Stress-induced hyperglycemia and hypoinsulinemia are suppressed by sulfonylurea. Predominant role of insulin

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Based on the in vitro blockade of adrenal catecholamines release by sulfonylurea, we searched for an anti-stress activity of this drug. Stress-induced hyperglycemia and insulin inhibition were employed as adrenergic stress indicators. A standard dose of the oral sulfonylurea glipizide (200 μg/100 g), administered 15 min before a 1-h restraint stress to intact or 80% pancreatectomized rats, produced total suppression of the stress-induced hyperglycemia-hypoinsulinemia, an effect followed by a significant post-stress hypoglycemia of 1 h duration. The latter effect was elicited by all the sulfonylureas assayed.

In the 80% pancreatectomized rats, glipizide nearly halved the increases in plasma catecholamines at 30 min of stress, but did not modify those attained at 60 min, when glycemia was decreasing and insulinemia was still increasing. Moreover, behavioral experiments in intact stressed rats showed that the adrenergic overt behavior inhibition caused by propranolol was not produced either by glipizide or insulin, reinforcing that glipizide effect was not mediated by catecholamine inhibition. These findings suggest a blockade of catecholamines hepatic receptors by the anticipated insulin release induced by sulfonylurea. Thus, insulin fully dominated when insulin and catecholamines were administered in a stress-like sequence. A confirmation of these findings in diabetic patients subjected to surgical stress would allow a new therapeutic application of sulfonylurea.

It is concluded that an anticipated insulin release plus an insulin dominant role over catecholamines activity might explain the anti-stress effect of sulfonylurea.

Key words: catecholamines, insulin, stress hyperglycemia, sulfonylurea.

INTRODUCTION
In the acute stress, hypoinsulinemia (12) and hyperglycemia are sensitive indicators of adrenergic activation in rat, monkey and human (1, 7, 12, 20). Catecholamines are known to play a main role in the development of such hyperglycemia (12). Although this appears to be a rather fixed reaction against various stressors (24), hemorrhage, cardiac puncture and urethral catheterization increased significantly the stress hyperglycemic response (2), inducing a transient diabetic condition (30), while tail tip pinching induced a 50% reduction of stress hyperglycemia through opioid stimulation (24).

The hyperglycemia and hypercatecholaminemia persisted under repeated stress (5, 7, 18) whereas cortisol attained normal values (19, 20). Therefore, an inhibition of stress induced hyperglycemia means inhibition of the most active stress component, i.e. adren­aline oversecretion. This latter hormone, the most powerful hyperglycemic agent, appears to be indispensable for the production of stress hyperglycemia, since this effect disappeared after adrenal demedullation (23). No other hormone was able to replace adrenaline, since
neither glucagon nor growth hormone nor cortisol changes were eliminated by this procedure.

Searching for a drug that could antagonize the stress-induced hyperglycemia-hypoinsulinemia, sulfonylureas were selected for their in vitro inhibitory effect on adrenal catecholamine release (14) and for the known in vivo insulin release (13), which could antagonize the stress-induced insulin inhibition. Therefore, we hypothesized that sulfonylureas would reduce the stress-induced catecholamine release, resulting in an inhibition of stress hyperglycemia. Glipizide (GPZ) was chosen among the sulfonylureas due to its rapid action (3).

To prevent the stress-induced hyperglycemia, an anticipated drug administration before the stressor application was advisable considering the time for drug intestinal absorption. Among the pre-stress times assayed, 15 min gave the best effect. The partial pancreatectomized rat was used as a more reactive stress hyperglycemic model, which may present glycosuria (5) and transient diabetes mellitus when subjected to acute stress (30).

METHODS

Animals

Experiments were performed on non-fasting male Sprague-Dawley rats reared in the Central Animal Facilities of our Faculty and maintained at 21°C room temperature, on a 12 h dark/light cycle (22:00 to 10:00 h). Both intact and 80% pancreatectomized (80%-P) rats were used. Eighty percent pancreatectomy was performed under ether anesthesia by blunt dissection of the pancreas paragastic region, leaving the paraduodenal portion (4). The rats were tested 7 days after the operation.

