Biochemical Pharmacology, Vol. 28, pp. 2807–2811.

0006-2952/79/0915-2807 $02.00/0

PROTECTIVE ACTIONS OF TAURINE AGAINST STREPTOZOTOCIN-INDUCED HYPERGLYCEMIA

HISASHI TOKUNAGA, YUKIO YONEDA and KINYA KURIYAMA
Department of Pharmacology, Kyoto Prefectural University of Medicine, Kyoto 602, Japan

(*Received* 13 *December* 1978; *accepted* 16 *April* 1979)

Abstract—The effect of continuous administration of taurine (2-aminoethanesulfonic acid) on streptozotocin (STZ)-induced hyperglycemia was examined.

Acute administration of STZ (250 mg/kg, i.v.) to ddy strain mice induced transient but significant increase in the serum level of glucose and decrease in the pancreatic immunoreactive insulin (IRI) content at 24 hr after the administration. These changes induced by acute STZ administration were suppressed significantly by the oral pretreatment of animals with taurine (7–9 g/kg/day, for 5–7 days). Similar preventive effects were also observed when methionine or cysteine was orally administered for 7 days prior to the sacrifice of STZ-administered animals, whereas isoleucine and valine had not such preventive properties. Since the administration of these three amino acids, which have a suppressive effect on STZ-induced hyperglycemia, significantly increased taurine content in the pancreas, it is suggested that the increase in pancreatic taurine may be intimately related to the protective effect against the pancreato-toxic action of STZ. In CD-1 strain mice, one of the STZ-sensitive strains, the continuous administration of taurine not only exhibited significant preventive effects on hyperglycemia and decrease in serum and pancreatic contents of IRI induced by acute administration of STZ (150 mg/kg, i.v.), but also suppressed the continuous hyperglycemia and fall in pancreatic IRI content caused by the repeated administration of STZ (50 mg/kg, i.p., for 5 days). In addition, it was also found that morphological alterations in the islets of Langerhans induced by STZ were considerably less in taurine-treated animals than those found in animals treated with STZ alone.

These results suggest that pancreatic taurine may play important physiological roles in maintaining the function and/or integrity of β cells in the islets of Langerhans and protecting these structures from pancreato-toxic substances such as STZ.

Although taurine (2-aminoethanesulfonic acid), a sulphur containing amino acid, is distributed in almost all mammalian tissues, its physiological roles are not clearly defined. It has been demonstrated that taurine possesses anti-arrhythmic effects on the epinephrine or digoxin-induced arrhythmia [1], depressant actions in the mammalian central nervous system (CNS) [2], membrane stabilizing actions on the sarcoplasmic reticulum [3] and anti-convulsive effects on experimental seizures [4]. In spite of the presence of a relatively large amount of taurine in the pancreas, physiological roles of this compound in the pancreas remain to be elucidated.

Streptozotocin (STZ), one of the antibiotics developed for the therapy of leukemia, has been found to cause hyperglycemia by inducing selective damage to β cells in the islets of Langerhans [5]. We have previously demonstrated that hyperglycemia induced by the exposure of rats to cold stress is suppressed significantly by the pretreatment of animals with taurine [6]. In obese-hyperglycemic mice, it was also found that a considerably high concentration of taurine is present in the islets of Langerhans [7]. Since these observations suggest that pancreatic taurine may play important physiological roles in the regulation of serum level of glucose and/or the maintenance of the function of the islets of Langerhans, we have examined the effect of taurine administration on hyperglycemic conditions induced by STZ.

MATERIALS AND METHODS

Male mice of STD-ddy or CD-1 strains weighing 25–30 g were used in all experiments. Animals were usually deprived of food for 15–16 hr prior to sacrifice.

