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Glycemic Response to Stress Stimulation by Ether Exposure in Adrenalectomized Rats[1]

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OLLER DO NASCIMENTO CURI, C. M., E. B. RIBEIRO, C. T. B. V. ZAIA AND M. S. DOLNIKOFF. *Glycemic response to stress stimulation by ether exposure in adrenalectomized rats.* PHARMACOL BIOCHEM BEHAV **37**(3) 399–403, 1990.—Stress was induced by short-term ether exposure (2 min) and tail vein puncture in normal (SO), adrenodemedullated (ADM), and adrenalectomized (ADR) rats. In ADM and SO rats stress provoked a significant hyperglycemic response with no change in plasma insulin levels. In ADR rats, on the other hand, the hyperglycemic response was not present. Actually, a significant rapid decrease in blood glucose, plasma insulin and hepatic glycogen content was observed. When the hypoglycemic effect of stress was prevented by glucose injection into ADR rats the decrease in plasma insulin and hepatic glycogen was not observed. The data suggest that the fall in plasma insulin and hepatic glycogen content observed in ADR animals result from an activation of the sympathetic nervous system induced by the decrease in blood glucose.

Glycemia Stress Adrenalectomy Adrenodemedullation Ether

ELEVATION of circulating glucose levels, at times to a marked degree, can occur during a variety of stressful situations, including anaesthesia (2).

It has been shown that short-term ether exposure (2 min) provokes stress associated with rapid and marked increase in the plasma levels of corticosterone (7,18). Although ether exposure has also been commonly used to induce short-term anaesthesia in laboratory animals (23), the acute hormonal and metabolic changes induced by this technique have not been hitherto investigated.

The experiments reported here were designed to investigate the acute alterations of carbohydrate metabolism following short-term ether anaesthesia as well as the role of the adrenal gland in this process. To this end, blood glucose, hepatic glycogen and plasma levels of corticosterone and insulin were determined in adrenalectomized (ADR), adrenodemedullated (ADM) and control rats (SO).

The results obtained show that, in contrast to its hyperglycemic effect in SO and ADM rats, short-term ether anaesthesia induces a significant decrease of blood glucose, plasma insulin levels and hepatic glycogen content in ADR rats, which could be prevented by glucose injection.

METHOD

Animals

Male Wistar rats weighing 150–200 g were used in this study. The animals were housed in individual cages for at least 7 days before the day of the experiment and were maintained under controlled conditions of lighting (lights on from 6:00 a.m. to 7:00 p.m.) and temperature (24 ± 1°C) with free access to Purina rat chow and water. Three groups of rats were studied: sham-operated (SO), adrenodemedullated (ADM) and adrenalectomized (ADR).

Surgical Procedures

Bilateral adrenalectomy was performed via the dorsal approach under pentobarbital anaesthesia (40 mg/kg); sham operations consisted of manipulation of both adrenal glands. Adrenalectomized rats were given 0.9% saline to drink, whereas sham-operated animals received tap water. Adrenalectomy was confirmed by inspection at autopsy as well as by measuring plasma corticosterone levels. For adrenal enucleation the gland was gently grasped with forceps, the capsule slit with microscissors and the

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content gently extruded with curved forceps. The ADM were utilized 15 days after surgery to allow for complete regeneration of cortical tissue (14) which was confirmed by normal basal levels of corticosterone. Histological analysis of adrenal gland confirmed the absence of medullary tissue.

Experimental Procedure

Animals were removed from the colony room and placed in a quiet chamber 2 hr before the experiment.

Exposure to stress. The ether stress consisted of rapidly removing the rats from their cages and placing them individually into a jar (20 cm diameter, 20 cm high) saturated with ether vapor (10 ml of diethyl ether-Qeel). After anaesthetization (2 min), 0.2 ml of saline were injected into the tail vein. The rats were returned to their cages and killed by decapitation after 5, 10 or 20 min. This procedure was performed 7 days after adrenalectomy or 15 days after adrenodemedullation, between 10:00 and 12:00 a.m. All experiments were performed with fed animals. A group of ADR rats was injected 0.2 ml of glucose (16 mg/kg) instead of saline. Animals decapitated immediately after removal from the cages were used as controls (basal time).

