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Effect of acute and repeated restraint stress on glucose oxidation to CO_2 in hippocampal and cerebral cortex slices

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Abstract

It has been suggested that glucocorticoids released during stress might impair neuronal function by decreasing glucose uptake by hippocampal neurons. Previous work has demonstrated that glucose uptake is reduced in hippocampal and cerebral cortex slices 24 h after exposure to acute stress, while no effect was observed after repeated stress. Here, we report the effect of acute and repeated restraint stress on glucose oxidation to CO_2 in hippocampal and cerebral cortex slices and on plasma glucose and corticosterone levels. Male adult Wistar rats were exposed to restraint 1 h/day for 50 days in the chronic model. In the acute model there was a single exposure. Immediately or 24 h after stress, the animals were sacrificed and the hippocampus and cerebral cortex were dissected, sliced, and incubated with Krebs buffer, pH 7.4, containing 5 mM glucose and 0.2 µCi D-[U-^{14}C] glucose. CO_2 production from glucose was estimated. Trunk blood was also collected, and both corticosterone and glucose were measured. The results showed that corticosterone levels after exposure to acute restraint were increased, but the increase was smaller when the animals were submitted to repeated stress. Blood glucose levels increased after both acute and repeated stress. However, glucose utilization, measured as CO_2 production in hippocampal and cerebral cortex slices, was the same in stressed and control groups under conditions of both acute and chronic stress. We conclude that, although stress may induce a decrease in glucose uptake, this effect is not sufficient to affect the energy metabolism of these cells.

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- Cerebral cortex

The acute secretion of glucocorticoids is critical for responding to stress. On the other hand, chronic stress produces deleterious effects (1,2). A classical catabolic action of glucocorticoids in numerous peripheral tissues is to inhibit glucose uptake into cells (3,4). Studies have shown that in hippocampal cell cultures glucocorticoids significantly inhibit glucose uptake and oxidation both by neurons and astrocytes (4,5).

It is well known that repeated exposure to the same aversive event during stress can lead to a process of adaptation to that stimulus. Hence, chronically stressed animals do

not show the same behavior and do not experience the same consequences as animals exposed to acute stress (6,7). Different neurotransmitter systems have been suggested to play a role in this stress desensitization process (e.g., 7,8). Probably, these neurotransmitters and glucocorticoids released during stress could modify glucose metabolism, leading to adaptation and to different glucose uptake and use.

The present study aimed to evaluate the consequences of acute or repeated exposure to restraint stress in rats on CO_2 production from glucose in slices of cerebral cortex and hippocampus at different times (0 or 24 h) after restraint stress.

Sixty-six adult male Wistar rats (60 days at the beginning of treatment) weighing 180-230 g were used. The experimentally naive animals were housed in groups of 6 to 8 rats in Plexiglas home cages (65 x 25 x 15 cm) with the floor covered with sawdust. The animals were maintained on a standard dark-light cycle (lights on between 7:00 and 19:00 h), at a room temperature of 22 ± 2°C. The rats had free access to food (standard lab rat chow) and water, except during the period when restraint stress was applied. In the experiment involving chronic stress the animals were divided into three groups: stressed, handled and control. The stressed group was taken to a different room, where restraint was carried out by placing the animal in a 25 x 7-cm plastic bottle and fixing it with plaster tape on the outside, so that it was unable to move. A 1-cm hole was left at one end for breathing. The animals were stressed 1 h/day, 5 days a week for 50 days. The immobilization procedure was performed between 10:00 and 12:00 h. The animals in the handled group were manipulated but not submitted to restraint. The animals were taken to a different room, also between 10:00 and 12:00 h. Handling consisted of gently manipulating each animal for 1 min. This group was included since handling may alter biochemical and behavioral parameters, both in young and adult rats (9-11). Control animals were kept undisturbed in their home cages. In the experiment involving acute stress, the animals were submitted to a single 1-h exposure and the control group was not stressed.

Animals were sacrificed by decapitation, immediately or 24 h after the last restraint session in the case of chronic treatment, and immediately after restraint in the case of acute stress. Immediately after decapitation, trunk blood was collected into heparinized tubes for corticosterone and glucose determination. Total corticosterone concentrations were analyzed directly in plasma by ^{125}I radioimmunoassay (Amersham Life Science Inc., Cleveland, OH, USA). Glucose was determined in plasma by the glucose oxidase method (Bio Diagnostica, Piraquara, PA, Brazil). The brains were quickly removed and the hippocampus and cerebral cortex were dissected out on ice. After removing the cerebral cortex with a scalpel and tweezers, it was possible to visualize the hippocampus, which was removed with small tweezers. The structures were weighed, and slices prepared within 5 min. Each tissue was cut into 0.3-mm slices using a McIlwain tissue chopper. About 50 mg of tissue slices were incubated in 1 ml Krebs-Ringer bicarbonate buffer, pH 7.4, containing 5 mM D-glucose and 0.2 µCi D-[U-^{14}C] glucose (specific activity: 3.0 mCi/mmol). Saturating glucose concentrations were used for CO_2 production. The content of the flasks was gassed with 95% O_2/5% CO_2 for 1 min and the flasks were sealed with rubber caps. The slices were incubated at 34°C for 1 h in a Dubnoff metabolic shaker (60 cycles/min). Incubation was stopped by adding 0.2 ml 50% TCA through the rubber cap. Then 0.2 ml of 1 M hyamine hydroxide was injected into the central wells to trap CO_2. These were small glass wells situated inside the flasks and above the level of the incubation medium. The flasks were shaken for a further 30 min at 34°C to trap CO_2, after which the center well content was transferred to

vials and assayed for CO_2 radioactivity in a liquid-scintillation counter (12).