Conditions for STZ administration. Acute administration: STZ was dissolved with physiological saline containing 10 mM citrate buffer (pH 4.5) and administered intravenously to the animals fasted for 15–16 hr. The STZ solution was prepared immediately before each use. After the injection, starvation was continued further for 1 hr and animals were then allowed to take food and water *ad lib.* for 7 hr. Seven hours later, animals were again deprived of food for 15–16 hr and then subjected to each analysis. Repeated administration: STZ was administered intraperitoneally once a day for 5 days to the animals allowed to take food and water *ad lib.*

Conditions for administration of taurine and other amino acids. Taurine, cysteine, methionine, valine and isoleucine were dissolved in drinking water and administered to animals *ad lib* until the sacrifice. The doses of these amino acids were 7–9 g/kg/day, 3–4 g/kg/day, 3–5 g/kg/day, 10–12 g/kg/day and 11–12 g/kg/day, respectively. Administration of these amino acids did not induce detectable changes in general behavior or mortality of the animals and the determinations described below were not influenced by these amino acids.

Determinations of serum levels of glucose and immunoreactive insulin (IRI), and pancreatic contents of IRI and taurine. Serum glucose was determined according to the *o*-toluidine method [8], and serum IRI was measured by double antibody radioimmunoassay using reagents obtained from the Dainabot Radioisotope Laboratory, Ltd. (Tokyo, Japan) and the porcine insulin as a standard. For measurement of pancreatic insu-

lin, the pancreas was frozen by dry ice–acetone immediately after decapitation, and homogenized with acid–ethanol solution [9]. After settling the homogenate for 24 hr at 4°C, and centrifugation, pancreatic IRI content in the supernatant was determined according to the radioimmunoassay procedures mentioned above. Pancreatic taurine content was also determined by the column chromatographic procedure described previously [10]. Determinations were always carried out in duplicate.

Histological examination of the pancreas. The pancreas was fixed in neutral formalin, and sections (thickness: 8 μm) were stained with hematoxylin and eosin, or aldehyde fuchsin.

Results were usually expressed as the mean ± S.E.M. and statistical significance was determined by Student's *t*-test.

STZ (Lot No. 1613-E) was kindly donated by Dr. W. Friis, Upjohn International Inc., Kalamazoo, MI.

RESULTS

Effect of taurine pretreatment on hyperglycemia observed at 24 hr after STZ administration to ddy mice

Twenty-four hours after the acute administration of STZ (250 mg/kg, i.v.) to male ddy mice, it was found that these animals exhibit a transient but significant hyperglycemia and a drastic fall in pancreatic IRI content, whereas serum level of IRI is not altered significantly in spite of the remarkable hyperglycemia (Fig. 1). These findings essentially agree with the report by Junod *et al.* [11]. Oral administration of taurine for 3 days (7–9 g/kg/day) prior to sacrifice of these STZ-administered animals showed a tendency to suppress the elevation of serum level of glucose, but this change was not statistically significant. The prolonged use of taurine (5–7 days), however, significantly inhibited the hyperglycemia induced by STZ. In addition to this suppressive effect on the hyperglycemia, taurine pretreatment for 7 days also significantly prevented the STZ-induced fall in pancreatic IRI content (untreated: 1040.7 ± 145.1 μU/mg, STZ: 5.45 ± 1.44 μU/mg, TAU + STZ: 24.9 ± 5.5 μU/mg, respectively). Since the hyperglycemia observed in animals injected with STZ dissolved in buffered saline alone and STZ plus 1 mM taurine dissolved in the same solution did not differ significantly (481.4 ± 4.7 mg/dl vs 481.5 ± 32.7 mg/dl), it is unlikely that these suppressive effects of taurine on the alterations in serum level of glucose and pancreatic IRI content induced by STZ are caused by direct chemical interactions between taurine and STZ.

Effect of other amino acids on hyperglycemia induced by STZ in ddy mice

To examine the specificity of this suppressive effect of taurine, effect of pretreatment with other amino acids for 7 days on STZ-induced hyperglycemia was investigated. Pretreatment of animals with methionine or cysteine, sulphur containing amino acid and precursors for the biosynthesis of taurine respectively, also significantly suppressed the elevation of serum level of glucose induced by STZ, whereas isoleucine or valine had no such property (Fig. 2). In addition, it was also found