Trunk blood was collected into heparinized tubes and 200 μl samples were immediately deproteinized for determination of glucose by the ortho-toluidine method (6). The remaining blood was centrifuged at 4°C and plasma stored at −20°C for insulin and corticosterone measurements. Insulin was determined in 100 μl of plasma by radioimmunoassay (25) using rat insulin (Novo) as standard and the sensitivity of the assay was 0.05 ng/ml. Plasma corticosterone was determined in 500 μl of plasma by a fluorimetric assay (11), with coefficients of variation for inter- and intraassay of 11.5 and 5% respectively. The sensitivity of the assay was 0.02 μg/dl.

Slices of liver were quickly prepared and 500 mg of tissue were transferred to tubes containing 2 ml of 30% KOH for digestion and subsequent extraction with ethanol. The glycogen was depolymerized and glucose was determined by the anthrone method (17).

Statistical Analysis

Statistical comparisons were performed using ANOVA. If analysis of variance indicated that a statistically significant difference existed, then Duncan's test was used to determine statistical significance among groups as well as among time points in the same group. Statistical significance was set at the $p<0.05$ level.

RESULTS

Blood Glucose and Hepatic Glycogen Response to Stress

The responses of blood glucose and hepatic glycogen to stress in SO, ADM and ADR rats are shown in Fig. 1A and B, respectively. Basal glucose levels of ADM (107.3±2.9 mg/dl) and ADR (111.8±3.9 mg/dl) rats did not differ significantly from SO, control animals (114.3±3.3 mg/dl) (Fig. 1A). Five min after stress a significant increase in glycemia was observed in SO, $F(3,20)=3.28$, 124.1±2.4 mg/dl, $p=0.05$, and ADM rats, $F(3,25)=8.02$, 128.7±6.2 mg/dl, $p=0.05$. In contrast, blood glucose levels of ADR rats decreased significantly 5 and 10 min, $F(3,28)=6.71$, 92.8±4.8 mg/dl and 97.6±2.6 mg/dl, $p=0.05$, after stress. Despite a tendency to hypoglycemia in ADM rats, blood glucose at 10 min (99.8±3.0 mg/dl) did not differ significantly from basal values ($p>0.05$).

Basal hepatic glycogen did not differ in SO and ADM rats (5.0±0.1 mg% and 4.8±0.2 mg%, respectively), while in the ADR group basal levels were significantly lower, $F(2,18)=25.52$,