Before the experiment, the rats were acclimatized to the laboratory room during 1 h to avoid novelty stress, with free access to water and pelleted food. The 60 min stress was performed according to standard immobilization procedures, allowing body and tail movements (23, 24).

Rats were divided into 9 experimental groups (see Results).

Drugs administration

All doses are expressed per 100 g b.w. Glipizide (GPZ) was given in a standard dose of 200 μg (0.4 mol) and the doses for the other sulfonylureas were calculated from human pharmacologic posology (11). Sulfonylureas (Sigma) were administered in single oral doses in 0.5 ml aqueous suspension, through an intragastric semielastic cannula, 1, 8 or 15 min before the stressor application. Previous controls with saline solution demonstrated that this procedure did not produce changes in glyceremia, but a new control was done with 0.5% methylcellulose (see 1st group in Results). Sulfonylureas suspensions were prepared in tridistilled water under permanent stirring immediately before administration. Rapid insulin (Nordisk) and catecholamines (Sigma) were injected intraperitoneally (ip), and propranolol and diazepam (Sigma) intracerebro-ventricularly (icv) in 1.0 μl saline solution, as described elsewhere (24). A two step surgical procedure was employed. In a first step, the skin section and needle puncture of the skull, made under ether anesthesia, increased glyceremia to 30 ± 5 mg/dl at 15 min, with rapid recovery. After 60 min, when normoglycemia had been reached, the icv injection was carefully made in conscious rats, without producing a significant hyperglycemia at 15 min.

Insulin and catecholamine administration to mimic the chronological hormone sequence of the sulfonylurea experimental model

The simulation was made in intact rats giving insulin 15 min before catecholamine administration (0 time). The first injection mimicked the initial sulfonylurea-induced insulin release and the second, the catecholamine discharge at stress onset. In other series, both hormones were injected at 0 time. Catecholamines were dissolved in 0.05 N hydrochloric acid and prepared just before their administration.

Behavioral experiments

In this group, the restrain stress lasted 30 min. Body movements, noise emissions and chewing movements were registered and
counted throughout the experiment, each rat being studied by one investigator. Results are expressed as the means of the total number of events.

Glycemia, glycosuria and hormone determinations

Glycemia was determined at times indicated in the figures by the Dextrostix-Dextrometer method (16, 24, 25) and the blood samples were obtained from one cut at the tail tip, without removing the rats from the cage to diminish handling stress.

For hormones determinations, blood samples were drawn from the abdominal aorta under general ether anesthesia. For catecholamine measurements, the samples were collected into prechilled vacutainers (Upjohn) at 0° C and assayed with CATA KIT of 1.8 pg/10 µl adrenaline sensitivity (21). Serum insulin was determined by radioimmunoanalysis (9). Hormones values are expressed as the means of two readings per sample.

Glycosuria was evaluated positive or negative using the glucose enzymatic test strip (Tes-Tape, Lilly) in a drop of urine taken from the urethra (28).

Statistical analyses

Results were analyzed by unpaired Student’s t tests or three-way factorial analysis of variance (ANOVA) (26), p < 0.05 being considered as statistically significant. The behavioral observations were analyzed by Wilcoxon’s tests (26). In 80%-P rats treated with GPZ and not submitted to stress, a linear regression was calculated for the data at 60 and 120 min.

RESULTS

Group 1. Hypoglycemic GPZ assay

This assay was performed on non-stressed 80%-P rats. Figure 1 illustrates the hypoglycemic effects produced by GPZ in single oral doses of 2, 20, 200 and 400 µg. A dose dependent effect for all doses was found, with a linear regression coefficient of 0.99 at 60 and 120 min. A control assay with oral methylcellulose 0.5% had no effect on glycemia (changes: + 2, -2, 0 and 0 mg/dl in 4 different rats), confirming previous controls with saline, proving that the oral procedure used did not provoke stress hyperglycemia.

According to the above results, GPZ 200 µg was suggested as the standard effective and reversible hypoglycemic dose (ANOVA, p < 0.005).