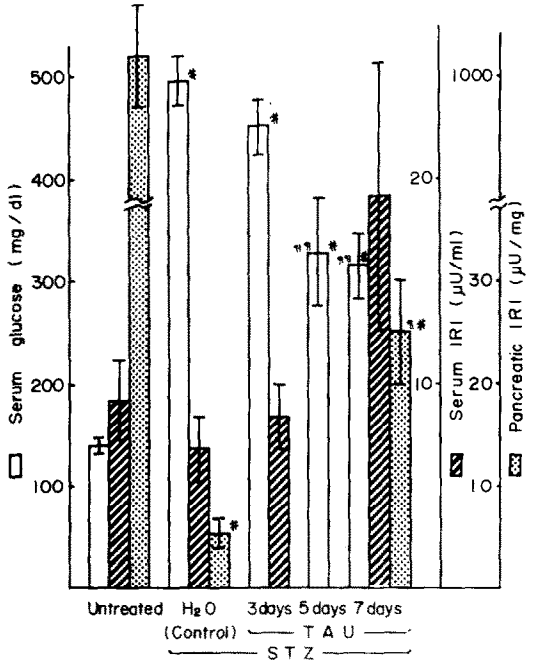

Fig. 1. Protective effect of taurine on STZ-induced alterations in serum levels of glucose and immunoreactive insulin (IRI), and pancreatic IRI content in ddy mice. Taurine was administered orally for 2, 4 or 6 days prior to the administration of STZ (250 mg/kg, i.v.) and was continued until the sacrifice of animals (24 hr after the STZ-administration). Each value represents the mean ± S.E.M. obtained from 5 to 24 separate experiments. #P < 0.01, Compared with each untreated value. ¶ P < 0.02, ¶¶ P < 0.01, Compared with each H_2O (control) value.

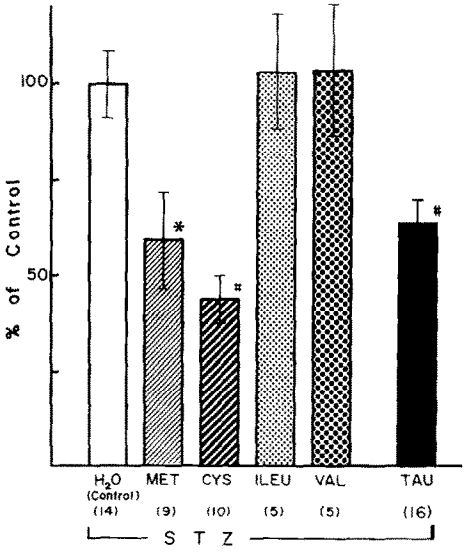

Fig. 2. Effect of various amino acids on STZ-induced hyperglycemia in ddy mice. Each amino acid was administered orally for 7 days prior to the sacrifice of STZ-administered animals. Each value represents the mean ± S.E.M. obtained from various numbers of separate experiments indicated in parentheses. * P < 0.05, #P < 0.01, Compared with the H_2O (control) value.

Table 1. Effect of oral administration of methionine (Met), cysteine (Cys) and taurine (Tau) on taurine contents in various organs

	Taurine (μmoles/g wet wt)			
	Pancreas	Liver	Diaphragm	Heart
Untreated	3.15 ± 0.22	6.63 ± 0.84	25.7 ± 1.1	35.7 ± 3.9
Met	5.50 ± 0.21†	7.81 ± 0.82	25.9 ± 1.9	37.0 ± 4.2
Cys	5.52 ± 0.28†	10.27 ± 1.04*	28.4 ± 2.2	42.6 ± 4.0
Tau	6.04 ± 0.69†	13.89 ± 0.67†	25.2 ± 1.8	34.0 ± 3.0

Each amino acid was administered for 7 days prior to the sacrifice of animals. Each value represents the mean ± S.E.M. obtained from 5 separate experiments. * $P < 0.05$, † $P < 0.01$, compared with each untreated value.

