whose molecular weight corresponds to the 135,000 dalton subunit of the insulin receptor. Second, hypotaurine competes for taurine binding and also stimulates glucose uptake while β-alanine has no effect on either system [14, 15]. Third, in the isolated perfused rat heart, taurine has been shown to potentiate the actions of insulin [12]. Fourth, taurine binding to isolated rat heart sarcolemma is inhibited by insulin [16]. Finally, preliminary observations have shown that taurine interacts with the purified insulin receptor [17].

Table 3. Effect of taurine-stimulated glycogen synthesis in fasted WKY rats*

	N	[^{3}H]Glucose incorporation (cpm/g liver)
Control	4	7,374 ± 830
Taurine	4	10,787 ± 477†

* Fasted WKY rats were injected with taurine (200 mg/kg) or vehicle. One-half hour later [^{3}H]glucose (800 mg/kg) was injected into the animals. One hour later a segment of the liver was removed and frozen on dry ice. A sample was weighed and assayed for liver glycogen as described in Materials and Methods. Values reported are means ± S.E.M.

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

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