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Research report

Effect of intrahippocampal dexamethasone on the levels of amino acid transmitters and neuronal excitability

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Abstract

Direct effect of type-II corticosteroid receptor agonist dexamethasone on extracellular amino acid levels and neuronal excitability in the hippocampus was studied by simultaneous application of in vivo microdialysis and recording hippocampal evoked responses in freely moving male rats. Microdialysis probes and hippocampal recording electrodes were implanted to the CA1–CA3 regions of dorsal hippocampus. Local dexamethasone infusion via microdialysis resulted in a transient increase in glutamate level at 30 min, while glutamine decreased by 30–40% throughout the 180-min sampling period. Taurine increased by 50% and remained elevated up to 180 min. No significant changes were detected in extracellular concentration of asparagine, arginine, glycine, threonine, alanine and serine. In contrast, dexamethasone infusion to the striatum had no effect on the extracellular levels of amino acid transmitters. Effect of dexamethasone injected via microdialysis on the neural activity elicited by perforant path stimulation was a decrease in population spikes after 60 min starting dexamethasone infusion. Steroid effect on neural excitability was reversible. Our data indicate that local infusion of type-II receptor agonist dexamethasone has a complex effect in the hippocampus, starts with a change in extracellular glutamate and glutamine concentration and followed by a reduced synaptic excitability.

Keywords: Dexamethasone; Amino acid; Hippocampus; Striatum; Population spike; Microdialysis; ACTH; Corticosterone

1. Introduction

Corticosteroids secreted from adrenal glands, particularly during stress easily get through the blood brain barrier. Radioligand binding studies [8,46], autoradiography [34], immunocytochemical [7,44,45] and in situ hybridization [35] methods have revealed that pyramidal and granule cells of the hippocampus are distinguished brain targets of corticosteroid hormones. Corticosterone binds to two types of intracellular corticoid receptors, type-I mineralocorticoid- and type-II glucocorticoid receptors with different affinity [33]. These intracellular receptors as ligand-activated transcription factors [6] influence expression of different genes in the target neurons. Functional implication of corticosteroids in the hippocampal formation involves their effect on mood, learning and memory [4], neuronal damage [49,38] and a feed-back regulation on the hypothalamo–pituitary–adrenal axis [37] inhibiting the corticotropin-releasing factor (CRF) synthesis [10,21] and secretion [22,32] regulating neuroendocrine stress-response.

Selective hippocampal cell loss due to ageing or overexcitation is responsible for hypersecretion of glucocorticoid hormones and delayed termination of stress-response [37]. As hormones, steroids affect cellular metabolism of glucose and amino acids. Corticosterone endangers the neurotoxicity caused by overexcitation in the hippocampus [39,47]. Chronic elevation of corticosterone results damage of apical dendrite of pyramidal neurons in hippocampus [49].

Corticosteroids are also known to affect hippocampal evoked responses. Extracellular recordings revealed that low level of corticosterone enhanced, while high corticosterone concentration reduced population spike in hippocampal slice preparations [16]. Cellular responses to adrenal steroids generally involve a mineralocorticoid re-

Abbreviations: Asp, aspartate; Glu, glutamate; Ser, serine; Asn, asparagine; Gln, glutamine; Gly, glycine; Thr, threonine; Ala, alanine; Arg, arginine; Tau, taurine; GABA, γ-aminobutyric acid; HPA, hypothalamo–pituitary–adrenal axis; AA, amino acids; HPLC, high-pressure liquid chromatography.

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ceptor (MR)-mediated stimulation and/or potentiation and a glucocorticoid receptor (GR)-mediated suppression of excitability of CA1 pyramidal neurons [14,15].

Although adrenal corticosteroids affect many different processes in the hippocampus, neurochemical mechanisms underlying its effects are relatively unknown. In understanding steroid effects in the hippocampus, one of the major question is the sequence of the neurochemical and synaptic event activated by injection of steroids. To study the timing of steroid activated processes, simultaneous recording of neurochemical and electrophysiological signals are needed. Thus, in the present study, the effect of infusion of dexamethasone into the hippocampus on the extracellular concentrations of amino acid transmitters and CA3 responses were studied in the same region by combined microdialysis and electrophysiological technique. As a control, experiments were also repeated in the striatum. Some of the findings have been presented in abstract form [1].

2. Materials and methods

2.1. Animals

Adult male Wistar rats (Charles River) were used in each group (weighing 350–400 g). They were housed at 24°C and 55–75% humidity with light–dark cycles (12/12 h) beginning at 06.00 h. Animals were given pelleted rat chow and tap water ad libitum.

2.2. Surgeries

All surgeries were performed under deep pentobarbital anaesthesia (Nembutal 20 mg/kg). An indwelling silastic cannula was placed into the right atrium through the external jugular vein and exteriorized using PE 50 cannula on the neck region. At the same time concentric microdialysis probe, made of Travenol hollow fibres as described elsewhere [18] (length 2 mm, diameter 0.2 mm, 50 000 Da mol.wt. cut off) was implanted into the right CA1–CA3 region dorsal hippocampus according to the Paxinos and Watson atlas [30] (flat skull position, from bregma and dura, hippocampus: A: −4.8, L: 4.5, V: 4.5, striatum: A: 0.2, L: 3.2, V: 7). The microdialysis probe together with jugular cannula was secured to the skull with dental acrylic. Inlet and outlet tubes of the probe together with jugular cannula were run through a liquid swivel to allow collecting dialysis and blood samples from freely moving conscious animals.

