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Effects of taurine in glucose and taurine administration*

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Summary. Taurine has several biological processes such as hypoglycemic action, antioxidation, detoxification, etc. To assess the effect of taurine administration on the guinea pigs with hyperglycemia, blood glucose, C-peptide levels together with morphologic alterations in the pancreatic ultrastructure were investigated in terms of hypoglycemic action and malondialdehyde and total sulfhydryl group levels with regard to oxidation-antioxidation relation. Animals were divided into four groups of six. Glucose supplementation group was administrated a single dose of glucose (400 mg/kg, i.p.) injection. Glucose and taurine supplementation group was administrated glucose treatment (a single dose, 400 mg/kg, i.p.) following taurine (a single dose, 200 mg/kg, i.p.). Taurine and glucose supplementation group was administered taurine treatment (a single dose, 200 mg/kg, i.p.) following glucose treatment (a single dose, 400 mg/kg, i.p.). Control animals received no treatment. Blood samples were collected at the end of the experiments for the determination of glucose, C-peptide (indicator of insulin secretion), lipid peroxidation (thiobarbituric acid reactive substances), and total sulfhydryl groups levels. Pancreatic tissue samples were then collected and processed for transmission electron microscopy. The findings showed that glucose supplementation following taurine administration significantly decreased blood glucose level by increasing C-peptide level and the pancreatic secretion stimulated morphologically and insignificantly changed thiobarbituric acid reactive substances and total sulfhydryl group levels. These observations suggest that taurine administration may be useful in hyperglycemia because of its hypoglycemic and protective effects.

Keywords: Taurine – Glucose – Lipid peroxidation – Pancreas

Introduction

Taurine (2-aminoethanesulfonic acid), a sulphur-containing β-amino acid, is the most prevalent free intracellular amino acid found in many human and animal tissues. The oldest known function of taurine is its conjugation with bile acids in the liver to form bile salts that are essential for fat absorption (Zelikovic and Chesney, 1989). It is known to play an important role in several biological processes, including inhibition of nerve impulses in marine invertebrates, energy storage in certain worms, elimination of cholesterol and regulation of excitability in various tissues. Several reports have suggested that taurine may have a vasodilatory effect and may increase heart rate in toads, frogs and rabbits (Dietric and Diacono, 1971). It has antioxidative action as well (Fang et al., 2002; Kaplan et al., 2003). It has been reported that it may be useful to use the dietary taurine in congestive heart failure to inhibit the degradative effect of free radicals (Kaplan et al., 1993). It also appears that this amino acid may induce moderate hypoglycemia in toads, frogs and rabbits possibly by potentiating the action of insulin (Dietric and Diacono, 1971). It has also affected the blood glucose level. C-peptide is produced in molar concentrations equal to that of insulin in the pancreas (Schlosser et al., 1987). In the present study, we determined C-peptide level, which is an index of insulin secretion. There is a considerable amount of taurine in the pancreas of rats, but its physiological roles in this organ are not entirely clear (Tokunaga et al., 1983). Taurine has both antioxidative and hypoglycemic actions. So lipid peroxidation formation (thiobarbituric acid reactive substances, TBARS) increases in some conditions with stress, such as fasting,

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injection stress, antioxidant demand of organism increases (Sardesai, 1995). Assessment of lipid peroxidation has included the analysis of lipid peroxides, isoprostanes, diene conjugates, and breakdown products of lipids. Among these products, malondialdehyde (MDA) is often used as a reliable marker of lipid peroxidation. Antioxidants (taurine, vitamin C etc.) and antioxidant enzymes (superoxide dismutase, catalase etc.) exert synergistic actions in scavenging free radicals (Fang et al., 2002; Pasantes-Morales et al., 1985). Major radical-trapping antioxidant potential in the human plasma is accounted for by ascorbate, urate, α-tocopherol, protein sulfhydryl groups (RSH), and bilirubin (Frei et al., 1988). Dincer et al. (1996) showed that the locally administered taurine significantly increased wound tensile strength by decreasing the MDA. We aimed to investigate the connection between blood glucose and lipid peroxidation levels with taurine administration in hyperglycemia. The present study was therefore carried out to investigate the effect of taurine administration on the glucose supplementation-induced hyperglycemia by determining the blood glucose level, C-peptide level, pancreatic ultrastructure in terms of hypoglycemic action and lipid peroxidation formation level, total sulfhydryl groups levels with regard to for oxidation-antioxidation relation.

