The role of taurine in the pathogenesis of the cardiomyopathy of insulin-dependent diabetes mellitus

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Abstract

The cellular and molecular physiology and pathology of insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM) are mostly studied and understood through the use of animal models. Fundamental differences between the IDDM and NIDDM animal models may help to explain the etiology behind diabetic cardiomyopathy, one of the most severe complications of IDDM. Experimental rat models of IDDM exhibit a characteristic increase in tissue levels of taurine in the heart, a change that is not seen in NIDDM rats. This article deals with the causes and possible consequences of this observation which may contribute to the development of diabetic cardiomyopathy. Modulation of pyruvate dehydrogenase (lipoamide) (PDH; EC 1.2.4.1) activity was found to be a possible mode for taurine involvement. PDH is a mitochondrial protein and is the rate-limiting step in the generation of acetyl CoA from glycolysis. In IDDM, PDH activity is decreased through a mechanism that includes the stimulation of the de novo synthesis of a kinase activator protein (KAP) which phosphorylates PDH and inactivates the enzyme. This lesion does not occur in NIDDM rat hearts. Taurine is known to inhibit the phosphorylation of PDH in vitro, and in taurine-depleted rats PDH phosphorylation is known to increase. Thus, the increased levels of taurine in the diabetic heart may be inhibiting this phosphorylation which in turn may be stimulating the synthesis of KAP through a negative feedback process. The main argument for this theory would be the lack of change in both the taurine levels and the activity of PDH in the NIDDM rat model.

Keywords: Cardiomyopathy; Diabetes; Energy metabolism; Protein phosphorylation

1. Introduction

Diabetes mellitus is a heterogeneous pathological condition involving altered metabolism of lipids, carbohydrates and proteins. It is identified by clinical hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Many of the complications ascribed to diabetes mellitus, such as renal failure, cardiomyopathy, vascular damage and visual degeneration, have been attributed to chronic hyperglycemia. In 1979, the National Diabetes Data Group of the National Institutes of Health authored a system of classification and diagnosis for diabetes mellitus and other similar pathologies of glucose intolerance [1]. The most important category is composed of those hyperglycemic conditions not secondary to other pathologies, which are commonly associated with the term diabetes mellitus: (1) insulin-dependent diabetes mellitus (IDDM) or Type I diabetes, formerly known as juvenile diabetes, juvenile onset-diabetes, juvenile-onset-type diabetes, ketosis-prone diabetes or brittle diabetes; and (2) non-insulin-dependent diabetes mellitus (NIDDM) or Type II, formerly known as adult diabetes, maturity-onset diabetes, maturity-onset-type diabetes, ketosis-resistant dia-
betes or stable diabetes. IDDM and NIDDM are probably the most dangerous forms of the disease and are definitely the most extensively researched. IDDM is best characterized by the severe depletion of insulin and generally results in the most severe complications among all the diabetic syndromes.

In 1997, a new classification for diabetes mellitus which endeavored to refine and eliminate the ambiguities of the original system was proposed [2]. In the new system, the use of the terms IDDM and NIDDM was discouraged and arabic numerals were used to replace the roman numerals for Type 1 and Type 2. However, for the purpose of this review, we will use the terms IDDM and NIDDM as many of the references originally used these terms, especially for the description of the animal models that were utilized.

Animal models of diabetes involve genetically diabetic animals and animals treated with drugs that compromise the ability of the pancreas to produce insulin [3]. The BB rat is a Wistar rat mutation, around 30% of which exhibit hyperglycemia, hypoinsulinemia and ketoadidosis. Human IDDM was found to be similar to this acute diabetic syndrome, and thus, the BB rat has been used in many diabetic studies. Alloxan and streptozotocin (STZ) are diabetogenic agents used frequently to induce diabetes in experimental animals.