All results are expressed considering the initial specific activity of the incubation medium, in such a way that for a given number of counts of CO_2 radioactivity per mg of tissue, the equivalent glucose consumed was calculated as pmol. The CO_2 production rate was constant over 30, 60 and 90 min of incubation.

Blood glucose and corticosterone concentrations after acute or chronic restraint stress are presented in Table 1. Corticosterone and glucose concentrations increased in plasma after 1-h acute restraint stress (Student *t*-test, $t = 6.45$, $P<0.001$ for corticosterone and $t = 4.16$, $P<0.001$ for glucose). After exposure to repeated restraint, there was no difference in plasma corticosterone or glucose between groups when the hormones were measured 24 h after the last restraint session (one-way ANOVA, $F = 1.790$, $P>0.05$ for corticosterone and $F = 0.535$, $P>0.05$ for glucose), and an increase in plasma glucose again occurred after another exposure to restraint (one-way ANOVA, $F = 8.784$, followed by the Student-Newman-Keuls test, $P<0.05$); plasma corticosterone was slightly increased when compared to the handled group but not when compared to the control group (one-way ANOVA, $F = 5.823$, followed by the Student-Newman-Keuls test, $P<0.05$).

The *in vitro* CO_2 production from glucose in cerebral cortex and hippocampus slices after 1-h restraint was measured in rats sacrificed immediately or 24 h later, and the results are shown in Figure 1A and B. When CO_2 production was measured immediately or 24 h after restraint, there was no significant difference between control and stressed rats (immediately after restraint: cortex: $t = 0.87$, hippocampus: $t = 0.084$; 24 h after stress: cortex: $t = 0.57$, hippocampus: $t = 2.17$; $P>0.05$ in all cases). Production of CO_2 from glucose was not affected by chronic restraint (Figure 1C) either in hippocampus or cerebral cortex slices, when the animals were sacrificed 24 h after exposure to stress ($P>0.05$, one-way ANOVA).

Glucose metabolism is essential for brain

Table 1 - Plasma levels of glucose and corticosterone in rats submitted to acute (1 h) or repeated restraint stress.

Data are reported as mean ± SEM and the number of animals is given in parentheses. *P<0.001 compared to control group (Student *t*-test); **P<0.05 compared to control and handled groups (Student-Newman-Keuls test); $^{+}$P<0.05 compared to handled group (Student-Newman-Keuls test); $^{\#}$no difference between groups (P>0.05, one-way ANOVA).

	Glucose (mg%)	Corticosterone (ng/ml)
After 1-h restraint (acute)		
Control	95.7 ± 2.42 (7)	246.0 ± 55.5 (6)
Stressed	116.1 ± 4.27* (7)	778.0 ± 61.1* (6)
24 h after a restraint session$^{\#}$		
Control	91.9 ± 2.5 (11)	356.8 ± 98.2 (10)
Handled	90.8 ± 3.5 (4)	189.3 ± 28.8 (9)
Stressed	87.3 ± 4.2 (6)	227.0 ± 32.2 (8)
Immediately after the last restraint session		
Control	90.1 ± 3.2 (9)	288.0 ± 50.3 (5)
Handled	85.7 ± 3.8 (9)	108.8 ± 32.9 (5)
Stressed	107.1 ± 4.3** (9)	232.0 ± 26.8^{+} (5)

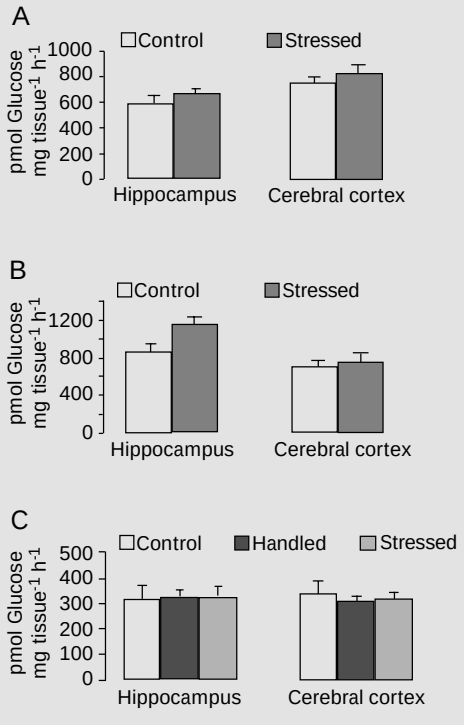

Figure 1 - Effect of acute and chronic stress on CO_2 production from glucose in slices of cerebral cortex and hippocampus. Data are reported as mean ± SEM. *A*, Immediately after 1-h restraint (acute) stress (N = 4-8 per group). *B*, Twenty-four hours after acute stress (N = 6-7 per group). *C*, Animals submitted to chronic stress and sacrificed 24 h after being submitted to the last restraint stress (N = 10-11 per group).

