GABA Agonists Differentially Modify Blood Glucose Levels of Diabetic Rats

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ABSTRACT—This study described the effects of GABA agonists on glucose plasma concentrations of streptozotocin-induced diabetic rats. Low doses of an indirect GABA agonist, AOAA (aminoxyacetic acid); a GABA$_A$ and a GABA$_B$ agent, THIP (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridone) and baclofen, respectively; and a benzodiazepine were administered to non-diabetic and to diabetic rats. Plasma glucose concentrations were estimated during fasting and after an oral glucose load. Diazepam (1 mg/kg), baclofen (1 mg/kg) and AOAA (30 mg/kg), significantly decreased glycemia after oral glucose overload of streptozotocin-induced diabetes. None of the GABA-acting agents tested changed fasting or glucose overload glycemia of normal rats. Diazepam was the only drug to increase the fasting blood glucose concentration of diabetic rats. Treatment with AOAA or diazepam was accompanied by increased insulin plasma concentrations in diabetic rats to levels similar to the ones of non-diabetic animals. These results demonstrate that benzodiazepines and other GABA drugs act the endocrine pancreas in vivo, ultimately increasing plasma insulin and decreasing high blood glucose levels of diabetic rats. The acute and prolonged effects of the multitude of drugs acting on the GABA$_A$-benzodiazepine-chloride ionophore complex remain to be broadly investigated as a therapeutic tool in diabetes.

Keywords: GABA$_A$, GABA$_B$, Hyperglycemia, Insulin

GABA is found in pancreatic islets of humans and other mammalian species in concentrations as high as those detected in cerebral tissue (1, 2). Interestingly, the apparatus to synthesize and release GABA in GABA and that to terminate GABA actions are also present in these endocrine cells. Synaptic-like microvesicles contain the amino acid inside the beta cells, and these vesicles are surrounded by glutamic acid decarboxylase (GAD), the GABA synthesizing enzyme (3). GABA-transaminase, the catalytic enzyme, is also detected in fairly high amounts in the cytosol of the beta cell (4, 5), while in a subpopulation of delta cells GABA-uptake systems may be activated to terminate the effect of extracellular GABA (6, 7).

Despite the identification of the complete metabolic pathway of GABA in pancreatic islets more than a decade ago, only very recently has information about GABA receptors in pancreatic cells been available. Specific high affinity binding of $^3$H-GABA was initially seen in the peripheral cells of neonatal pancreatic islets (8), but no binding of this ligand to GABA$_A$ or GABA$_B$ receptors could be demonstrated in the endocrine or exocrine pancreas of adult rats (6). However, nowadays it seems more evident that hormonal secretion induced by GABA in the pancreas of different species may be due to its interaction with specific binding sites. Cell lines derived from the endocrine pancreas have functional GABA$_A$ receptors suited for positive modulation (9). Membranes prepared from pig pancreatic islets bind benzodiazepines, and these exogenous agents induce insulin secretion from isolated islets (10). Using human endocrine pancreases, mRNA shows the expression of $\alpha_2\beta_3\gamma_1$ subunits (11) and $\alpha_{1-3}\beta_1-3\gamma_7$ subtypes are derived from rat pancreas (12), leading to the conclusion that ligand sites for both GABA$_A$-type and for benzodiazepines are represented in this endocrine organ.

The importance of the GABA system for the function of hormonal secretion still needs to be elucidated in greater detail. GABA is decreased in endocrine pancreatic tissue in experimental and human diabetes. Also, the role of the GABA system in the endocrine pancreas should not
be underestimated since there is a connection of autoimmune mechanisms related to GAD and the origin of diabetes mellitus (13). It was our interest to explore the effects of GABA agonists with different mechanisms of action on experimental diabetes. This study was planned to evaluate the effects of some GABA_A and GABA_B agents on fasting and overload blood glucose of streptozotocin-induced diabetic rats with moderate or severe hyperglycemia.

MATERIALS AND METHODS

Animals
Female Wistar rats from the colony kept in the Animal Facility of the Division of Pharmacology, Fundação Faculdade Federal de Ciências Médicas (Porto Alegre, Brazil) were used when 90-day-old. The animals were maintained in groups of 6 in white Plexiglas boxes, under a 12-hr light cycle (lights on from 7 a.m. to 7 p.m.) and temperature-controlled conditions (22±2°C). Water and food were available at all times except on the 10th day after diabetes induction, when they were fasted for 12 hr before the experiments. Rearing, maintenance and experimental procedures conform to the guidelines of the Society of Neuroscience and of the Brazilian College for Animal Experimentation.