Material and methods

This study was performed on 24 adult male guinea pigs (Cavia aperea porsellus) weighing 802 ± 25 g. All procedures were carried out in accordance with the 1975 Helsinki Declaration. The taurine used for the study was purchased from ICN Biomedical Inc., USA. The animals were divided into four groups of six. In the glucose supplemented group (G, n:6), glucose (a single dose, 400 mg/kg, i.p.) was administered. In this group, hyperglycemia occurred. In the glucose plus taurine group (G + T, n:6), glucose was administered intraperitoneally in a single dose (400 mg/kg) following taurine administration (a single dose, 200 mg/kg, i.p.). Thus, taurine after hyperglycemia was administrated in this group. In the taurine plus glucose group (T + G, n:6), taurine was administered intraperitoneally in a single dose (200 mg/kg) following glucose administration (a single dose, 400 mg/kg, i.p.). Therefore, hyperglycemia after taurine treatment occurred in this group. The control group (C, n:6), received no treatment. At the end of the experiments, each guinea pig in all four groups was anesthetized with pentobarbital sodium (0.03 mg/kg i.p., Abbott, Compoverde di Aprilia [LT] Italy). Blood samples were collected for the determination of glucose and C-peptide levels. Glucose level was determined using the glucose oxidase method (Bauer, 1982). C-peptide level was determined by insulin level measurement. It was assayed because guinea pig insulin cannot be assayed by using commercially available methods. Since guinea pig C-peptide has a primary structure similar to that of other mammals (e.g. humans), this peptide can be measured using a commercial assay method (Schlosser et al., 1987). C-peptide level was measured by competitive chemiluminescent enzyme immunoassay method (Identifier code: 1040, Catalog no: LKPE1). Blood glucose and C-peptide levels were immediately determined.

Lipid peroxidation was quantified by measuring the formation of thiobarbituric acid reactive substances (TBARS) in plasma (Kaplan, 1995). For the determination of plasma total sulfhydryl groups (RSH) were measured by the method of Kurtel et al. (1992). Blood was immediately centrifuged (Kurtel et al., 1992). The serum was stored in liquid nitrogen for subsequent use.

After blood collection, each animal was injected with an overdose of pentobarbital sodium anaesthesia. Upon opening the abdomen was opened, and the pancreas was removed. Tissue samples were immediately placed in 1:15 phosphate buffer containing 2.5% gluteraldehyde, and then postfixed to a 1% osmium tetroxide solution. The specimens were initially embedded in dodencenyl succinic anhydride, Araldite CY212 (1:1, v/v) and benzyldimethyl amine. An ultramicrotome was used to cut 1-μm (thick section) and 0.05-μm (thin section) sections from the blocks. The thick sections were stained with 0.5% toluidine blue for light microscopy, and the thin sections were stained with uranyl acetate and lead citrate for examination in a LEO 906E transmission electron microscope.

Statistical analysis

All values are reported as means $\pm$SE. The difference between groups were statistically analyzed using the Mann-Whitney U test. P value of < 0.05 was considered as significant.

Table 1. Serum glucose and C-peptide levels in the control and experimental groups

Groups	n	Serum glucose (mg/dl)	Serum C-peptide (ng/dl)
C	6	144.8 ± 4.4	46 ± 11
G	6	$222.8 \pm 20.6^{*}$	134 ± 0.31
G + T	6	$166 \pm 13^{\circ}$	$306 \pm 26^{*,\circ}$
T + G	6	218.6 ± 12.5	$170 \pm 19^{*,\oslash}$

$P < 0.05$, significantly different from the *control group, $^{\circ}$glucose supplementation group, $^{\oslash}$glucose supplementation following taurine administration. *C*, Control group; *G*, glucose was supplemented 400 mg/kg, i.p., as a single dose. *G + T*, glucose supplementation (400 mg/kg, i.p., as a single dose) following taurine administration (200 mg/kg, i.p., as a single dose) group; *T + G*, taurine administration (200 mg/kg, i.p., as a single dose) following glucose supplementation (400 mg/kg, i.p., as a single dose) group