Taurine (2-aminoethanesulfonic acid) is a free amino acid found in millimolar concentrations in all mammalian tissues [4]. Taurine levels in the heart were consistently found to be between 20 and 26 μmol g⁻¹ wet weight [5–7], values that suggest taurine concentration levels of at least 20 mM within the heart. Among tissues sampled from control rats, taurine levels were found to be highest in heart tissue, with lung, spleen, kidney, adrenal, brain and liver tissues exhibiting decreasing levels, respectively [5]. Taurine levels appeared to be particularly low in the liver as compared to the heart (<10%). Goodman and Shihabi [7] reported very similar findings, with skeletal muscle also exhibiting lower taurine levels than the heart and the liver with the least amount of taurine compared to all tissues studied. The trend is significant in that the role of taurine in terms of cellular physiology and pathophysiology may then be more important in the heart than in the other tissues, specifically the liver. Among others, taurine is thought to produce important physiologic effects through osmoregulation, calcium modulation and phospholipid interaction [3]. Taurine is thought to modulate the movement of ions across the sarcolemma, particularly sodium, providing some protection against abrupt ion balance changes that may lead to cell damage [8]. It has also been reported that taurine modulates protein phosphorylation and Ca²⁺ movement in heart tissue [9,10].

This review aims to study the possible role taurine may play in the physiology and pathology of the diabetic heart. Cardiovascular diseases represent the most devastating prognoses in diabetes mellitus [11,12]. Diabetes mellitus manifests a plethora of acute and chronic cardiac complications which are debilitating and even fatal. The most important complication is accelerated atherosclerosis which results in myocardial infarction, stroke, and gangrene. In fact, about 20% of diabetic deaths are due to myocardial infarction. There are many types of cardiac complications in diabetes mellitus, and for this review, we will limit ourselves to the discussion of diabetic cardiomyopathy, itself a major diabetic pathology. Specifically, the cardiomyopathy of IDDM and the possible involvement of taurine in its pathogenesis will be discussed.

### 2. Taurine and energy metabolism

The regulation of energy metabolism is impaired in the diabetic heart and may be related to the development of diabetic cardiomyopathy [13–15]. The physiologic role of taurine in the pathogenesis of heart disease, whether in the diabetic state or in other pathologic conditions, has not been adequately addressed in the scientific literature. There are a few interesting experimental studies in which taurine can be easily observed to have a significant modulatory function in cardiac energy utilization.

Lampson et al. [16] performed a series of experiments which used taurine as a potentiating factor of the in vitro insulin effects on the control heart. Taurine was shown to potentiate (Table 1) the stimulatory effects of insulin on glucose utilization at virtually every concentration of insulin that was tested. In these experiments, a time- and concentration-dependent increase in glucose utilization was observed with taurine in the presence of 2.5 U 1⁻¹ insulin. The investigators suggested that this increase was due to the increased activity of 6-phosphofructokinase (PFK; EC 2.7.1.11), the rate-limiting enzyme in glycolysis. Taurine was reported to have no direct effects on the enzyme, and so the increase in PFK activity was attributed to the effects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Insulin-dependent diabetes</th>
<th>Perfusion: Insulin treatment (vs. control rats)</th>
<th>Perfusion: Taurine depletion (vs. control rats)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose utilization/glucose oxidation</td>
<td>Decreased</td>
<td>Increased (effect potentiated by taurine)</td>
<td>Increased</td>
</tr>
<tr>
<td>Lactate production</td>
<td>Decreased</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Citrate levels</td>
<td>Increased</td>
<td>not measured</td>
<td>Decreased</td>
</tr>
<tr>
<td>6-Phosphofructokinase activity</td>
<td>Decreased</td>
<td>Increased</td>
<td>Increased</td>
</tr>
</tbody>
</table>

Table 1

Heart metabolism under various conditions [16,17,40]
of taurine on insulin activity. Apparently as a consequence of the increased glucose oxidation, a corresponding increase in lactate production was also observed. These changes occurred without a large increase in contractile function.