Drugs
Streptozotocin (STZ) (Sigma Co., St. Louis, MO, USA) was diluted in citrate buffer (pH 4.5) in concentrations of 30 and 60 mg/ml. Diazepam at 1 mg/ml (Roche, São Paulo, Brazil) was suspended on propyleneglycol. Baclofen at 1 mg/ml (Biogalênia, São Paulo, Brazil) and aminoxyacetic acid (AOAA) at 30 mg/ml (Sigma Co.) were diluted in distilled water. THIP (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridone) at 1 mg/ml (RBI, Natick, MA, USA) was diluted in acidified distilled water (pH 4). Control animals received the respective diluting solutions. All drug solutions were prepared immediately before use and were injected i.p. in a fixed volume of 1 ml/kg.

Experimental design
To evaluate the consequences of different intensities of hyperglycemia, groups of rats were injected either with STZ at 30 or 60 mg/kg. These doses were previously seen in our laboratory to reproduce, respectively, a) a diabetic state characterized by normal fasting glycemia and hyperglycemia after a glucose load which is called herein as moderate hyperglycemia or b) a state in which fasting and glucose overload hyperglycemia were higher than 140 mg/dl and was taken to indicate severe hyperglycemia (14). Control groups received 1 ml/kg of buffer solution and were shown not to present hyperglycemia at any time. On the 10th day after streptozotocin administration, the rats were fasted for 12 hr and blood was collected by retro-ocular venous puncture under light ether anesthesia. Plasma was used to estimate fasting glycemia. Immediately after this first blood collection, 30 mg/kg of a glucose solution (30 mg/ml) was administered by gavage. A second blood sample was collected after 60 min to measure glycemia after the oral glucose overload.

The GABA agents, diazepam (1 mg/kg), baclofen (1 mg/kg), THIP (1 mg/kg) or AOAA (30 mg/kg), or their respective control solutions (1 ml/kg) were injected to the diabetic rats and to control animals 30 min before the fasting blood collection. These drug doses were chosen because they were the lower amounts to produce anxiolytic-like changes of behavior in previous studies in our laboratory (15).

Plasma glucose concentration was estimated by the glucose oxidase method, using commercial kits (Labtest, Lagoa Santa, Brazil). Insulin and glucagon concentrations were estimated, during fasting, in the plasma of non-diabetic and diabetic animals treated or not treated with AOAA and diazepam by double antibody radioimmunoassay, using anti-pork insulin and glucagon serum (Pharmacia Kit; Pharmacia, Uppsala, Sweden).

Statistical analyses
Analysis was performed with a two-way analysis of variance taking into account the diabetic condition and the drug treatment as independent factors. Further comparisons between groups were made by the Student-Newman-Keuls’ test. Differences with P<0.05 were considered statistically significant.

RESULTS
Figure 1-1 shows that diazepam, THIP, baclofen and AAOA do not change fasting or glucose overload glycemias of non-diabetic rats (STZ 0).

As may be observed in Fig. 1-2, fasting glycemias of rats previously administered with STZ at 30 mg/kg was similar to the fasting glycemias of animals injected with streptozotocin-control solution (STZ 0). These same animals (Fig. 1-2) presented plasma glucose levels higher than 140 mg/dl, 60 min after the oral glucose overload (F_{2,93} = 36.04, P < 0.001). Rats that had been administered STZ at 60 mg/kg (STZ 60) (Fig. 1-3) had significantly higher fasting plasma glucose concentrations (F_{2,93} = 87.73, P < 0.001) and overload plasma glucose (F_{2,93} = 36.04, P < 0.001) than STZ 0 treated rats.