Table 2. Plasma thiobarbituric acid reactive substances (TBARS) and total sulfhydryl groups (RSH) levels in the control and experimental groups

Groups	n	TBARS (abs)	RSH (abs)
C	6	0.037 ± 0.002	1.812 ± 0.046
G	6	0.038 ± 0.002	$1.937 \pm 0.042^{*}$
G + T	6	0.039 ± 0.001	1.822 ± 0.065
T + G	6	$0.049 \pm 0.002^{*,\oslash,\oslash\oslash}$	$1.780 \pm 0.036^{\oslash}$

$P < 0.05$, significantly different from the *control group, $^{\oslash}$glucose supplementation group, $^{\oslash\oslash}$glucose supplementation following taurine administration. *C*, Control group; *G*, glucose was supplemented 400 mg/kg, i.p., as a single dose. *G + T*, glucose supplementation (400 mg/kg, i.p., as a single dose) following taurine administration (200 mg/kg, i.p., as a single dose) group; *T + G*, taurine administration (200 mg/kg, i.p., as a single dose) following glucose supplementation (400 mg/kg, i.p., as a single dose) group

Results

The serum glucose and C-peptide findings of the four groups are shown in Table 1. The glucose level in glucose supplementation group was significantly higher than that of the control group ($P<0.05$) and glucose supplementation following taurine administration group ($P<0.05$). Blood glucose level significantly low in glucose supplementation following taurine administration group compared with glucose supplementation group ($P<0.025$). The serum glucose level was lower (but not significantly) in the taurine administration following glucose supplementation group compared with the glucose supplementation group ($P>0.05$).

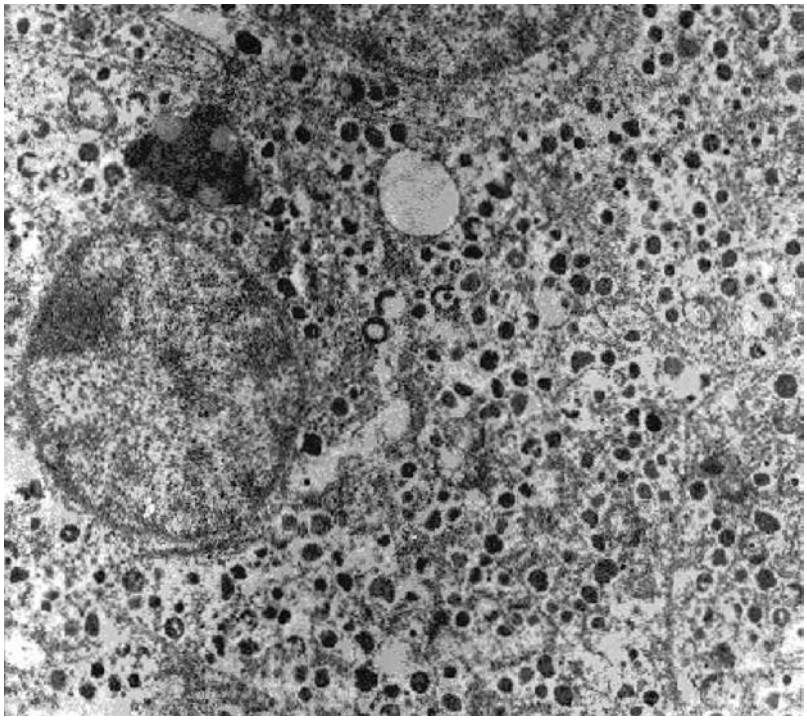

Fig. 1. Control group: The beta cells in the islets of langerhans exhibited normal ultrastructure. (Uranyl acetate, lead citrate × 6000)