The lower values of glycemia evoked by 2, 20 and 200 µg GPZ doses occurred at 180 min, whereas they were observed at 60 min after the 400 µg GPZ dose (Fig 1). Under stress conditions (Fig 3), these lower values were displaced to the left, indicating a more rapid recovery, which for the 200 µg dose attained normal level 1 h before the control.

Group 2. Influence of interval between GPZ administration and stress on antihyperglycemic effect

These experiments were performed on 80%-P rats subjected to 1 h restraint stress. Glipizide was administered per os in the single standard dose of 200 µg. As illustrated in figure 2, the hyperglycemic effect of stress was clearly manifested at the middle and end of the stress period. This hyperglycemic effect was minimally modified by GPZ given 1 min before the stress, but was nearly halved when the drug was given 8 min prior to the stress. GPZ presented its maximal suppressor effect when administered 15 min before the stressor, reversing the hyperglycemia into a mild hypoglycemia at the end of the stress period. Thus, this optimal interval was used as the standard time for all the following experiments.

![Fig 1. Reversible hypoglycemic response to single oral GPZ administration in non-stressed 80%-P rats. Doses indicated in the figure. Note incomplete recovery at 240 min. Ordinate, changes in glycemia (in mg/dl); abscissa, time (in min). Means ± SEM's. n = 4 rats for each dose.](image-url)
Fig 2. Influence of interval between drug administration and stress-induced hyperglycemia in 80%-P rats. GPZ 200 μg given at 1, 8 and 15 min before stressor application for 1 h. Suppressor hyperglycemic effect only when given at -15 min. Ordinate, changes in glycemia (in mg/dl); abscissa, time (in min). Means ± SEM's. n = 3 rats for stressed control and each interval of pretreatment.

Group 3. Dose-dependency of GPZ antihyperglycemic effect in stress

These experiments were also performed on 80%-P rats subjected to 1 h restraint stress. Figure 3 shows that the hyperglycemic effect of stress is prolonged for 2 h following stress. Doses of 2, 20 and 200 μg of GPZ, administered 15 min before the stressor, suppressed the stress and post-stress hyperglycemia. The 200 μg suppressive dose caused a poststress hypoglycemia (p < 0.005) of 1 h duration, whereas a 2 μg dose abolished hyperglycemia without inducing hypoglycemia.

Group 4. GPZ antihyperglycemic effect in stressed intact rats

When intact rats were subjected to 1 h restraint stress, the hyperglycemia resolved within 1 h post-stress (fig 4). When the single standard dose of 200 μg GPZ was administered 15 min before stress, we observed a significant suppression of the hyperglycemic effect of stress, followed by post-stress hypoglycemia similar to the one obtained in the 80%-P rat. This antihyperglycemic effect of GPZ 200 μg was closely mimicked by a single injection of insulin 0.3 IU given 15 min before the stress.

Fig 3. Suppression of stress-induced hyperglycemia by different doses of GPZ given 15 min before 1 h restraint stress in 80%-P rats. 1. Control (filled circles and continuous line). 2. GPZ 2 μg (open circles and dotted line). 3. GPZ 20 μg (open circles and interrupted line). 4. GPZ 200 μg (filled circles and continuous line), which caused post-stress hypoglycemia with fast recovery at 180 min. Ordinate, changes in glycemia (in mg/dl); abscissa, time (in min). Means ± SEM's. n = 10 rats for each GPZ dose; n = pool of 30 rats for control.

Fig 4. Suppression of stress-induced hyperglycemia by single oral dose of GPZ or insulin injection. Drugs given 15 min (at arrow) before 1 h restraint stress. Data from intact rats, but similar effects were obtained in 80%-P rats. 1. Stressed control (filled circles and continuous line). 2. GPZ 200 μg (filled circles and continuous line). 3. Insulin 0.3 IU (filled circles and dotted line), which effects closely mimic those of the hyperglycemic suppressive action of GPZ. Ordinate, changes in glycemia (in mg/dl); abscissa, time (in min). Means ± SEM's. n = 3 rats for control and each treatment.
Group 5. Plasmatic levels of catecholamines during stress.

