Short communication

Changes in hippocampal amino acid concentrations after chronic administration of corticosterone

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Abstract:
The effects of acute rise in corticosterone concentration upon the levels of hippocampal glutamate (Glu) are well described. Much less is known about the effect of chronic elevation of glucocorticoids on hippocampal glutamate. This is an important question, given the role of glutamate in the neurodegenerative and cognitive effects of chronic stressors. To this end, we have compared the effects of acute and chronic (25 days), administration of corticosterone on the concentration of glutamate, and gamma-aminobutyric acid (GABA), in the dorsal hippocampus, in freely moving rats. The acute administration of corticosterone (20 mg/kg) produced an expected increase in hippocampal concentration of extracellular glutamate and a smaller but significant enhancement of local concentration of GABA. The Glu/GABA ratio remained unchanged, indicating that the balance between excitatory and inhibitory processes was not affected. In the chronically treated animals, the baseline concentration of glutamate and the Glu/GABA ratio were increased. Most interestingly, a challenge dose of corticosterone given to the animals chronically pretreated with this hormone almost completely depleted hippocampal glutamate, and decreased the Glu/GABA ratio. In summary, the present study showed that chronic administration of corticosterone increased the hippocampal concentration of glutamate. Possible implications of this phenomenon are discussed.

Key words: corticosterone, hippocampus, microdialysis, glutamate, GABA, rat


Introduction
Circulating glucocorticoids, along with excitatory amino acids, are involved in the mechanism leading to neuronal atrophy. The coincidence between decre-
ments in cytoskeletal proteins and glutamate release in the rat hippocampus suggests possible links between high glucocorticoid levels, dendritic atrophy and the cognitive impairment reported in patients suffering from Cushing’s disease and depression [4, 6]. Chronic corticosterone (CORT) impairs also hippocampal brain-derived neurotrophic factor (BDNF) function, what is potentially relevant to the hippocampal atrophy reported in major depression [10]. In preclinical studies, chronic administration of CORT induced a significant impairment of inhibitory avoidance learning, decrease in hippocampal glucocorticoid receptor density, and in hippocampal CA3 total dendritic length [3]. It also decreased hippocampal long-term potentiation, even when measured 48 h after cessation of CORT treatment [14].

It is known that an increase in circulating and brain CORT, induced by either stress or intraperitoneal injections of this hormone, elicits an increase in the level of hippocampal glutamate, thus possibly contributing to the deleterious effects of stress on hippocampal neurons [1, 6, 12, 22, 25]. Much less is known about the effect of chronic elevation of glucocorticoids on hippocampal amino acid concentrations. To our surprise, the review of literature data on this topic (MEDLINE/PubMed) revealed that there was no information available on the effects of chronic elevation of circulating CORT on the hippocampal concentration of amino acids. This is an important gap, given the above-described role of glutamate in the neurodegenerative and cognitive effects of chronic stressors. Thus, in the present study, we aimed to analyze the effects of acute and chronic (25-day) administration of CORT on glutamate and GABA concentrations in the dorsal hippocampus in freely moving rats.

Materials and Methods

Animals

The experiment was performed on a cohort of 30 animals. Adult male Wistar rats (200 ± 20 g) were bought from a licensed breeder (the Górzkowska’s farm, Warszawa, Poland). Animals were housed 5 per cage in standard laboratory conditions under 12 h : 12 h light : dark cycle (lights on at 7 a.m.) at a constant temperature (21 ± 2°C) and 70% humidity. The rats were given free access to food and water. The experiments were performed in accordance with the European Communities Council Directive of 24 of November 1986 (86/609 EEC). The Local Committee for Animal Care and Use at Warsaw Medical University approved all experimental procedures using animal subjects.

Drugs

The corticosterone (Sigma-Aldrich, Poland) was suspended in sesame oil at a volume adjusted to 1 ml/kg of body weight and administered subcutaneously (sc) to the nape of the neck. The dose of 20 mg/kg was selected according to the previous study [20]. For vehicle injections, sesame oil (d = 0.92 g/cm³, heavy metals content < 0.001%; Sigma-Aldrich, Poland) alone was used in the same volume.

Treatments

Depending on the experiment, CORT injections were given either acutely (a single injection of 20 mg/kg) or chronically (daily injections of 20 mg/kg for 25 consecutive days). Separate groups of animals were used in the experiment with acute and repeated administration of CORT.