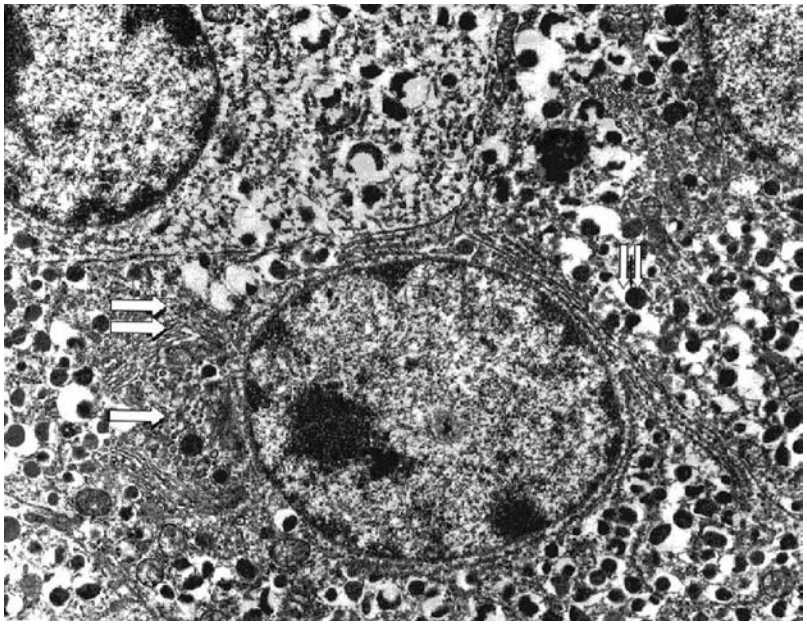

Fig. 2. Glucose supplemented group: The beta cells were filled with secretion granules (thick single arrow). Golgi complexes were active (thick double arrow), and numerous newly produced secretion granules (thin double arrow) were observed. (Uranyl acetate, lead citrate × 6000)

Serum C-peptide level increased significantly in all of the groups compared with the control group ($P < 0.05$). The highest C-peptide level was observed in the glucose supplementation following taurine administration group.

The plasma TBARS and RSH findings for the four groups are shown in Table 2. In the taurine administration following glucose supplementation group, plasma TBARS level increased compared with the others. Plasma RSH level increased significantly in the glucose supplementation group compared with the control group ($P = 0.076$). Conversely, in the taurine administration following glucose supplementation, plasma RSH level was decreased

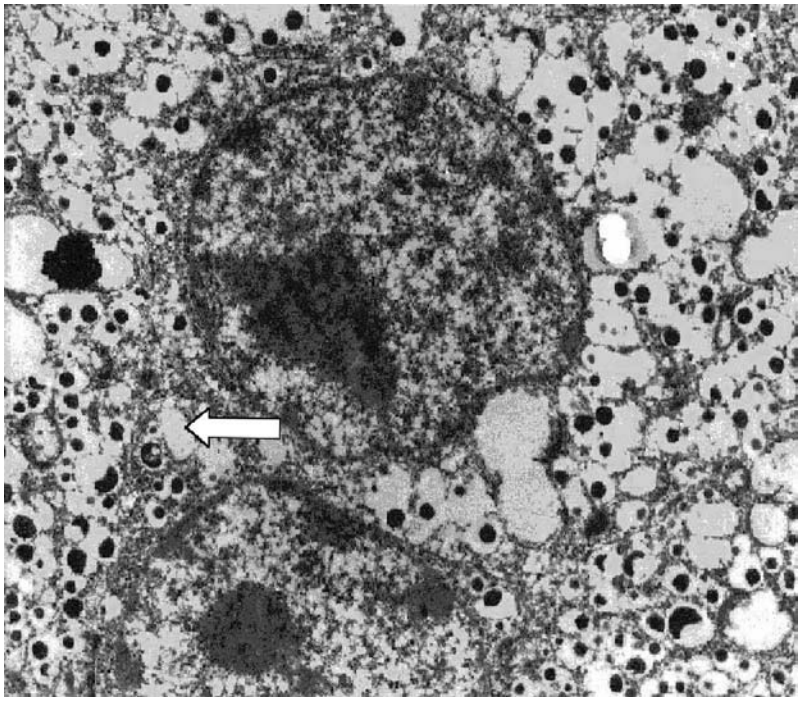

Fig. 3. Glucose supplementation following taurine administration group: Most of the secretion granules in the beta cell cytoplasm were empty (thick single arrow). (Uranyl acetate, lead citrate × 6000)