Figure 5 illustrates the changes in the plasmatic levels of catecholamines observed in control 80%-P rats (open bars) subjected to 1 h restraint stress: adrenaline and noradrenaline were markedly increased at 30 and 60 min, while dopamine only increased at 60 min. The standard dose of 200 µg of GPZ (striped bars), administered 15 min before the beginning of the stress, nearly halved the increase of adrenaline and reduced to nearly one third that of control stressed rats at 60 min. Contrariwise, counter-regulatory noradrenaline and dopamine increases were found at 60 min of stress, significantly larger than those of control stressed rats (p < 0.001). These late changes in catecholamines levels occurred when glycemia was already decreasing.

Group 6. Inverse changes in glycemia and insulinemia during stress.

Simultaneous determinations of glucose and insulin levels in plasma were performed along 1 h restraint stress in 80%-P rats. As illustrated in figure 6, GPZ given in the single oral standard dose of 200 µg 15 min prior to the beginning of the stress caused a complete reversal of the changes in insulinemia and glycemia of the control. Thus, insulinemia increased 200% over control values (ANOVA, p < 0.005), while glycemia decreased (p < 0.0005).

Group 7. Insulin antagonism of hyperglycemic catecholamines effect

Figure 7 illustrates the hyperglycemic effects of single injections of noradrenaline 200 µg or adrenaline 20 µg in non-stressed intact rats, to mimic the catecholamine liberation observed under stress. Adrenaline at a dosage 10 times lower than noradrenaline proved to be 260% more hyperglycemic than noradrenaline (calculated by averaged changes in glycemia).

When insulin 0.3 IU was given 15 min before the administration of noradrenaline 200µg, the noradrenaline hyperglycemic effect was completely superseded by the insulin hypoglycemic effect (ANOVA, p < 0.005) (fig 7A). A similar result was found when the same dose of insulin preceded by the same interval the administration of adrenaline 20 µg (fig 7B). Furthermore, when the same doses of adrenaline and insulin were simultaneously injected, the hypoglycemic effect of insulin predominated (ANOVA, p < 0.005) (fig 7C).

Group 8. Comparison of antihyperglycemic effects of sulfonylureas

These experiments were performed on 80%-P rats subjected to 1 h restraint stress. Figure 8
Fig 7. Insulin effect upon the hyperglycemic response to catecholamines in non-stressed intact rats. I, insulin 0.3 IU; NA, noradrenaline 200 μg; A, adrenaline 20 μg. 1. Controls injected with catecholamines at time 0 (dots and continuous line). 2. Insulin given 15 min before (A and B) or simultaneously (C) with catecholamines (dots and interrupted line). Ordinates, glycemia (in mg/dl); abscissae, time (in min). Means ± SEM’s. n = 3 rats for each control and each treatment schedule.

Fig 8. Glipizide-like effect of other sulfonylureas on stress-induced hyperglycemia. One hour restraint stress in 80%-P rats. 1. Control (filled circles and continuous line) (n = pool of 11 rats). 2. Tolbutamide 1.4 mg (open circles and interrupted line) (n = 3 rats). 3. Glibenclamide 200 μg (filled circles and continuous line) (n = 3 rats). 4. Chlorpropamide 700 μg (open circles and interrupted line) (n = 5 rats). Doses equivalent to 200 μg of GPZ; administered orally 15 min before (arrow) the stressor application. Ordinate, changes in glycemia (in mg/dl); abscissa, time (in min). Means ± SEM’s. n = 3 rats for each control and each treatment schedule.

This illustrates the changes in glycemia observed along the period of stress and the following 1 h post-stress. We studied the effects of single oral administration of three sulfonylureas in hypoglycemic doses equivalent to that of GPZ 200 μg. We observed that glibenclamide 200 μg (0.4 μmol), chlorpropamide 700 μg (2.5 μmol) and tolbutamide 1.4 mg (5.2 μmol) were capable of abolishing the hyperglycemia induced by stress (ANOVA, p < 0.005). Their post-stress glycemas fell below basal levels, with similar intensities as those observed after GPZ.