In the part of experiment with acutely administered corticosterone, after 4 days of acclimatization to the vivarium, the animals were divided into two experimental groups: veh-a – animals treated acutely with sesame oil; CORT 20a – rats given acutely CORT at the dose of 20 mg/kg. Next, the rats were handled for 7 days prior to experiment. During the first 4 days of handling the animals were removed from their home cages and held by an experimenter in the same way as during drug administration for 1 min. Days 5–7 of handling included subcutaneous injection of saline (0.9% NaCl) at a volume of 1 ml/kg. On the last day of handling, 90 min after saline administration, the rats were implanted with a dialysis probe. On the experimental day, the rats received injection of CORT or vehicle (Fig. 1A).

In the part of experiment with chronically administered CORT, after 4 days of habituation to the vivarium, the animals were divided into two experimental groups: veh-ch – animals treated chronically with sesame oil; CORT 20ch – rats given chronically CORT, at the dose of 20 mg/kg. Next, the rats were handled for 7 days prior to experiment. During the first 4 days of handling the animals were removed from their home cages and held by an experimenter in the same way as during drug administration for 1 min. Days 5–7 of handling included subcutaneous injection of saline (0.9% NaCl) at a volume of 1 ml/kg. On the last day of handling, 90 min after saline administration, the rats were implanted with a dialysis probe. On the experimental day, the rats received injection of CORT or vehicle (Fig. 1A).
day prior to dialysis experiment, 90 min after CORT or vehicle administration, the rats were implanted with the dialysis probe. On the experimental day, the rats received the final injection of CORT or vehicle (Fig. 1B).

Surgery and microdialysis procedure

The rats were anesthetized by intraperitoneal injection of ketamine (100 mg/kg), and fixed in a stereotaxic apparatus (Stoelting & Co., USA). The dialysis probe (hand-made dialysis probe, membrane loop of 6 mm length, U-shaped – 3 mm the tip length, pore diameter 0.8–2.0 μm, 30 kDa cut-off) [8], was implanted into the right dorsal part of the hippocampus, according to the coordinates from the atlas of rat brain [15]: 2.8 mm posterior to bregma, 2.5 mm lateral to bregma and 3.5 mm ventral to dura. The mean in vitro recovery of amino acids using the dialysis probes was about 25%. The dialysis probe was fixed to the skull with jewelry screws and dental acrylic cement. Figure 2 shows the location of the probe in the hippocampus. The microdialysis procedure started one day after surgery. The dialysis probe was perfused by Ringer’s solution (mM): sodium chloride – 147, potassium chloride – 4, calcium chloride – 2.4, pH = 6, at a flow rate of 2 μl/min with a help of the micro-syringe pump (BAS, USA) in the conscious, freely moving rats. Starting the following day after surgery, 90 min after perfusion stabilization, three consecutive samples...
were collected at 20-min intervals to measure basal levels of amino acids before CORT or sesame oil administration. Subsequently, a challenge injection of CORT (20 mg/kg, sc) or vehicle was given. Perfusate samples were collected at 20-min intervals for the next 220 min into polypropylene microcentrifuge vials and stored at –70°C until analysis. The extracellular concentrations of amino acids (Glu and GABA) were determined by a fully automated high performance liquid chromatography (HPLC) system with electrochemical detection. Additionally, the Glu/GABA ratio was calculated (the theoretical parameter defining local neuronal excitation ratio).

**Histology**

After experiment the animals were deeply anesthetized with Morbital (200 mg/kg, Biowet, Poland) and decapitated. The brains were removed, frozen in dry-ice cooled cyclopentane, and stored at –70°C. Next, the brains were sectioned with a cryostat and stained with 0.5% cresyl violet for verification of the probe placement. Probe placement was determined by microscopic examination.

**Biochemical analysis of amino acid concentrations**

HPLC analysis of amino acids was performed using a Luna 5 µm C18(2) 100A, 250 × 4.6 mm, reverse phase column, according to the procedure described previously [24]. Compounds were eluted isocratically with mobile phase delivered at 0.70 ml/min using a Shimadzu Clas VP LC 10AD pump. Electrochemical detector with a flow-through cell (Intro-Antec Leyden), linked to Shimadzu Class VP Integrator SCL-10 Avp, was used. A high-density glassy carbon-working electrode (Antec) operated at +0.85 V. Rheodyne injection valve with a 20 µl sample loops was used to manually inject the samples. Preparation of the mobile phase and the derivatizing agents was based on the method of Rowley et al. with some modifications [17]. The mobile phase consisted of 45 mM disodium phosphate and 0.15 mM ethylenediaminetetraacetic acid (EDTA) with 24% methanol (v/v) water adjusted to pH = 3.9 with 0.2 M citric acid. It was then filtered through 0.45 µm filters and degassed for 15 min. Stock solutions (0.01 M) of amino acid standards were prepared in polyethylene vials. Working solutions were prepared daily by dilutions of the stock solution. To obtain agents for derivatization; o-phthaldialdehyde (OPA, 22 mg, Fluka) was dissolved in 0.5 ml of 1 M sodium sulfite, 0.5 ml of methanol, and 0.9 ml of sodium tetraborate buffer (0.1 M) adjusted to the pH 10.4 with 5 M sodium hydroxide. The reaction of derivatization was performed at room temperature. Derivatizing agent (20 µl) reacted with 1 ml of amino acid standard for 15 min in polyethylene vial before injection onto the column. For reaction with microdialysis samples (20 µl), the volume of derivatizing agent was reduced to 0.4 µl in order to eliminate contamination of chromatogram by excessive reagent, which is electroactive. The concentration of amino acids was calculated as µM.