Diazepam (1 mg/kg) significantly increased fasting glycemias of STZ 60 diabetic rats (F_{1,93} = 11.21, P = 0.001) (Fig. 1-3D). Interaction between diazepam treatment and the diabetic state was significant (F_{2,93} = 6.06, P < 0.05)
since the drug did not modify fasting glycemia of non-diabetic (STZ 0) and STZ (30 mg/kg) (STZ 30)-treated animals. Post-glucose overload glycemia of the STZ 30 and STZ 60 diabetic rats (Fig. 1-2D and Fig. 1-3D, respectively) were significantly decreased after diazepam ($F_{1,93}=12.07$, $P<0.001$). A significant interaction was detected ($F_{2,93}=5.08$, $P<0.008$). Diazepam treatment eliminated the great difference between fasting and glucose overload glycemia of STZ 30-treated rats. This group behaved in a similar way as the STZ 0 animals with respect to blood glucose. Mean glucose overload glycemia of STZ 60 diabetic rats was decreased to levels similar to non-treatment fasting glycemia when diazepam was the treatment.

The hypoglycemic effect of THIP was very mild in this study and could only be seen in the moderately hyperglycemic rats. A significant interaction between THIP treatment (1 mg/kg) and the diabetic state was detected ($F_{2,86}=3.11$, $P=0.05$) due to the normal glucose overload glycemia in the group previously administered STZ at 30 mg/kg (Fig. 1-2T).

Baclofen at 1 mg/kg did not change fasting plasma glucose concentrations, and fasting hyperglycemia was only present in animals administered STZ at 60 mg/kg ($F_{2,92}=76.56$, $P<0.001$) (Fig. 1-3B), as expected. Glycemias after the glucose load were decreased in both STZ 30 and STZ 60 groups (Fig. 1-2B and Fig 1-3B, respectively) when treated with baclofen ($F_{1,92}=21.04$, $P<0.001$) in comparison to controls. Consequently, overload plasma glucose of the STZ 30 group was similar to that of non-diabetics (Fig. 1-1B), and the glycemies of the STZ 60 was reduced to levels similar to fasting glycemias ($F_{2,92}=3.55$, $P=0.03$).

A0AA at 30 mg/kg did not change fasting plasma glucose concentration, and hyperglycemia was only present in the STZ 60 group ($F_{2,97}=90.69$, $P<0.001$) (Fig. 1-3A). Hyperglycemia after the oral glucose load also was decreased in the STZ 30 and STZ 60 groups of animals (Fig. 1-2A and Fig 1-3A, respectively) after treatment with A0AA ($F_{1,97}=13.25$, $P<0.001$), with the same pattern of effects as the one seen after baclofen.

Insulin plasma concentrations of diabetic and non-diabetic animals previously treated with A0AA or diazepam is shown in Table 1. Insulin concentration was dose-dependently decreased in rats administered with the different doses of streptozotocin ($F_{2,36}=4.968$, $P<0.05$). Both A0AA ($F_{1,39}=46.14$, $P<0.05$) and diazepam ($F_{1,36}=36.624$, $P<0.001$) increased insulin concentrations to values at least 100% higher than those of non-treated animals. The concentration of insulin after these treatments was similar in diabetic and non-diabetic rats.

Glucagon concentrations did not change in diabetic rats (STZ 30 = 86.1 ± 2.5 pg/ml and STZ 60 = 87.5 ± 3.8 pg/ml) when compared to the non-diabetic ones (100.3 ± 4.0 pg/ml). Treatment with A0AA at 30 mg/kg ($F_{1,28}=7.839$, $P<0.05$) or with diazepam at 1 mg/kg ($F_{1,30}=1.252$, $P<0.05$) mildly increased glucagon concentrations by about 15% when compared to non-treated rats (data not shown).

![Figure 1. The effects of control treatment (C); diazepam, 1 mg/kg (D); THIP, 1 mg/kg (T); baclofen, 1 mg/kg (B); or A0AA, 1 mg/kg (A) on blood glucose concentration of rats pretreated with control solution (STZ 0) (1), streptozotocin at 30 mg/kg (STZ 30) (2) and streptozotocin at 60 mg/kg (STZ 60) (3), during fasting and after oral glucose. The values represent the mean ± S.E.M.; each group was composed of 6–10 rats. After a two-way ANOVA, a significant difference was seen between groups with the diabetic condition and *P < 0.05, differs from STZ 0 and STZ 30 groups from the same condition; †: P < 0.05, differs from STZ 0 and STZ 60 groups from the same condition; drug treatment showed significantly different effects on glycemia, and ‡P < 0.05, differs from the controls (C) under the same condition/treatment.](image-url)
Table 1. Plasma insulin concentrations (mean±S.E.M., μU/ml) of diabetic rats induced with STZ at 30 mg/kg and STZ at 60 mg/kg are decreased 60 min after glucose overload, and AOAA at 30 mg/kg and diazepam at 1 mg/kg significantly increased insulin plasma concentrations