A second study to determine the effects of taurine on cardiac metabolism was accomplished by partially depleting rats of their tissue levels of taurine (Table 1) [17]. In these experiments, control rats were partially depleted of taurine through the in vivo administration of guanidinoethylsulfonate (GES) or β-alanine [18,19]. After 3 weeks of treatment, taurine levels in the heart were reduced by 50% in these control rats and metabolic rates were observed to be significantly altered. For example, the rate of glycolysis was significantly stimulated, and lactate and pyruvate production were also increased (50%). Measurements of various glycolytic intermediates suggested that the increase in glycolysis was due to the increased activity of PFK. Mozaffari et al. [17] also found that citrate levels had decreased (Table 1), and the increased activity of PFK was attributed to this decrease, in as much as PFK is known to be inhibited by citrate. Other explanations, though, are certainly possible. These two studies [16,17] demonstrated that taurine probably has an important endogenous role in the modulation of energy metabolism in the control heart.

### 3. Pyruvate dehydrogenase (lipoamide) (PDH; EC 1.2.4.1) activity and the development of diabetic cardiomyopathy

PDH is a mitochondrial enzyme that catalyzes the key irreversible step (pyruvate→acetyl CoA) in carbohydrate oxidation. Interestingly, PDH activity is known to be reduced in IDDM hearts [20]. The decrease in PDH activity is observed both in drug-induced IDDM rats and in genetic strains of diabetic rats [21–23]. Consequently, this decrease in PDH activity leads to abnormalities in energy metabolism which impact greatly on the development of diabetic dysfunction in diabetes. The proportion of active PDH, i.e. the dephosphorylated enzyme, decreases in IDDM as the phosphorylation of the enzyme by [pyruvate dehydrogenase (lipoamide)] kinase (PDH kinase; EC 2.7.1.99) increases [24,25]. Diabetic hyperlipemia, specifically the elevation of plasma-free fatty acid (FFA), has been identified as the main culprit responsible for the decreased PDH activity [Fig. 1(A)]. A cascade of events thus follows. The increase in β-oxidation [Fig. 1(B)] of FFA produces higher ratios of acetyl CoA/CoA, NADH/NAD⁺ and ATP/ADP which allosterically stimulate PDH kinase [Fig. 1(C)]. Consequently, the phosphorylation of PDH is increased [Fig. 1(D)] and the enzyme is deactivated. In addition, the kinase responsible for the phosphorylation of PDH is inhibited by pyruvate, an inhibition enhanced by increased levels of ADP [26]. PDH phosphorylation is reversible through the activity of the [pyruvate dehydrogenase (lipoamide)]–phosphatase (PDH phosphatase; EC 3.1.3.43), an enzyme susceptible to stimulation by increased Ca²⁺ in the presence of magnesium [27].

PDH activity decreases in alloxan-treated rats and normalizes with in vivo insulin treatment [28,29]. This decrease is attributed mostly to an increase in PDH kinase activity [24,25], and insulin has been widely considered as an inhibitor of PDH kinase expression in IDDM [30]. Tonic inhibition by insulin probably involves the suppression of the PDH kinase gene as insulin is known to reverse the increase in PDH kinase mRNA and protein levels observed in rats treated with STZ [31]. In animal models of IDDM, cardiomyopathy has also been shown to be reversible with in vivo insulin therapy [32,33] and thus the observed cardiomyopathy has been largely attributed to insulin deficiency [13,30]. Metabolic changes resulting from insulin deficiency have been described to be major contributors to the pathogenesis of this cardiomyopathy [13,30]. Specifically, the downregulation of PDH activity in IDDM may be an important factor.

However, insulin treatment is thought to be only partially successful, if not inadequate, in the reversal of cardiac dysfunction in IDDM [34,35]. There are, in fact, some reports that demonstrate the lack of effect of insulin in treating the functional and metabolic deficits of the diabetic heart [36] or that prove that the benefits of in vivo insulin treatment are not due to direct myocardial effects [37]. In addition, diabetes-induced PDH lesions are known to persist even when substrate and insulin levels are...
4. Metabolic lesions in IDDM: systemic vs. phenotypic changes