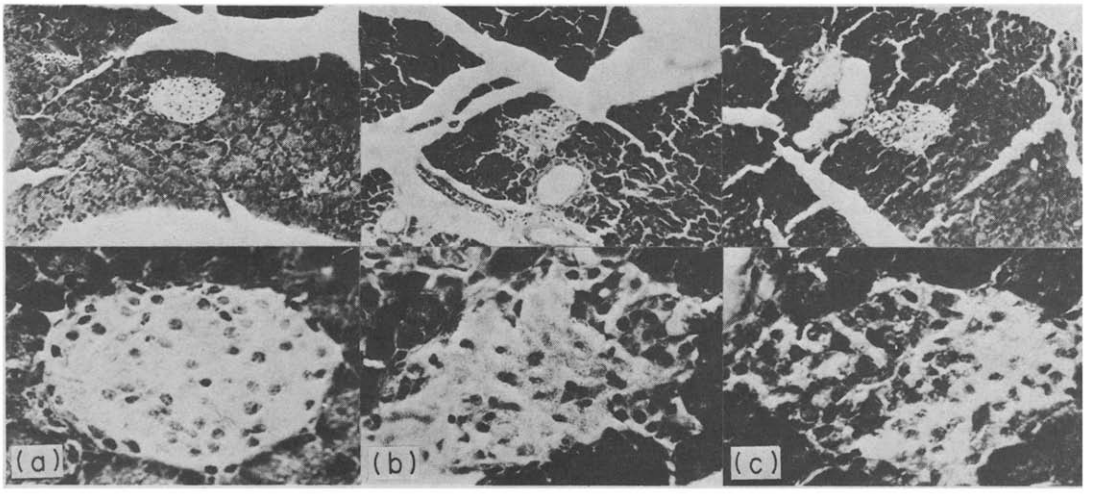

Fig. 3. Light microscopic observations of pancreatic islets in ddy mice (hematoxylin-eosin stain). a: Control, b: STZ alone, c: TAU + STZ. Upper raw: × 100, Lower raw: × 400. Animals were sacrificed 24 hr after the administration of vehicle or STZ.

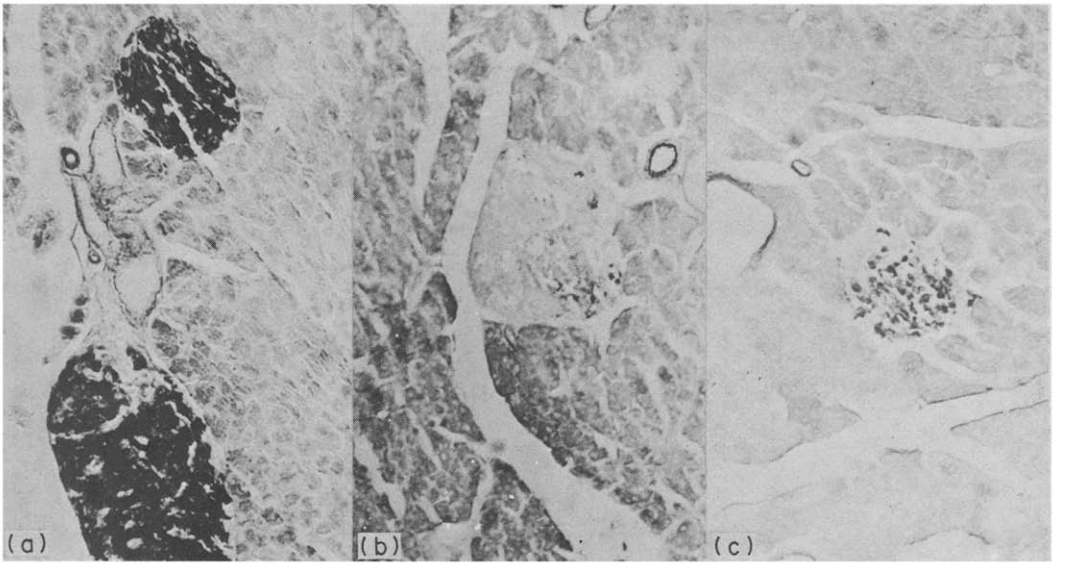

Fig. 4. Light microscopic observations of pancreatic islets in ddy mice (aldehyde-fuchsin stain, × 400). a: Control, b: STZ alone, c: TAU + STZ. Animals were sacrificed 24 hr after the administration of vehicle or STZ.

that pancreatic taurine contents are increased significantly in animals pretreated with taurine, methionine or cysteine for 7 days (Table 1). Considering these results, it can be said the the increase in pancreatic taurine content may have a cross correlation with the suppressive effect on the hyperglycemia induced by STZ.