**Data analysis**

Dialysate data are shown in relative values, as the means ± standard error of the mean (SEM) of the percentage changes in relation to the baseline level according to the following formula: the average of the three samples preceding a challenge injection of corticosterone or vehicle was defined as 100% and used as a baseline for the following 11 samples. Basal levels of amino acids (µM) are presented as the mean ± SEM. Basal values of amino acids in veh-ch group vs. CORT 20ch group were compared using the Student’s t-test. The other data were analyzed by two-way ANOVA with repeated measures, followed by post-hoc least significant difference method (Fisher’s LSD test). For the total effect, the data are shown as the means ± SEM of the sum of percentage changes in concentrations of amino acids in dialysates (Student’s t-test). A probability value of p < 0.05 was considered significant in this study. Statistical analysis was performed with the use of Stat-Soft Statistica 7.0 for Windows (StatSoft Inc., USA).

**Results**

**Histology**

Two animals had to be excluded from evaluation due to misplacement of the probes. The probes of all other
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Fig. 3. The effect of acute administration of corticosterone at the dose 20 mg/kg on concentrations of amino acids (Glu, GABA), and Glu/GABA ratio, in the right dorsal hippocampus. The data are shown as the means ± SEM of percentage changes in relation to baseline concentration. The total effects are also shown as the means ± SEM of the sum of percentage changes in concentrations of amino acids in dialysates. Veh-a – animals pretreated acutely with sesame oil (n = 6); CORT 20a – rats given acutely corticosterone at the dose of 20 mg/kg (n = 6). * differs from veh-a. ** p < 0.01; *** p < 0.001
Animals were situated within the dentate gyrus of the hippocampus (Fig. 2B).

**Effects of acute corticosterone administration on the concentrations of amino acids**

ANOVA with repeated measures showed significant differences between veh-a and CORT 20a groups in Glu concentration: group effect \( [F(1, 10) = 31.88, (p < 0.01)] \), time effect \( [F(10, 100) = 15.59, (p < 0.01)] \), group × time interaction \( [F(10, 100) = 2.26, (p < 0.05)] \); in GABA concentration: group effect \( [F(1, 10) = 32.44, (p < 0.01)] \), time effect \( [F(10, 100) = 5.31, (p < 0.01)] \), group × time interaction \( [F(10, 100) = 2.09, (p < 0.05)] \); in Glu/GABA ratio: time effect \( [F(10, 100) = 3.60, (p < 0.01)] \), group × time interaction \( [F(10, 100) = 1.96, (p < 0.05)] \) (Fig. 3).

Post-hoc analysis revealed an increased Glu concentration in CORT 20a group in comparison to veh-a group, 60, 80, 160 and 220 min after hormone administration \( (p < 0.05) \) (Fig. 3A). Post-hoc analysis showed also an increase in GABA concentration in CORT 20a group in comparison to veh-a group, 40 and 220 min after drug administration \( (p < 0.05) \) (Fig. 3B). Post-hoc analysis of the Glu/GABA ratio did not reveal significant changes between experimental and control groups (Fig. 3C).

For the total effect, the Student’s t-test revealed a significant increase in Glu and GABA concentration in CORT 20a group \( (t = 5.65, df = 10, p < 0.01; \) and \( t = 5.70, df = 10, p < 0.01, \) respectively) (Fig. 3A, B).

**Effects of chronic corticosterone administration on the concentrations of amino acids**

**Basal levels**

Student’s t-test showed a significant increase in Glu concentration \( (t = 3.96, df = 13, p < 0.01) \), and in Glu/GABA ratio \( (t = 3.37, df = 13, p < 0.01) \) in CORT 20ch group, in comparison with veh-ch group (Fig. 4A, C). Baseline GABA concentration did not differ between experimental groups \( (t = 1.60, df = 13, p > 0.1) \) (Fig. 4B).