As expected from a pathology primarily defined by metabolic derangements, a wide array of changes in both enzyme or transport activities and in the plasma milieu has been described in IDDM [15,20]. These changes can be thought of as either phenotypic alterations, as in the case of the former (changes in enzyme or transport activities), or as systemic abnormalities, as in the case of the latter (changes in plasma milieu). Both types of changes impact greatly on the metabolic pathology found in the IDDM heart. In general, systemic abnormalities, most specifically hyperglycemia but also including elevated circulating FFA, have been the main focus of the study of diabetic cardiomyopathy as many of the symptoms of the disease can be attributed to changes in metabolic substrate availability [13–15]. However, phenotypic changes also provide important clues to the understanding of the development of the disease, especially with respect to the role of taurine.

4.1. Systemic changes in IDDM: glucose oxidation and the citric acid cycle

It has been demonstrated that metabolic adaptation in the IDDM heart is a continuous prolonged process in that the metabolic changes in acute and in chronic diabetes are reported to be different [40]. The classical signs of IDDM, such as hyperglycemia and hyperketonemia (elevated levels of acetoacetate and 3-hydroxybutyrate), are observed in both acute and chronic rat models, but hyperlipemia (elevated levels of FFA, triacylglycerol and cholesterol) is evident only in chronically diabetic rats. Similarly, only with chronic diabetes are increases in 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) activity and decreases in succinate dehydrogenase (EC 1.3.99.1) activity reported, changes which should lead to an increase in fatty acid oxidation and cause impaired aerobic metabolism through the citric acid cycle.

In the IDDM heart, glucose oxidation has been reported to be consistently impaired (Table 1). This effect is probably due to decreased glucose flux through PFK [40]. In turn, this decrease in glucose oxidation is partly attributed to allosteric inhibition of PFK by increased levels of citrate, although other mechanisms of action may also play a role. It is interesting to note that changes in glucose utilization, PFK activity and citrate levels in the IDDM heart are the complete opposite of the changes observed in control rats that are depleted of taurine [17] (Table 1). Taking into consideration the insulin-potentiation effects of taurine [16] and the insulin-deficient nature of IDDM, the possibility arises that taurine in the IDDM heart may be involved in the development of these important metabolic lesions. While current data do not provide adequate answers to this question, it is clear that the role taurine performs relative to the metabolic lesions in the IDDM heart should be investigated. The specific phenotypic changes in PDH activity reported in IDDM hearts provide the best clues to the importance of taurine in the development of energy deficits in the diabetic heart and, in turn, in the pathogenesis of diabetic cardiomyopathy.

4.2. Kinase activator protein (KAP): phenotypic change in diabetes and reverse feedback mechanism

PDH kinase is tightly integrated into the PDH enzyme complex in the mitochondrial membrane [24,25]. Although it is clear that systemic abnormalities like hyperlipemia provide an acceptable explanation for the increase in PDH kinase activity found in IDDM, numerous studies demonstrate that some factor other than concentration ratios of acetyl CoA/CoA, NADH/NADH and ATP/ADP is involved [21,41]. Isolated mitochondria from diabetic hearts exhibit this increase in PDH kinase activity, obviously independent of allosteric modulation by metabolic substrates and cofactors [42,43]. Consequently, a phenotypic adaptation has been discovered to occur in IDDM that would also explain the decrease in PDH kinase activity. In the diabetic rat heart, it has been reported that a PDH kinase activator protein (KAP) is present and active in the high-speed supernatant of the mitochondrial fraction [43], a protein which is not found in control hearts [Fig. I(E)] [24,25].

This long-term regulation of PDH by KAP is interesting in that it appears to represent a classic feedback mechanism in reverse. Usually the activation or stimulation of an enzyme results in the downregulation of its activity. In the case of IDDM, systemic abnormalities have been definitively shown to activate PDH kinase, albeit transiently, and thus cause decreases in PDH activity. A normal feedback response would result in the downregulation of PDH kinase, but strangely, this is not the case. Instead, KAP is activated, producing a permanent metabolic lesion wherein PDH phosphorylation is increased and PDH is deactivated even more.