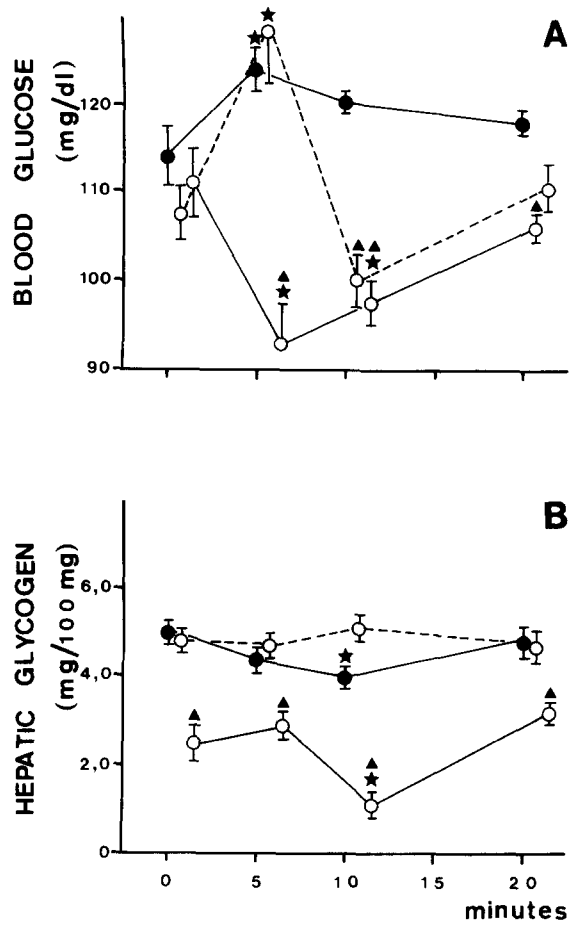

FIG. 1. Response of blood glucose concentration (A) and hepatic glycogen content (B) as a function of time before (zero time) and after stress stimulation. (●—●) SO, (○---○) ADM and (○—○) ADR rats. Values are mean±SEM of 6–8 animals for each time point. ⋆$p<0.05$, as compared to basal values. ▲$p<0.05$ as compared to control group.

2.5±0.4 mg%, $p=0.05$ (Fig. 1B). After 10 min of stress hepatic glycogen in SO, $F(3,28)=3.61$, 4.0±0.2 mg%, and ADR, $F(3,28)=9.03$, 1.1±0.3 mg%, decreased ($p<0.05$), but did not change significantly in ADM rats. However, return to basal values was observed after 20 min in both SO and ADR rats (Fig. 1B).

Plasma Insulin and Corticosterone Response to Stress

The time courses of plasma insulin and corticosterone are shown in Fig. 2A and B, respectively. Basal insulin levels in SO rats (3.0±0.4 ng/ml) were significantly higher than in ADM and ADR animals, $F(2,18)=4.33$, 1.5±0.2 and 1.6±0.4 ng/ml, $p=0.05$. After ether exposure, plasma insulin levels fell only in ADR rats (68.75% after 10 min), while ADM and SO animals showed no significant changes. As shown in Figs. 2A and 1B, the fall in plasma insulin levels in ADR rats paralleled the fall in hepatic glycogen content. These changes occurred 5 min after blood glucose levels had reached the lowest values.

Baseline plasma corticosterone levels (Fig. 2B) were similar in SO and ADM groups (10.0±1.9 and 9.5±0.5 μg/dl, respectively). Stress produced a significant elevation in plasma corticosterone concentration which persisted until the end of experimental

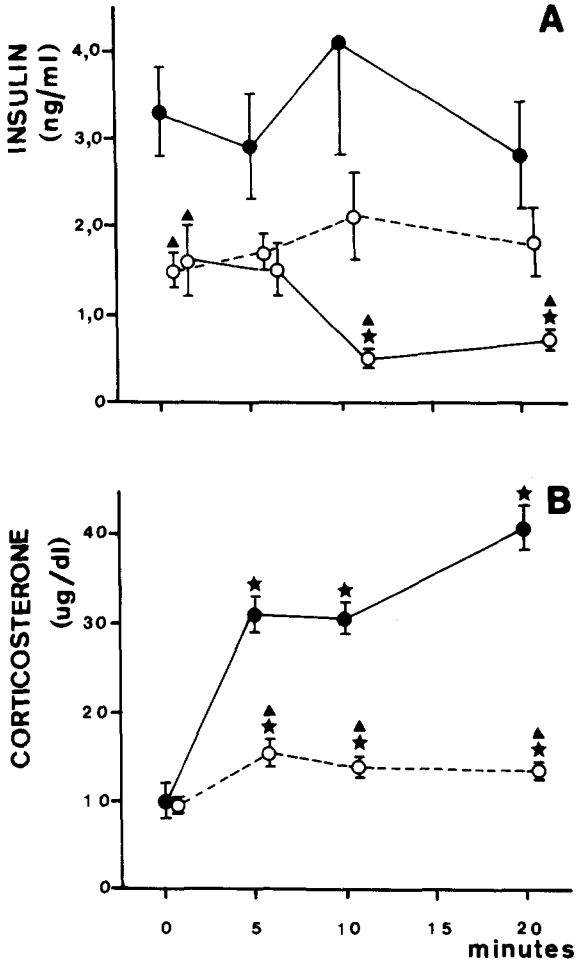

FIG. 2. Response of insulinemia (A) and corticosteronemia (B) as a function of time before (zero time) and after stress stimulation. (●—●) SO, (○---○) ADM and (○—○) ADR rats. Values are mean ± SEM of 6–8 animals for each time point. ⋆$p<0.05$, as compared to basal values. ▲$p<0.05$ as compared to control group.