Morphological alterations in pancreas of ddy mice

Light microscopic observations of pancreatic sections stained by hematoxylin-eosin revealed that in the STZ-administered animals, there existed strong modifications such as necrosis extended over the islets of Langerhans, fusion or granular coagulation of cytoplasm and disappearance of nuclei in β cells in spite of the normal appearance of exocrine cells. On the other hand, taurine pretreatment induced a significant preventive effect on these morphological changes in the pancreas induced by STZ administration. In taurine-pretreated animals, it was observed that relatively large numbers of normal islets remained despite the presence of slightly damaged figures such as cell degeneration and the fusion of cytoplasm as compared with those found in untreated animals (Fig. 3). In aldehyde-fuchsin stained sections of the pancreas, it was also found that granules stained by aldehyde-fuchsin, namely β-granules, completely disappeared in STZ treated animals, whereas many granules (thought to be functioning normally) stained by aldehyde-fuchsin still remained in the pancreas of animals pretreated with taurine for 7 days (Fig. 4). These results clearly indicate that the action site for protective effect of taurine on STZ-induced hyperglycemia is indeed present in the pancreas.

Effect of taurine pretreatment on hyperglycemia induced by acute administration of STZ in CD-1 mice. Since it has been demonstrated that a remarkable strain difference is present in the sensitivity to STZ in mice [12], effect of taurine pretreatment on the hyperglycemic conditions induced by acute STZ administration was further studied using CD-1 strain mice, one of the STZ-sensitive strains. Acute administration of STZ (150 mg/kg, i.v.) to these animals caused a significant increase in serum level of glucose and remarkable decreases in serum and pancreatic contents of IRI.

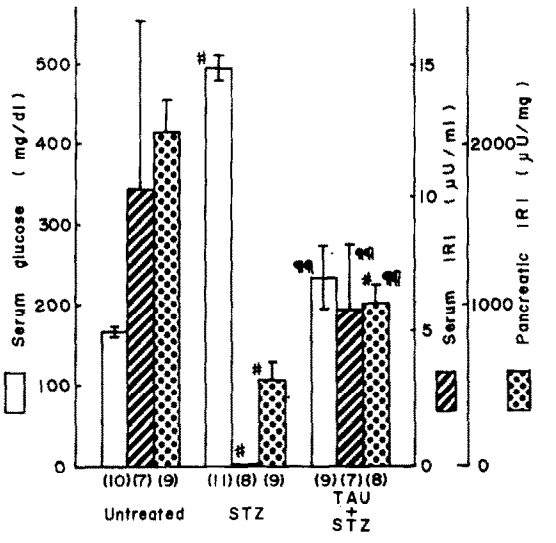

Fig. 5. Protective effect of taurine on hyperglycemic conditions induced by acute administration of STZ in CD-1 mice. Taurine was administered orally for 6 days prior to the administration of STZ (150 mg/kg, i.v.) and was continued until the sacrifice of animals (24 hours after the STZ administration). Each value represents the mean ± S.E.M. obtained from various numbers of separate experiments indicated in parenthesis. # $P < 0.01$, Compared with each untreated value. ¶¶ $P < 0.02$, Compared with each STZ value.

These alterations were also suppressed significantly by the pretreatment of animals with taurine for 7 days (Fig. 5). These results indicate that the protective action of taurine on STZ-induced hyperglycemia occurs independently of strain difference or the susceptibility to STZ.

Morphological alterations in pancreas of CD-1 mice. Similar to the observations in ddy mice, it was also found in CD-1 mice that many β granules still remain intact in the pancreas of the animals pretreated with taurine for 7 days compared with the case of animals treated with STZ alone (Fig. 6).