function and structure. In the normal state, glucose is the only significant substrate for energy metabolism in the brain. The actual glucose oxidation to CO_2 is not only sufficient to account for total O_2 consumption but is in excess. The excess glucose is probably used to produce other intermediates of carbohydrate metabolism, or used for synthesis of the chemical constituents of the brain rather than for energy production (13). The present experiments were conducted to evaluate the consequences of acute and repeated exposure to restraint stress in rats on CO_2 production from glucose in slices of cerebral cortex and hippocampus. These measurements are directly related to energy production from glucose.

Changes in the hippocampus after exposure to stress have been reported by several authors (1,2). The human hippocampus has been shown to present atrophy in different pathologic circumstances related to high cortisol levels (14,15). Moreover, a previous stress history was found to be related to impairment of cognitive functions in aging rats (16). These findings led to the development of the glucocorticoid hypothesis of brain aging and degeneration.

The mechanism by which glucocorticoids may exert their damaging effects on hippocampal neurons is thought to involve disruption of the energy metabolism of hippocampus neurons (5), and it has been suggested that a critical feature of the action of glucocorticoids on neurons might be the inhibition of glucose oxidation (4,5). Some reports suggest that glucocorticoids inhibit glucose uptake in the brain both *in vivo* (17,18) and in cultured hippocampal neurons and glia (4,5,19). Previous results showed that when glucose uptake was measured soon after restraint stress (1 h) there was no significant difference between control and stressed rats in glucose uptake, but when measured 24 h after the stress session there was a significant decrease in glucose uptake in both structures (cortex and hippocampus) in stressed rats (18). This effect is probably related to a corticosteroid-dependent effect, since many of the primary effects of adrenal steroids involve transcriptional regulation of a subset of genes in specific cell types that needs some time to be resumed. These results are in agreement with those obtained by other authors with cultures containing both neurons and glia (4,5). These authors found a time-dependent effect, with more than 4 h of steroid exposure needed for the inhibition of glucose uptake to be observed. This disruptive glucocorticoid effect could be mediated by a decreased expression of glucose transporter molecules on the cell surface. It has been shown that treatment of fibroblasts with dexamethasone for several hours reduces glucose transporters (19).

Nevertheless, there is little direct evidence to indicate whether brain energy metabolism itself is compromised after exposure to excess corticosterone or to stress. In the present study there was no difference in CO_2 production from glucose in slices of cerebral cortex or hippocampus from rats submitted to a restraint stress session when compared to the control group either 1 h or 24 h later. This result suggests that, although there may be decreased glucose uptake, as suggested by several investigators (4,5,17-19), the energy production is adequate. This fact suggests that the possible effects of glucocorticoid release on glucose transport are not sufficient to generate a physiologically important energy deficit under normal conditions. It is possible, however, that other metabolic routes are being affected (e.g., synthesis of lipids, glucoproteins, and glycogen).

The present study agrees with other reports showing that treatment with corticosterone did not influence normoxic ATP levels but potentiated the loss of ATP following metabolic insults. Using fetal neuronal cultures, Lawrence and Sapolsky (20) observed that glucocorticoid treatment alone did not affect neuronal ATP content, but acceler-

ated the rate of ATP loss in neurons in response to cyanide and aglycemia.

When rats were submitted to repeated restraint and sacrificed 24 h after exposure to stress no difference was observed in CO_2 production. It is important to note that the levels of corticosterone observed after acute restraint stress are higher than those observed after repeated restraint. In the chronic model, plasma levels of corticosterone present a modest increase immediately after restraint compared to the handled group but not when compared to the control group.

Handling is known to induce biochemical and behavioral alterations, and an increased anxiogenic response in animals naive to handling has been suggested (9,10). Although corticosterone levels did not differ significantly in chronically treated animals 24 h after the last restraint session, they were lower in the handled and stressed groups, showing that daily handling may possibly lead to an adaptation in hormonal release. Handling during the neonatal period causes alterations in the response to stress, with reduced levels of glucocorticoids, and increased density of glucocorticoid receptors in the hippocampus (11), but similar findings have not been reported in adults.

In conclusion, the present study demonstrates that although changes in glucose uptake by hippocampal and cerebral cortex cells may be part of the brain's response to stress, as demonstrated by other authors, this effect does not imply a decreased energy production from glucose, at least under these conditions, when the cells were not submitted to any other insult. Moreover, repeated exposure to the same stressful event has no effect on glucose oxidation to CO_2.

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