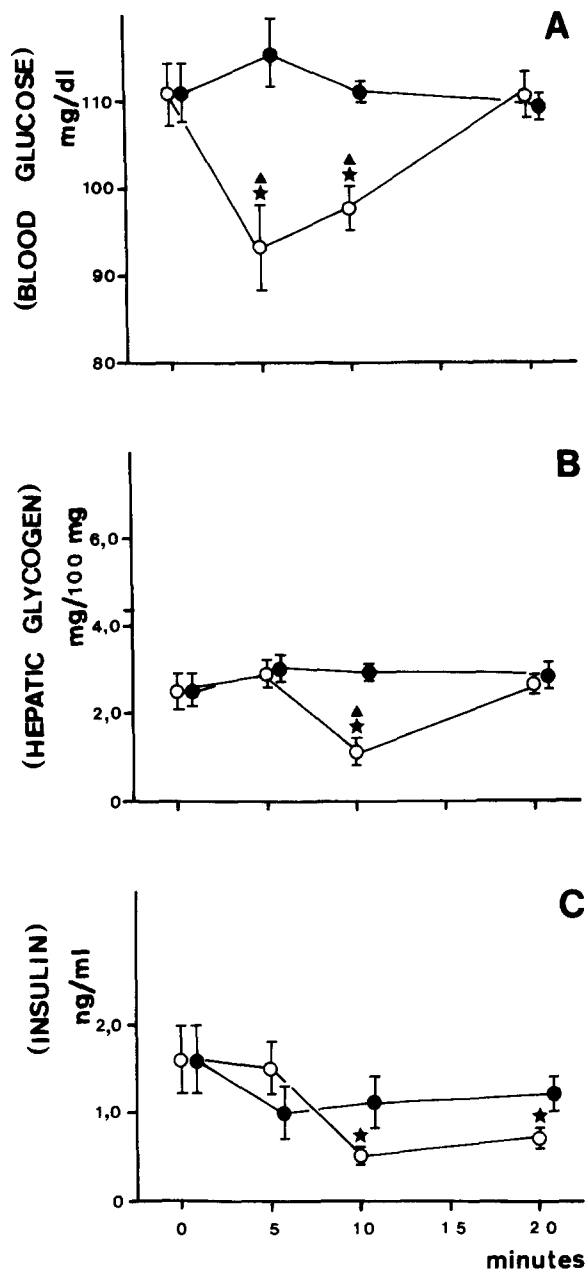

FIG. 3. Response of blood glucose concentration (A), hepatic glycogen content (B) and insulinemia (C) as a function of time in ADR rats before (zero time) and after ether exposure associated with saline (○—○) or glucose injection (●—●). Values are mean ± SEM of 6–8 animals for each time point. ⋆$p<0.05$, as compared to basal values. ▲$p<0.05$ as compared to control group.

period in both groups (Fig. 2B). However, SO rats showed a 306% increase in corticosterone levels 20 min after stress and in ADM rats the increase was of only 40%.

The basal plasma corticosterone level in the ADR animals was 2.79 ± 0.77 μg/dl. This value represents the nonsteroidal residual fluorescence inherent to the fluorescence determination (11). In ADR rats there was no change in corticosterone levels after exposure to stress (data not shown).

Blood Glucose, Liver Glycogen and Plasma Insulin Responses in ADR Rats After IV Injection of Either Saline or Glucose

As shown in Fig. 3A, glucose administration prevented the decrease in blood glucose levels in response to stress in ADR animals. In this situation, both hepatic glycogen (Fig. 3B) and plasma insulin concentration (Fig. 3C) were kept quite constant until the end of the experimental period.