Continuous hyperglycemia induced by repeated administration of STZ in CD-1 mice. Repeated adminis-

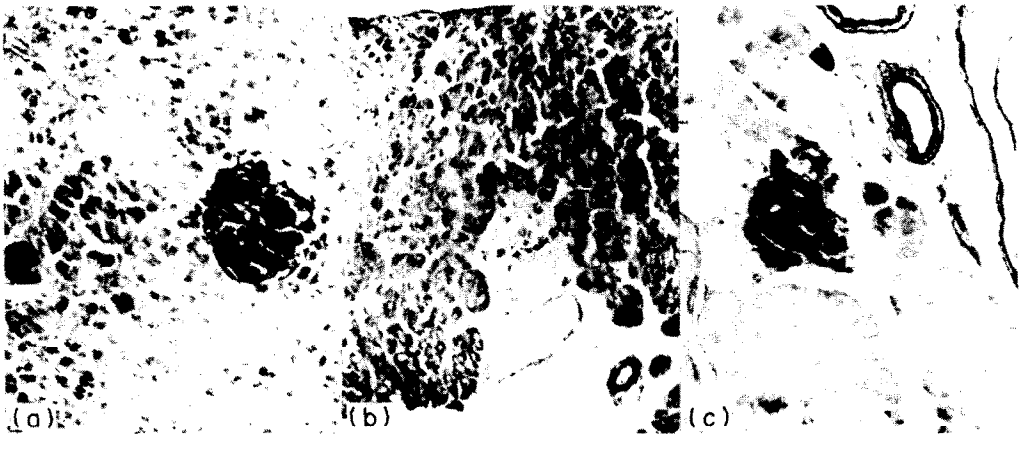

Fig. 6. Light microscopic observations of pancreatic islets in CD-1 mice (aldehyde-fuchsin stain, × 400). a: Control, b: STZ alone, c: TAU + STZ. Animals were sacrificed 24 hr after the administration of vehicle or STZ.

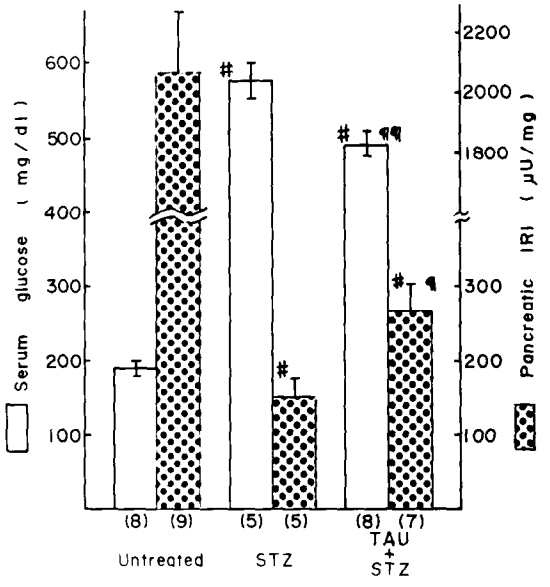

Fig. 7. Protective effect of taurine on continuous hyperglycemia induced by repeated administration of STZ in CD-1 mice. STZ was intraperitoneally administered (50 mg/kg) daily for 5 days and animals were decapitated 2 weeks after the initiation of STZ administration. Taurine was orally administered from the 7 days before the initiation of STZ administration to the day of sacrifice (for 21 days). Each vlaue represents the mean ± S.E.M. obtained from various numbers of separate experiments indicated in parentheses.

$P < 0.01$, Compared with each untreated value. ¶ $P < 0.05$, ¶¶ $P < 0.02$, Compared with each STZ value.

trations of a lower dose of STZ (50 mg/kg, i.p.) induced continuous hyperglycemia as previously reported [13]. The serum level of glucose was found to increase significantly 1 week after the initiation of repeated administration and this hyperglycemia was continued for at least 3 weeks (untreated: 189.4 ± 8.7 vs treated for 1 week: 244.8 ± 21.5, 2 weeks: 537.6 ± 46.5, and 3 weeks: 565.5 ± 27.2, respectively). Pretreatment and continuous administrations of taurine also significantly suppressed not only this continuous hyperglycemia, but also a drastic fall in the pancreatic IRI content (Fig. 7). Considering these results, it is conclusive that taurine has a protective action on both transient and continuous hyperglycemic conditions induced by STZ in CD-1 mice.

DISCUSSION

Several reports are available which indicate a possible role of taurine in the regulation of serum levels of glucose, such as the enhancement of hypoglycemic action of insulin [14], protective effects on hyperglycemia induced by alloxan [15], dehydroascorbate [15], or cold stress [6], and hypoglycemic action of taurine [16], but its mechanisms are not clearly defined.