4.3. Molecular identification of KAP in the rat heart

PDH kinase has been identified as a heterodimer in the
bovine kidney, composed of a ~48 kDa subunit (α) and a 45 kDa subunit (β) [44]. Differential protein digestion procedures provided evidence that the kinase activity was specific to the α subunit. In the rat, however, the α and β subunits have been cloned using a rat heart cDNA library and both recombinant proteins were discovered to be active PDH kinase isofoms [45,46]. Subsequently, the α and β subunits were designated as PDH kinase I (PDK1) and PDH kinase II (PDK2), respectively. This difference in kinase activity between bovine and rat PDH kinase subunits may be a real phenotypic distinction between species or a result of differences in methodologies used.

Four PDK isoforms have been identified in human tissues and two isoforms have been discovered to be encoded from cDNAs which were almost identical with the DNA of rat PDKI and PDKII [47,48]. Another rat PDK isoform was discovered using the DNA of the human PDK4 isoform as a probe and was thus designated rat PDK4 isoform [49]. Probing various rat tissues with the cDNAs of rat PDK1, PDK2 and PDK4, and of human PDK3, the same study also determined that four distinct types of PDK mRNA are expressed in the rat, with mRNA of the four isoforms being differentially expressed in the rat tissues. The mRNA for PDK1 was almost exclusively expressed in the heart and the mRNA for PDK4 was predominantly expressed in skeletal muscle and heart. PDK2 mRNA was expressed in all tissues tested. PDK3 mRNA was found largely in the testis. Though both PDK2 and PDK4 mRNA were expressed in the liver, PDK2 expression was much more abundant than PDK4.

The N-terminal amino acid sequence of KAP, as induced by starvation in the rat liver, was reported by Priestman et al. [50]. The purified protein presented as a single band of Mr 45 kDa. Using N-terminal sequence analysis, this amino acid sequence was compared to the predicted amino acid sequence of the cloned p45 subunit (PDK2 isoform) [46]. The analysis concluded that the KAP isolated by Priestman et al. [50] was very similar if not identical to the p45 subunit (PDK2) cloned by Popov et al. from the rat heart [46].

However, it appears that this KAP activity measured in the rat liver after starvation may not be identical to the KAP activity that is expressed in the rat heart after induction of diabetes with STZ. It was discovered that in the rat heart, the increase in PDH kinase activity found in both starved and diabetic rats correlates to increases in the expression of PDK4, not PDK2, mRNA and protein [31]. It may be that, as the mRNA of the four PDK isoforms are unevenly distributed among different tissue types, the isoform identity of KAP activity expressed in the liver is different from the isoform responsible for the KAP activity measured in the rat heart. As only KAP from starved rat liver has been purified and sequenced, it is not known what the amino acid sequence of KAP from the diabetic rat heart is. Moreover, the present data do not preclude the existence of other isoforms of PDK and it is possible that KAP activity corresponds to an isoform of PDK so far unidentified.

5. Taurine levels in the diabetic heart

5.1. Taurine levels increase in the Type 1 insulin-dependent diabetes mellitus (IDDM) heart

Diabetic cardiomyopathy presents probably the most interesting case study of taurine involvement in a diabetic anomaly, mainly because of the unique response of taurine levels in IDDM animal models. Taurine levels in the heart, as well as in skeletal muscle, are observed to increase in Type 1 insulin-dependent diabetes mellitus (IDDM) animal models, in contrast to other organ systems studied (Table 2) [6,7,51–55]. In particular, taurine levels in the liver, eye and sciatic nerve either decreased or remained unchanged. Data for the kidney were ambiguous.

Alloxan- or STZ-treated adult rats exhibit fasting hyperglycemia and increased cardiac taurine content as early as 48 h after the start of diabetogenic drug treatment. Taurine levels rise at least 30% compared to control levels and are observed to be elevated even 55 days after STZ treatment. Thus, the increase in cardiac taurine levels in IDDM can be assumed to be significant and persistent. Whether this specific increase occurs in human diabetic conditions is currently unknown, but it is known that the decrease in plasma taurine levels observed in STZ-treated rats is also observed in human patients suffering IDDM and undergoing insulin therapy [56,57]. It has also been that reported that in these human subjects, the decrease in plasma taurine and the negative effects of IDDM on platelet aggregation are reversed by taurine supplementation in the diet.