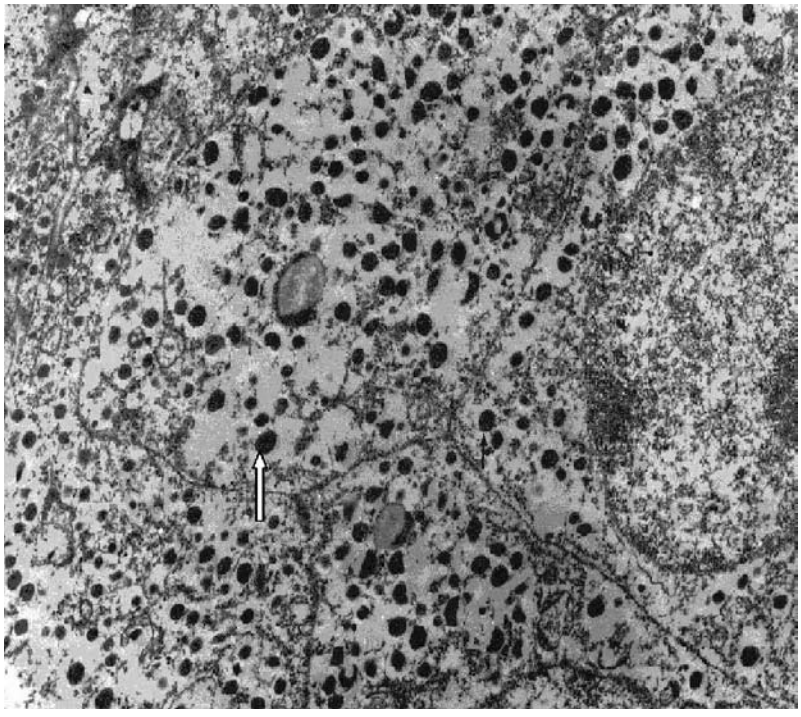

Fig. 4. Taurine administration following glucose supplementation group: The specimens showed fewer secretion granules in the beta cells compared to glucose supplementation following taurine administration and newly forming secretion granule were reduced in number (thin single arrow). (Uranyl acetate, lead citrate × 6000)

significantly compared with the glucose supplementation group ($P = 0.037$).

The morphological changes in the four groups are shown in figures 1, 2, 3, 4, respectively. In the control group, beta cells had round nucleus full of cytoplasmic granules (Fig. 1). As for the glucose supplementation group, insulin synthesize could be observed due to the accumulation of insulin secretion granules in the beta cell. Nuclear chromatin appearance of nucleus was regular (Fig. 2). In the glucose supplementation following taurine administration, a lot of secretion granules in the beta cells were empty. There was a few newly forming secretion granules (Fig. 3). In the taurine administration following glucose supplementation group, there was not increase in the secretion granules which were empty. In the same group, secretion granules were fewer than those of the glucose supplementation following taurine administration group. A few newly forming secretion granules were also observed in this group as in glucose supplementation following taurine administration group (Fig. 4).

Discussion

Taurine influences various biological and physiological functions, including brain and retinal development (Sturman, 1986), cell membrane stabilization (Pasantes-Morales et al., 1987), antioxidation (Nakamura et al., 1993; Kaplan et al., 2003) detoxification (Nakashima et al., 1982), osmoregulation (Huxtable, 1992), hypoglycemic action (Kulalowski and Maturo, 1984) and neuromodulation (Wu and Xu, 2003; Yun-Zhong et al., 2002). We investigated hypoglycemic and antioxidative actions of taurine on hyperglycemia by assessing blood glucose level, C-peptide level, pancreatic ultrastructure and lipid peroxide formation levels, total sulfhydryl groups.