The present study also clearly indicates that continuous pretreatments of mice with taurine significantly suppress streptozotocin (STZ)-induced hyperglycemia. Since these animals pretreated with taurine exhibited significantly less decrease of immunoreactive insulin (IRI) in the pancreas and less damage of β cells in the islets of Langerhans than those found in animals treated with STZ alone, it is likely that the site of protective action of taurine resides at the islets of Langerhans in the pancreas *per se*. Although mechanisms underlying protective actions of taurine against the damage of β cells by STZ are not clear at present, it may be related to the well known membrane stabilizing action of taurine. In fact, there is much evidence to support the theory that taurine acts as a membrane stabilizer, such as anti-arrhythmic action on epinephrine or digoxin-induced arrhythmia [1], anti-convulsant effect on experimentally induced epilepsy [4,17], protective effect on the rate of calcium loss from phospholipase C-treated sacroplasmic reticulum [3], and suppressive effects on the releases of epinephrine from adrenal medullary chromaffin granules and of ^{45}Ca from cerebral crude synaptosomal fraction [18], respectively. Considering the present results together with these previous findings, it is suggested that the elevation of taurine level in the pancreas may induce membrane stabilizing actions on β granules in the islets of Langerhans and thereby exhibits a preventive action on the STZ-induced damages on these pancreatic structures. Exact molecular mechanisms underlying these actions of taurine in the pancreas remain to be elucidated.

Acknowledgement—This work was supported in part by research grants [Nos. 377088 and 321942 (1978)] from the Ministry of Education, Science and Culture, Japan.

REFERENCES

1. W. O. Read and J. D. Welty, *J. Pharmac. exp. Ther.* **139**, 283 (1963).
2. D. R. Curtis and J. C. Watkins, *J. Neurochem.* **6**, 117 (1960).
3. R. Huxtable and R. Bressler, *Biochim. biophys. Acta* **323**, 573 (1973).
4. N. M. Van Gelder, *Brain Res.* **47**, 157 (1972).
5. N. Rakieten, M. L. Rakieten and M. V. Nadkrni, *Cancer. Chemother. Rep.* **29**, 91 (1963).
6. K. Nakagawa and K. Kuriyama, *Jap. J. Pharmac.* **25**, 737 (1975).
7. G. Briel, E. Gylfe, B. Hellman and V. Neuhoff, *Acta physiol. scand.* **84**, 247 (1972).
8. A. Hyvarinen and E. A. Nikkila, *Clinica chim. Acta* **7**, 140 (1962).
9. J. C. Sodoyez and R. F. Sodoyez-Goffaux, *Metabolism* **22**, 1389 (1973).
10. Y. Yoneda, S. Takashima, K. Hirai, E. Kurihara, Y. Yukawa, H. Tokunaga and K. Kuriyama, *Jap. J. Pharmac.* **27**, 881 (1977).
11. A. Junod, A. E. Lambert, L. Orci, R. Pictet, A. E. Gonet and A. E. Renold, *Proc. Soc. exp. Biol. Med.* **126**, 201 (1967).
12. A. A. Rossini, M. C. Appel, R. M. Williams and A. A. Like, *Diabetes* **26**, 916 (1977).
13. A. A. Like and A. A. Rossini, *Science, N.Y.* **193**, 415 (1976).
14. G. Donadio and P. Fromageot, *Bull. Soc. Chim. Biol.* **46**, 293 (1964).
15. T. Shimizu, Y. Shimizu, S. Nagami and M. Wada, *Niigata Med. J.* **74**, 931 (1960) (in Japanese).
16. D. Ackermann and H. A. Heinsen, *Z. physiol. Chem.* **235**, 115 (1935).
17. K. Izumi, H. Igisu and T. Fukuda, *Brain Res.* **76**, 171 (1974).
18. K. Kuriyama, M. Muramatsu, K. Nakagawa and K. Kakita, in *Taurine and Neurological Disorders* (Eds. A. Barbeau and R. J. Huxtable), p. 201, Raven Press, New York (1978).