**Challenge with corticosterone**

ANOVA with repeated measures showed significant differences between veh-ch and CORT 20ch groups in Glu concentration: group effect \( [F(1, 13) = 54.23, (p < 0.01)] \), time effect \( [F(10, 100) = 15.59, (p < 0.01)] \), group × time interaction \( [F(10, 100) = 2.26, (p < 0.05)] \); in GABA concentration: group effect \( [F(1, 10) = 32.44, (p < 0.01)] \), time effect \( [F(10, 100) = 5.31, (p < 0.01)] \), group × time interaction \( [F(10, 100) = 2.09, (p < 0.05)] \); in Glu/GABA ratio: time effect \( [F(10, 100) = 3.60, (p < 0.01)] \), group × time interaction \( [F(10, 100) = 1.96, (p < 0.05)] \) (Fig. 4).

Post-hoc analysis revealed an increased Glu concentration in CORT 20ch group in comparison to veh-a group, 60, 80, 160 and 220 min after hormone administration \( (p < 0.05) \) (Fig. 4A). Post-hoc analysis showed also an increase in GABA concentration in CORT 20ch group in comparison to veh-a group, 40 and 220 min after drug administration \( (p < 0.05) \) (Fig. 4B). Post-hoc analysis of the Glu/GABA ratio did not reveal significant changes between experimental and control groups (Fig. 4C).

For the total effect, the Student’s t-test revealed a significant increase in Glu and GABA concentration in CORT 20ch group \( (t = 5.65, df = 10, p < 0.01; \) and \( t = 5.70, df = 10, p < 0.01, \) respectively) (Fig. 4A, B).
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**Fig. 5.** The effect of chronic administration of corticosterone at the dose 20 mg/kg on concentrations of amino acids (Glu, GABA), and Glu/GABA ratio, in the right dorsal hippocampus. The data are shown as the means ± SEM of percentage changes in relation to baseline concentration. The total effects are also shown as the means ± SEM of the sum of percentage changes in concentrations of amino acids in daily/rates. Veh-ch = animals pretreated chronically with sesame oil (n = 7); CORT 20ch = rats given chronically corticosterone at the dose of 20 mg/kg (n = 8). *p < 0.05; **p < 0.01
(p < 0.01)], time effect \[F(10, 130) = 3.51, (p < 0.01)\], group × time interaction \[F(10, 130) = 3.00, (p < 0.01)\]; in GABA concentration: group × time interaction \[F(10, 130) = 3.93, (p < 0.01)\]; and in Glu/GABA ratio: group effect \[F(1, 13) = 77.38, (p < 0.01)\], time effect \[F(10, 130) = 3.12, (p < 0.01)\], group × time interaction \[F(10, 130) = 2.40 (p < 0.05)\] (Fig. 5).

Post-hoc analysis revealed a significant decrease in Glu concentration in CORT 20ch group, in comparison to veh-ch group, at all time points from 120 to 200 min (p < 0.05), and at 220 min (p < 0.01), after hormone administration (Fig. 5A). Post-hoc analysis did not reveal any significant changes in GABA concentration between experimental and control groups (Fig. 5B). Post-hoc analysis revealed a decrease in Glu/GABA ratio in CORT 20ch group in comparison to veh-ch group, 20 min (p < 0.01), 40 min (p < 0.05), 120 min (p < 0.01), 140 min (p < 0.05), 160 min (p < 0.01), 180 min (p < 0.01), 200 min (p < 0.05), and 220 min (p < 0.01) post hormone administration (Fig. 5C).

For the total effect, the Student’s t-test revealed a significant difference between veh-ch and CORT 20ch groups: a decrease in Glu concentration (t = 7.36, df = 13, p < 0.01), and in the Glu/GABA ratio (t = 8.80, df = 13, p < 0.01) (Fig. 5A, C).