In our study, we induced hyperglycemia with glucose supplementation. It is known that insulin secretion increases in the hyperglycemia. In addition, insulin along with equimolar amounts of C-peptide accounts for 90–97% of the product released from the beta cells of pancreatic islet cells (Schlosser et al., 1987). Ray and Rolek (1975) reported that taurine did not alter blood glucose concentration significantly but it did prevent oubain-induced hypoglycemia. In the previous study, we found out that its hypoglycemic action was insignificant in the taurine administration following hyperglycemia group. Our findings are consistent with Ray's and Rolek's reports (1975). However, its hypoglycemic action markedly increased in the hyperglycemia (glucose supplementation-induced hyperglycemia) following taurine administration. Our results demonstrate that after the taurine administration followed by i.p. injection of glucose, there was a decrease in the granular content whereas the number of empty granules increased. Thus, this may be an important modulator for the insulin secretion. However, taurine administration prior to glucose did not cause an increase in the granule discharge.

In the present study, these results indicate that C-peptide secretion from beta cell of langerhans islets of pancreas was tenaciously stimulated in the hyperglycemia following taurine administration group. Its hypoglycemic action was much stronger in the glucose supplementation following taurine administration group than it was in the taurine administration following glucose supplementation group. Chang and Kwon (2000) demonstrated higher insulin levels in the beta cells of diabetic patients treated with injection 1–2% taurine than in the control group. Chang and Kwon (2000) suggested that immunohistochemical localization of insulin increases in the pancreatic beta cells of taurine supplemented or taurine depleted diabetic rats. Our results do not support the findings of Chang and Kwon (2000). In the present study, the number of secretory granules (an index of stored insulin) in beta cells increased only in the glucose supplemented group. On the other hand, in the glucose supplementation following taurine administration group, the number of secretion granules decreased along with their content. These results indicate that taurine has stimulated pancreatic secretion in the hyperglycemia. It was reported that insulin secretion is stimulated by taurine in the various situations (Cherif et al., 1998; Cherif et al., 1996), which is consistent with our observations. When taurine was used at concentrations higher than 1–2%, morphology of secretion granules, granular endoplasmic reticulum and mitochondria of the beta cells were found to be improved (Chang, 2000). Our results are congruent with the findings of Chang (2000). Kulalowski and Maturo (1984) reported that the hypoglycemic action of taurine may be related to its direct action on both liver and muscle plasma cell membranes at or near the insulin receptor, which is not supported by our findings. According to our biochemical and histological results, hypoglycemic action of taurine may have been increased by increasing pancreatic insulin secretion in the hyperglycemia following taurine administration group.

Lipid peroxidation is a process in which the unsatured fatty acids of the cell membrane react with free radicals and cause oxidative damage (Ginsberg et al., 1988; Basaga, 1990). It has been shown that taurine possesses antioxidant effects (Alvarez et al., 1983) and/or

suppresses lipid peroxidation (Nakashima et al., 1982). The beneficial effects of taurine against oxidant induced tissue injury have been attributed to its ability to stabilize biomembranes (Huxtable, 1992; You et al., 1998) and scavenge reactive oxygen derivates including hypochlorous acid (Wright et al., 1986; Marquez et al., 1994; Marcinkiewicz et al., 2000). Chang and Kwon (2000) showed that taurine protects pancreatic beta cells against destruction by streptozotocin injection in a dose-dependent manner. However, the insulin levels in the non-diabetic group treated with 3% taurine were close to the levels in the untreated-diabetic rats. These findings led researchers to suggest that taurine protects against damage caused by streptozotocin because of its antioxidative action (Tokunaga et al., 1979). Chang (2000) showed that damage of pancreatic beta cells in streptozotocin treated rats was significantly reduced by administration of 1% taurine. Lim et al. (1998) reported that intraperitoneal injection of taurine protects against lipid peroxidation in type I diabetic mice. In our study we showed that the injection stress did not play an important role in lipid peroxidation. In the taurine application following glucose supplementation neither the antioxidative nor the hypoglycemic actions of taurine were significant. These results are not consistent with Lim's results (1998). We believe that the taurine and glucose application method made such on effect. Our results indicate that in the taurine administration without hyperglycemia, neither taurine nor total sulfhydryl groups could prevent lipid peroxide formation. These findings suggest that taurine has relative antioxidative and hypoglycemic actions in the taurine administration following glucose supplementation.

In view of our results, it might be suggested that glucose-induced hyperglycemia may be regarded as an important contributing factor in taurine-induced insulin secretion.

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