DISCUSSION

The finding in the present experiments of lower basal insulinemia in ADM and ADR rats in the presence of normal blood glucose levels has been reported by others (10). It has been proposed that this could be due to an inhibition of insulin secretion by increased levels of norepinephrine (27,28). Also, the observation of normal and reduced basal levels of hepatic glycogen in ADM and ADR rats, respectively, has been previously reported (20, 26, 28). In fact, stimulation of hepatic glycogen synthesis is one of the best established actions of glucocorticoids (5,15).

The data of the present study show that exposure to the stressful stimulus of a short-term ether anaesthesia and injection in a tail vein, which are common procedures in experimental research, induces significant glycemic alterations in rats. Both control and ADM rats showed a hyperglycemic response to stress. In contrast, ADR rats showed a significant decrease in blood glucose levels (Fig. 1A). It is well known that hyperglycemia occurring after a stress situation results from an integrated response of autonomic stimulation and counterregulatory hormones release (12). The regulation of blood glucose concentration is also a well-recognized function of endocrine system, involving at least five hyperglycemic hormones: glucagon, epinephrine, norepinephrine, growth hormone and cortisol. All these hormones are known to be released during many stress situations like hypoglycemia (9), exercise (13) and anaesthesia (21). Thus, these hyperglycemic factors are potentially important glucose counterregulatory factors and the synergistic nature of these hormone-hormone interactions with respect to raising circulating plasma glucose levels may constitute a mechanism of stress hyperglycemia (8).

The fact that SO and ADM rats showed similar hyperglycemic response 5 min after stress (Fig. 1A) suggests that the acute hyperglycemic response is not mediated by the adrenal medulla. On the other hand, the significant decrease in blood glucose observed in ADR rats indicates that the presence of corticosterone is essential for the hyperglycemic response induced by a short-term ether anaesthesia (Fig. 1A). In fact, it is well known that corticosterone exerts a permissive effect upon the hyperglycemic action of other hormones, particularly glucagon and epinephrine (19). It has also been demonstrated that adrenalectomy impairs the gluconeogenic action of glucagon in the liver of fasted rats (4). Therefore, in our experiments, the lack of the permissive effect of corticosterone could result in a lower rate of hepatic glucose production in ADR than in SO and ADM rats.

The finding of lower blood glucose levels in ADM than in SO rats after 10 min of stress (Fig. 1A) is consistent with the observation that, at this same time point, the hepatic glycogen remained unchanged in ADM rats but was significantly reduced in SO rats (Fig. 1B). These results are in agreement with previous work showing the importance of adrenal medullary epinephrine secretion for the stress-induced hepatic glycogenolysis (1). Moreover, they suggest that, in addition to liver glycogen mobilization, other glucose raising mechanisms such as gluconeogenesis probably participated in the hyperglycemic response to stress observed in both SO and ADM rats. In fact, in absence of glucocorticoids, as found in ADR rats, a low rate of gluconeogenesis (20) could explain the significative decrease in blood glucose levels observed after ether stress.

It is important to note that the decrease of blood glucose levels in ADR rats was followed by a 56% decrease in hepatic glycogen and a 68.75% decrease in plasma insulin levels (Figs. 1B and 2A). The likeliest explanation for this finding is that the hypoglycemia induced a marked stimulation of the sympathetic nervous system (24,27) with resulting inhibition of insulin secretion and activation of hepatic glycogenolysis which were responsible for the recovering of blood glucose in ADR rats at 20 min. In fact, as shown in Fig. 3A, B and C, glucose administration to ADR rats prevented the decrease of both plasma insulin and hepatic glycogen levels.

The higher corticosterone response found in SO rats than in ADM rats (Fig. 2B) is in agreement with previous data (3, 16, 18). It has been proposed that the smaller response of the adrenal gland to stress in ADM rats results from the lack of stimulation of ACTH secretion by epinephrine (22). It has also been suggested that adrenal responsiveness to ACTH may be chronically impaired after adrenal enucleation (14).

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