The GPZ curve (not illustrated in fig 8) occupies a place between curves 2 (tolbutamide) and 3 (glibenclamide). No statistical differences between the hyperglycemic suppressor effects of these drugs and GPZ were encountered. That the GPZ and glibenclamide curves produced by 200 μg doses were almost identical confirms in this model their equivalent pharmacological activities.

Group 9. Comparison of stress-induced changes in behavior and glycemia

Figure 9 illustrates the changes in overt behavior induced by 30 min restraint stress in intact rats, and the changes in glycemia observed at the end of the stress period and 90 min post-stress. Intracerebroventricular injections of diazepam 1.0 μg or propranolol 50 ng inhibited the overt behavioral response to stress (p < 0.02), but only propranolol inhibited the stress-induced hyperglycemia (p < 0.02), which recovered at 120 min.

Contrarily to the above, pretreatment with GPZ 200 μg or insulin 0.3 IU did not modify the pattern of stress behavior, while both substances produced the same marked hypoglycemic effects, even 90 min after the end of the stress (p < 0.001).

Group 10. GPZ inhibition of chlorpromazine induced hyperglycemia

The icv administration of chlorpromazine 50 μg produces an hyperglycemic response in un-stressed intact rats, peaking at 30 min following the injection and prolonged for at least 120 min, as illustrated in figure 10. The icv administration of GPZ 2 μg halved the hyperglycemia induced by the icv injection of such high dose of chlorpromazine (p < 0.001 at 30 min; ANOVA, p < 0.05). Thus, GPZ acting centrally is able to inhibit the hyperglycemia induced by the central application of chlorpromazine.

Glycosuria

Glycosuria was not found in the GPZ treated 80%-P rats subjected to restraint stress. This
Fig 9. Propranolol and sulfonylurea effects upon stress overt behavior and their correlations with changes in glycemia. Intact rats subjected to 30 min restraint stress. Controls (open bars). In upper part (A), rats receiving icv diazepam 1 μg (hatched bars) or propranolol 50 μg (horizontally striped bars). In lower part (B), rats receiving GPZ 200 μg (cross-hatched bars) or insulin 0.3 IU (horizontally striped bars). Rats receiving GPZ follow closely the insulin pattern, whereas propranolol and diazepam treated rats exhibit an inhibition of overt behavior. Asterisks, significantly different from respective controls (p<0.05, at least). Ordinates, number of movements (left) and glycemia (in mg/dl) (right); abscissae for glycemia, time (in min). Means ± SEM’s. n = 7 rats for each control and each treatment.

finding must be compared with the glycosuria observed in 85% of the untreated stressed control rats (not illustrated).

DISCUSSION

The suppression of the stress-induced hyperglycemia and glycosuria observed in the 80%-P rat treated with sulfonylureas (figs 2, 3, 6 and 8) implies abolition of the stress-induced transient diabetes mellitus of the untreated control rat (30). The working hypothesis to explain this sulfonylurea effect was based on the assumption that adrenaline and insulin were the main factors responsible for such effect. It was considered that: a) adrenaline is the most powerful hyperglycemic agent (23; see also fig 7); b) other potentially hyperglycemic stress hormones like cortisol or glucagon cannot substitute this hyperglycemic effect of adrenaline, as evidenced by the stress provoked in adrenal-medullectomized rats (23); c) stress-induced hyperglycemia tends to inhibit glucagon secretion (12); d) other stress hormones like ACTH, MSH, prolactin and β-endorphin are not hyperglycemic agents, and growth hormone produces hyperglycemia but only after 2 h (22); e) no changes in somatomedin-C occur during insulin-induced hypoglycemia) (29), though somatomedin-C is an insulin-like substance.