Animal models of IDDM always exhibit reduced weight gain 21, 30 and 44 days after STZ treatment [40,54], suggesting reduced food intake and a semi-starved condition for the experimental animals. It is known that with 21-day-old non-diabetic rats which were fed a limited 7 g day⁻¹ stock diet, taurine levels in the heart increased (~80%) compared to rats fed ad libitum [58]. Similar findings were observed in skeletal muscle, kidney, spleen, liver and plasma. However, the heart exhibited the highest absolute change and the highest final levels in tissue taurine content, mainly because taurine levels were highest in the heart in the control animals as compared to the other tissues. Fasting data exhibited a similar increasing trend in taurine levels but of less statistical significance [59]. However, fasted rats are not a good representation of actual food intake found in IDDM models as diabetic rats in these models are fed ad libitum. Thus, taurine increases in the diabetic rat heart may be attributed in part to the limited food intake of the animals, although other factors are surely involved, too.
Table 2
Taurine levels in experimental rat models of IDDM [6,7,51–55]*

<table>
<thead>
<tr>
<th>Treatment, duration</th>
<th>Rat strain</th>
<th>Taurine supplementation</th>
<th>Tissue or sample</th>
<th>Experimental effect vs. control</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALX** 60 mg kg⁻¹, 48 h</td>
<td>SD†</td>
<td>None</td>
<td>Heart</td>
<td>Increased 30%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Skeletal muscle</td>
<td>Increased 20%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liver</td>
<td>Decreased 25%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Kidney</td>
<td>Decreased 40%</td>
</tr>
<tr>
<td>STZ* 75 mg kg⁻¹, 2 weeks</td>
<td>SD</td>
<td>None</td>
<td>Heart</td>
<td>Increased 48%</td>
</tr>
<tr>
<td>STZ 50 mg kg⁻¹, 55 days</td>
<td>SD</td>
<td>None</td>
<td>Plasma</td>
<td>Decreased 50%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Urine</td>
<td>(Decreased 38%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Heart</td>
<td>Increased 40%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Skeletal muscle</td>
<td>Increased 17%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Kidney</td>
<td>Decreased 52%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liver</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Plasma</td>
<td>(Decreased 19%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Urine</td>
<td>Increased 719%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Heart</td>
<td>Increased 34%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Skeletal muscle</td>
<td>Increased 26%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Kidney</td>
<td>Decreased 34%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liver</td>
<td>None</td>
</tr>
<tr>
<td>STZ 60 mg kg⁻¹, 40 weeks</td>
<td>SD</td>
<td>None</td>
<td>Renal tissue</td>
<td>Increased 70%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1% in drinking water</td>
<td>Renal tissue</td>
<td>Increased 240%</td>
</tr>
<tr>
<td>STZ 65 mg kg⁻¹, 20 days</td>
<td>Long Evans</td>
<td>None</td>
<td>Retina</td>
<td>(Decreased 17%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RPE‡</td>
<td>Decreased 54%</td>
</tr>
<tr>
<td>STZ 65 mg kg⁻¹, 21 days, 44 days</td>
<td>SD</td>
<td>None</td>
<td>Lens</td>
<td>Decreased 68%</td>
</tr>
<tr>
<td>STZ 60 mg kg⁻¹, 21 days</td>
<td>Wistar</td>
<td>None</td>
<td>Sciatic nerve</td>
<td>Decreased 32%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5% in rat chow</td>
<td>Sciatic nerve</td>
<td>None</td>
</tr>
</tbody>
</table>

* Values in parentheses with $P$ values $>0.05$.
** ALX, alloxan.
† STZ, streptozotocin.
‡ SD, Sprague Dawley.
§ RPE, retinal pigment epithelium.