Discussion

The acute administration of CORT at the dose of 20 mg/kg produced an expected increase in hippocampal concentration of extracellular glutamate, and a smaller but significant enhancement of local concentration of GABA. The Glu/GABA ratio remained unchanged, thus indicating that the balance between excitatory and inhibitory processes in the hippocampus, was not affected. These findings are similar to the other authors’ reports on changes in hippocampal neurotransmitter system activity after a single dose of CORT or acute stress [1, 12, 22, 25]. For example, it was found that intraperitoneal or intrahippocampal administration of CORT or dexamethasone (a synthetic glucocorticoid) induced a rapid and transient increase in extracellular amino acid level. This effect was not inhibited by the specific antagonists of the two types of glucocorticoid receptors nor by a protein synthesis inhibitor, anisomycin. Such result suggests that glucocorticoids produced an increase in hippocampal concentration of amino acid levels through a non-genomic mechanism of action [19, 25]. The mechanism by which CORT might induce rapid increases in glutamate is currently unknown. Different studies have indicated that glucocorticoids could exert their rapid effects through an action upon the neuronal membrane [7], interaction with the GABA_Ψ receptor-coupled chloride channel [11] or stimulation of calcium uptake in cortical synaptosomes upon depolarization by K⁺ [23]. Furthermore, extracellular concentration of GABA was also significantly increased after acute CORT administration. Stress- and corticosterone administration-induced extracellular elevation of hippocampal glutamate may enhance the activity of the perinuclear GABAergic neurons, which modulate hypothalamic-pituitary-adrenal (HPA) axis activity by inhibiting corticotrophin releasing factor-secreting parvocellular neurons in the paraventricular hypothalamic nucleus (PVN) [1]. In our experiment, the CORT-mediated rise in glutamate levels was delayed (within 60 min) and upheld for 160 min, because the oil suspension of CORT was absorbed from the injection site at a slower rate than water-based solution, and single subcutaneous injection of a glucocorticoid hormone suspended in sesame oil produced prolonged elevation in plasma hormone concentration (for about 8 h) [5, 9].

It has been previously found that acute treatment of rats with CORT (5 and 20 mg/kg) disinhibited rat behavior controlled by fear [2, 20]. The effect of CORT was accompanied by a selective enhancement of the aversive context-induced c-Fos expression in the cingulated cortex, and some nuclei of the amygdala and hypothalamus as well as an increase in the concentration of aversive context-induced endogenous serum glucocorticoid [21]. The lack of specific changes in c-Fos expression and in the Glu/GABA ratio in the hippocampus after acute corticosterone administration [21, and this report], indicates that the presently reported increase in hippocampal glutamate concentration probably does not contribute to the effects of this hormone on rat behavior.

The more potent and characteristic changes appeared after repeated administration of CORT for 25 days. In animals chronically pretreated with CORT, the baseline concentrations of glutamate as well as the Glu/GABA ratio were increased in comparison to the control animals, indicating an enhancement of excitatory processes in the hippocampus. This is a new and
interesting finding that can explain neural atrophy in the hippocampus and the cognitive impairment reported in animal models, and in patients with hypercortisolemia. We previously found that repeatedly administered CORT (5 and 20 mg/kg) inhibited rat exploratory behavior, enhanced freezing response on retest in the conditioned fear test, and attenuated aversive context-induced c-Fos expression in some brain nuclei, including dentate gyrus [20]. It is conceivable, therefore, that the long-lasting enhancement of glutamate concentration in the hippocampus with ensuing degeneration of local neuronal circuits, could be responsible for the weaker excitatory effect of a stressor on neurons in the hippocampus (c-Fos), due to a decrease in the number or diminished reactivity of local neuronal populations. Similar mechanism could also participate in the down-regulation of the hippocampal control over the HPA axis activity, as evidenced by a significant decrease in plasma concentration of endogenous CORT in CORT-pretreated animals (20 mg/kg daily for 25 days) [20].

Most interestingly, a challenge dose of CORT given to animals chronically pretreated with this hormone almost completely depleted hippocampal glutamate, and decreased the Glu/GABA ratio. These phenomena probably reflect an enhancement of local feedback mechanisms, operating to eliminate excess of extracellular glutamate from the synaptic cleft to maintain the equilibrium between the excitatory and inhibitory processes in the hippocampus. When extracellular glutamate levels rise to about normal, glutamate overactivates glutamate receptors, triggering a multitude of intracellular events in the postsynaptic neuron, which ultimately result in neuronal cell death. This phenomenon is known as excitotoxicity and is the underlying mechanism of a number of neurodegenerative diseases. A dysfunction of the glutamate transporters is thought to contribute to cell death during excitotoxicity [18]. To allow for an efficient signaling to occur, glutamate levels in the synaptic cleft have to be maintained at the very low levels. This process is regulated by glutamate transporters, which remove excess of extracellular amino acid via a sodium-potassium coupled uptake mechanism [16, 18]. It is possible, therefore, that glutamate depletion might be due to an enhancement of glutamate acid transporters activity, and increased glutamate uptake into neuronal and glial cells [13].

In summary, the present study showed that chronic administration of the glucocorticoid hormone leads to profound changes in hippocampal concentration of glutamate. It can be also assumed that chronic corticosterone-associated increase in hippocampal concentration of glutamate is accompanied by activation of local protective mechanisms, i.e. an increased activity of glutamate transporters.

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