The sulfonylureas-induced post-stress hypoglycemia observed in our experiments (figs 3, 4 and 8) appeared not to be related to sympathoadrenal inhibition, because: a) central propranolol adrenergic blockade (1) or peripheral adrenal demedullation (1, 23) abolished stress-induced hyperglycemia without producing post-stress hypoglycemia (fig 9A), indicating that the sulfonylurea effect cannot be explained by the sole adrenergic inhibitory mechanism; b) sulfonylurea inhibited the catecholamine surge at 30 min stress, but not those observed at 60 min (fig 5), just when hypoglycemia started and hyperinsulinism reached a high level, supporting the predominant role of insulin in post-stress hypoglycemia; c) when insulin and catecholamine were administered mimicking the chronology that would occur under sulfonylurea treatment (i.e., first insulin and then the catecholamine), insulin fully dominated, an effect still observed when both hormones were administered at the same time; these powerful

Fig 10. Central GPZ inhibition of chlorpromazine induced hyperglycemia in intact rats. 1. Controls showing hyperglycemia induced by chlorpromazine 50 μg icv (triangles and interrupted line). 2. Rats receiving GPZ 2 μg icv plus chlorpromazine 50 μg icv (dots and continuous line). ANOVA, p < 0.05. Ordinate, glycemia (in mg/dl); abscissa, time (in min). Means ± SEM’s. n = 8 rats for control and combined treatment.
insulin effects help to understand why the early sulfonylurea-induced insulin release was able to neutralize the hyperglycemic effect of catecholamines; d) upon the overt stress behavior, GPZ followed the insulin pattern and not the propranolol one. On the basis that adrenal catecholamines contribute to the full behavior response, this last result provides another argument against an in vivo sulfonylurea inhibitory action on adrenal catecholamines release, as it occurred in isolated cat adrenal glands (14), where the insulin antagonistic factor is absent, a demonstrative example of in vitro and in vivo model differences.

The predominant role of insulin was definitely favored by the 15 min pre-stress timing of GPZ administration, which covered the absorption time and produced the complete suppression of the stress-induced hyperglycemic effect. Results support the hypothesis that the insulin released by GPZ would arrive earlier to the hepatic insulin receptors, blocking the liver glycogenolytic adrenergic activity. In addition, the insulin effect after 0 time would be facilitated by the simultaneous transient inhibition of catecholamine found at 30 min, which would afford insulin a free opportunity to act without an adrenaline competitor.

The predominant role of insulin puts into evidence that the stress-induced hypoinsulinemia is necessary to avoid a risky post-stress hypoglycemia. Therefore, the sulfonylurea effect represents an interesting rupture of the typical adaptive stress-induced glucose and insulin responses. It clearly suggests that if insulinemia and glycemia would maintain their physiological parallel responses, proper of the glucose ingestion, the insulin increased concentration would dominate upon the stress-induced catecholaminergic activity.

The anti-stress activity of sulfonylureas suggests an extra-pancreatic mechanism of action through inhibition of the growth hormones (GH), because: a) sulfonylureas inhibit GH secretion in human diabetics (15) and this represents a central effect; b) icv administration of GPZ 2 µg inhibits the hyperglycemia induced by icv chlorpromazine 50 µg (fig 10), known to be accompanied by GH secretion (17); c) GH administered in adequate doses presents an hyperglycemic effect in diabetic patients (22), inhibits in vivo the tissue glucose uptake (8), shows insulin antagonistic activity (10) and is an important counterregulatory mechanism during hypoglycemia in normal subjects (6). These antecedents support a central GH-induced inhibition by sulfonylureas, that would decrease GH insulin antagonism, allowing an enhancement of the sulfonylurea suppression of the stress-induced hyperglycemic effect.

The sulfonylurea effect observed in the 80%-P rat prompts for an application in diabetic type II patients, so prone to develop surgical stress hyperglycemia and insulin resistance (12). In case of a trial of this drug in surgical stress, the age of the patients and the duration of the disease must be considered, because of the hypoglycemic risk due to adrenergic neuropathy (31). The rebound catecholamine response found at 60 min stress may have had influence in accelerating the recovery of glycemia, since it appeared more than 1 h earlier when the stress was added (figs 1 and 3). Since GPZ 2 µg inhibited the stress-induced hyperglycemia without producing post-stress hypoglycemia, there is an ample dosage range to be assayed.

It is concluded that the anticipated insulin release plus the insulin dominant role over catecholamines might be a main factor in the suppressive sulfonylurea effect on stress-induced hyperglycemia and hypoinsulinemia of the rat, the transient 30 min catecholamines inhibition being not responsible for the general suppressive effect. A complementary mechanism of action based on a probable GH inhibition was also discussed.

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