5.2. Taurine levels are unchanged in the NIDDM heart

A rat model for Type 2 non-insulin-dependent diabetes mellitus (NIDDM) has been developed which produces diabetic rats exhibiting consistent normoglycemia with simultaneous glucose intolerance during adulthood [60,61]. Thus, these animals are radically different from their more common hyperglycemic IDDM counterparts. Theoretically, the drug regimen for producing the NIDDM model (diabeticogenic drug treatment is administered to neonatal rats instead of adult rats) allows for some degree of post-natal pancreatic development and, in turn, insulin secretion. The animals, in fact, are initially hypersecretory in response to a glucose challenge, and then there is a shift to a hyposecretory state sometime between 6 and 14 months of age which coincides with the development of insulin resistance [62]. These rats exhibit a more delayed onset of mechanical and metabolic cardiac lesions that are more similar to NIDDM than to IDDM [63]. Myocardial mechanical defects have been demonstrated to be due to the redistribution of the myosin isozyme content to the least active $V_3$ form and also to impaired Ca$^{2+}$ handling [64].

Interestingly enough, the tissue levels of taurine are not changed in every organ system studied in these NIDDM rats, including the heart (Table 3) (Schaffer, unpubl. data). Taurine measurements were performed approximately 12 months after the start of STZ treatment. Significantly, diabetic rats in this NIDDM model exhibited no difference in weight gain after 10–12 months as compared to control rats [65,66], implying adequate food intake. The data underscore two fundamental differences between the IDDM and NIDDM rat models, that of weight gain and
Table 3
Taurine levels in experimental rat model of NIDDM (12 months of treatment)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Taurine levels (μmoles taurine g⁻¹ wet weight, mean (S.E.M), N=4–5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>NIDDM</td>
</tr>
<tr>
<td>Muscle (gastrocnemius)</td>
<td>16.8±1.1</td>
</tr>
<tr>
<td>Kidney</td>
<td>8.2±0.5</td>
</tr>
<tr>
<td>Spleen</td>
<td>13.8±0.9</td>
</tr>
<tr>
<td>Liver</td>
<td>4.2±1.8</td>
</tr>
<tr>
<td>Heart</td>
<td>24.3±1.3</td>
</tr>
</tbody>
</table>

*Previously unpublished data.

Tissue taurine changes, and suggest that in diabetic rats the correlation between starvation and changes in taurine levels may be significant. These fundamental differences provide a comparative model by which the effects of taurine may be evaluated in diabetic animals.

6. The possible role of increased levels of taurine in the development of metabolic changes in the IDDM heart

6.1. Taurine modulates PDH phosphorylation

Lombardini [67] has demonstrated that taurine acutely inhibits the phosphorylation of a ~44 kDa protein in the rat heart mitochondria in vitro, and further identified the protein as PDH by isolation on PAGE gels and amino acid sequence analyses [Figs. 1(F) and 2(A)] [68]. Available data demonstrate that the phosphorylation process of the ~44 kDa protein is inhibited by calmodulin antagonism [69]. Also, it was determined by Lombardini that the phosphorylation of the mitochondrial ~44 kDa protein occurs exclusively on serine residues, similar to the phosphorylation of PDH by PDH kinase [70].

However, the mechanism behind the inhibition of PDH phosphorylation by taurine is as yet unknown. Increased PDH phosphatase activity would decrease PDH phosphorylation. PDH phosphatase is activated by Ca²⁺ [27] and taurine is known to stimulate Ca²⁺ uptake into the mitochondria [9]. However, in as much as the phosphorylation reaction was performed in Ca²⁺-free conditions [67], it is highly unlikely that taurine is acting in a Ca²⁺-dependent manner to modulate PDH phosphorylation. The short-term (6 min) in vitro phosphorylation conditions also eliminate the possibility of de novo synthesis of modulatory proteins. It is, thus, assumed that the action of taurine is direct and Ca²⁺-independent.