<table>
<thead>
<tr>
<th>Drug</th>
<th>Group</th>
<th>n</th>
<th>Insulin (μU/ml)</th>
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<tr>
<td>Control</td>
<td>Non-diabetic</td>
<td>10</td>
<td>10.4±1.7</td>
</tr>
<tr>
<td></td>
<td>STZ 30</td>
<td>10</td>
<td>5.4±2.1</td>
</tr>
<tr>
<td></td>
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<td>5</td>
<td>3.5±0.6*</td>
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<td>AOAA</td>
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<td>21.2±3.2*</td>
</tr>
<tr>
<td></td>
<td>STZ 30</td>
<td>6</td>
<td>17.3±2.6*</td>
</tr>
<tr>
<td></td>
<td>STZ 60</td>
<td>5</td>
<td>17.4±3.4*</td>
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<tr>
<td>Diazepam</td>
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<td>10</td>
<td>17.4±4.4*</td>
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<td></td>
<td>STZ 30</td>
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<td></td>
<td>STZ 60</td>
<td>5</td>
<td>16.9±7.7*</td>
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*Student-Newman-Keuls test: differs from drug-control non-diabetic, P < 0.05. 

With the estimates of plasma insulin concentrations in animals pretreated with AOAA or diazepam, this “in vivo” study confirms the recent molecular biological findings of functional GABA_4 receptors in pancreas islets (9). These GABA receptors may induce insulin release from isolated islets and are suited to bind positive modulators such as benzodiazepines (10). The physiological function of GABA in the endocrine pancreas still is matter of conjecture. Previous studies proposed GABA to be a mere source of energy to the islet cells, through the GABA shunt (4, 6). There is, however, evidence of GABAergic neuronal input (20) and the aminoacid neurotransmitter might be modulating the synthesis of pro-insulin or regulating insulin release (16, 18). Although it was not an objective of this study, it should not be forgotten that GABA acts as a modulator of the function of alpha and delta cells, regulating glucagon release (19, 21) and inhibiting somatostatin release (7, 16, 22). In favor of its paracrine function, GABA was shown to be co-localized and released from beta cells at the same time as insulin (20) and to inhibit the secretion of glucagon from alpha cells by increased chloride influx, which could be inhibited by bicuculline and potentiated by diazepam (7, 19, 21). In preliminary “in vivo” studies in our laboratory, we could not find significant changes in glucagon levels of animals treated with AOAA or diazepam (unpublished results). Further studies should be planned to elucidate whether the increase of insulin concentrations induced by the GABA agonists is secondary to suppression of somatostatin secretion.

It is amazing to realize that even though we utilized agents with different signal transduction mechanisms such as diazepam, THIP and baclofen that are known to act on different ligand sites, benzodiazepine site, GABA_A and GABA_B receptors, respectively, the final effect on hyper-
glycemia of diabetic rats was similar. These results are quite difficult to explain since studies published during the last decade depict that through GABA_A action, there are increased releases of insulin and of glucagon (19), and GABA_A receptors present at the alpha cells are responsible for lower glucagon release (21). Many of these studies were obtained using “in vitro” techniques and might reflect the action of an agent on a sole hormonal system in the isolated tissue and not allowing counter regulatory mechanisms to be concomitantly evaluated. Additionally, only few recent studies used novel techniques to study the function of GABA_A receptors in beta cells (as reviewed in 23).

One more effect for diazepam is depicted by this experiment; it increases insulin blood levels and decreases blood glucose levels of diabetic rats. Recently obtained details regarding the pentameric configuration of GABA receptors derived from endocrine pancreas (11, 12) leads to the conclusion that multiple ligand sites for GABA_A agonists and for benzodiazepines are represented in this endocrine organ of different species. Whether the different GABA_A receptor subtypes are represented in distinct islet cells remains to be studied. Because chemical agents known as ligands to these sub-units are capable of influencing the function of the endocrine pancreas of diabetic animals, as shown herein, further studies will be necessary to elucidate if the effect is maintained after prolonged treatment with GABA agonists and if it has any therapeutic value.

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