6.2. Classic feedback activation of KAP

In the IDDM heart, the levels of taurine increase as early as 48 h after treatment with diabetogenic drugs [Figs. 1(G) and 2(B)] [51]. The increased taurine levels provide an acute inhibitory effect on PDH phosphorylation within the same time frame as the expression of KAP activity. Thus, considering taurine inhibition of PDH phosphorylation as a focal point, the expression of KAP activity may actually be a classic feedback response [Figs. 1(H) and 2(C)].

The same phenomenon of increased PDH kinase activity has been observed in partially taurine-depleted rats (Fig. 2) [70]. This observation strengthens the idea that the feedback response to PDH kinase inhibition may lead to the induction of KAP activity (Figs. 1 and 2). Rats were partially depleted of their tissue levels of taurine through the use of GES, a taurine transport inhibitor [18,19]; heart taurine levels dropped by as much as 85%. Pyruvate production in the heart is also known to increase significantly in these rats (50%) [Fig. 2(G)] [17]. Under these conditions of partial taurine depletion, it was demonstrated that in vitro phosphorylation of the ~44 kDa protein (PDH) was markedly increased [Fig. 2(D)–2(F)], indicating increased PDH kinase activity, perhaps through the upregulation of KAP by a feedback mechanism. In this experimental design, it would not be the increased levels of taurine that would provide the PDH kinase inhibitory stress that results in the activation of KAP. Rather, it would be the increased levels of pyruvate known to exist in cases of taurine depletion [Fig. 2(G)] [17] that would provide allosteric inhibition of PDH kinase [Fig. 2(G)–2(H)] [26]. It is thus possible that in the partially taurine-depleted...
heart, KAP phosphorylation activity is induced as in IDDM, although the activity of KAP was not specifically studied and other explanations are certainly possible.

6.3. Semi-starved and NIDDM rat models: experimental evidence for the possible feedback stimulation of KAP by taurine

There are no direct data demonstrating taurine regulation of PDH kinase in the rat heart. Experiments designed to test taurine regulation of PDH kinase would entail the stimulation of taurine uptake into control rat hearts and the measurement of KAP activity. However, data from experiments using the fasted and NIDDM rat models indirectly support the idea that increased levels of taurine in the heart may be producing effects on the PDH activity in IDDM hearts.

During complete starvation, PDH kinase activity in the heart is increased acutely [30,71–73], a lesion that has been associated specifically with an increase in PDK4 gene expression [31]. Insulin deficiency is the most likely mechanism behind this effect as this model of starvation is associated with decreased expression of insulin receptors [30] and with at least an acute decrease in insulin levels [74]. The changes in PDH kinase activity in the hearts of semi-starved non-diabetic rats have not been studied, but the effect on PDH kinase activity may be similar to fasted rats. The IDDM animal model involves rats that are considered to be semi-starved, as previously discussed. Thus, it is possible that semi-starvation may be partly responsible for the changes in PDH kinase activity in the IDDM rat heart. This effect, in turn, may be dependent on the increases in taurine levels in the heart as semi-starvation is known to result in increases in tissue levels of taurine [58].

NIDDM rat hearts show no alterations in taurine content, as previously discussed (Table 3) (Schaffer, unpubl. data). The reasons for the lack of changes in taurine content are yet unclear, although normal food intake may be an important factor (discussed previously). The NIDDM rat is glucose intolerant; glucose transport and glycolysis are impaired. ATP synthesis, both from glucose and from palmitate, is also decreased. In the NIDDM model, metabolic and functional derangements correlate with the onset of insulin hyposcretion between 6 and 14 months after initiation of STZ treatment [62]. However, at 12 months, glucose flux through PDH remains unaffected and PDH activity remains normal [38]. The NIDDM model then indirectly supports the concept that increases in taurine content may be linked to the depression of PDH activity.

Thus, in this review, taurine is considered to be a significant factor in the development of diabetic myocardial metabolic lesions, specifically those of PDH and PDH kinase activity, warranting further studies of the role of taurine in diabetic cardiomyopathy and in diabetes in general.

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References