2.3. Microdialysis and blood sampling

Dialysis was started 24 h after implantation. Artificial cerebrospinal fluid (ACSF) (containing 147 mM Na^+, 3.5 mM K^+, 2 mM Ca^{2+}, 1 mM Mg^{2+}, pH 7.1) with or without dexamethasone (concentration: 400 ng/μl, Oradexon®, Organon, Oss, Netherlands) or cholesterol (concentration: 400 ng/μl, Sigma) was dialysed through the probe at constant flow rate (1 μl/min) in freely moving rats. Cholesterol was dissolved in ethanol, subsequently ACSF was added to reach the appropriate concentration. The final concentration of ethanol was 0.01%. The in vitro recovery of the amino acids was approximately 30%. The outflow of the dexamethasone and cholesterol was estimated on the basis of the in vivo concentration difference between inflowing and outflowing perfusates. It was approximately 45%. After 60 min equilibration period, two dialysis samples with 30 min sampling period and one blood sample were collected to estimate baseline values at 0 min. The baseline of dialysis is derived from average of the two baseline measures. Then animals were perfused with ACSF containing 400 ng/l dexamethasone or cholesterol, dialysates and blood samples were collected at 30, 60, 90, 120, 150, 180 min. Microdialysis samples were immediately frozen, blood was collected on pre-chilled tubes containing 20% K-EDTA, centrifuged and plasma was stored at −20°C until hormone measurement.

At the end of the experiments the brain was removed and fixed in 10% formaline solution. The locations of the probes and neuronal cell loss as well as the glial reaction were verified on frozen sections after crezyl violet staining.

2.4. Amino acid analysis

Extracellular amino acid levels were determined by HPLC analysis of ortho-phtalaldehyde (OPA) derivatives of amino acids. The OPA derived amino acids were detected fluorometrically at 305–395 nm excitation and 430–570 nm emission filters. Because of the instability of OPA derivatives, the HPLC technique was automated on a Pharmacia AminoSys chromatography system specialized for amino acid analysis. Separation of amino acids was done by HP Hypersil ODS C18 reversed phase columns (200 × 2.1 mm). Eluent A was 0.1 M phosphate buffer, pH 7.2; eluent B was 70% acetonitrile in 0.1 M phosphate buffer: 0% B at 0 min, 22% B at 17 min, 50% B at 21 min, 100% B at 25 min, 100% B at 30 min, 0% B at 35 min. The column was equilibrated for 10 min in 100% B and for 10 min in 100% A. Aspartate (Asp), glutamate (Glu), asparagine (Asn), serine (Ser), glutamine (Gln), glycine (Gly), threonine (Thr), alanine (Ala), arginine (Arg), taurine (Tau), γ-aminobutyric acid (GABA) were appropriately separated (Fig. 1). Chromatograms were evaluated by PE Nelson 2000 software, the sensitivity was 0.5 pmol/sample. Infiltration of dexamethasone and cholesterol through the dialysis membrane was checked by measuring the concentration difference between in and outflow fluids using small bore chromatograph (SMART, Pharmacia LKB). Eluent A was 20 mM phosphate at pH 7 and the eluent B was 20 mM phosphate at pH 7 in 50% iso-

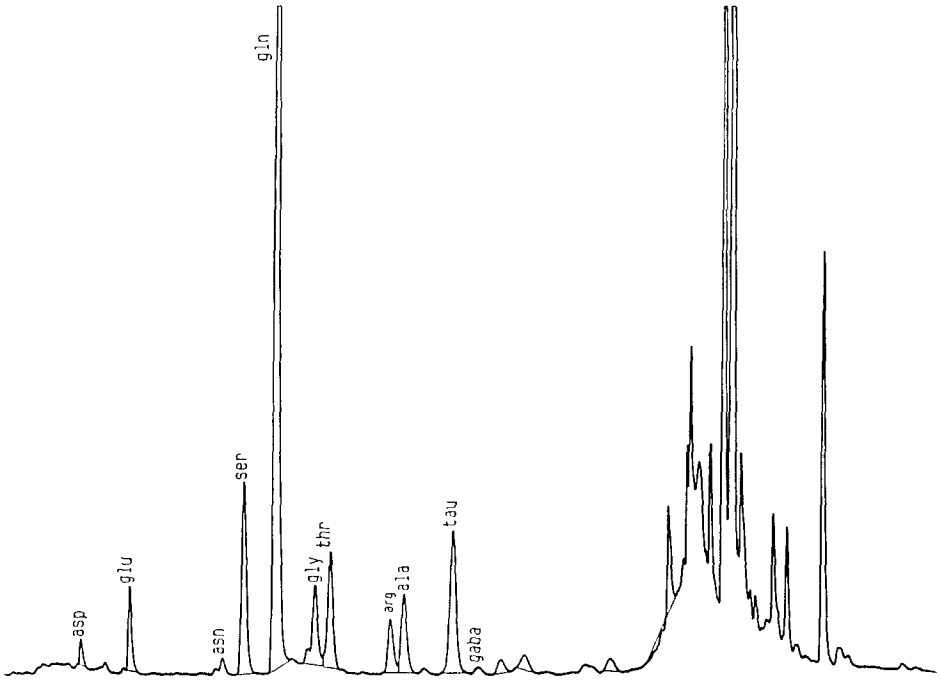

Fig. 1. Representative chromatogram of basal dialysate from hippocampus of freely moving rat. Amino acid was measured by HPLC analysis of ortho-phtalaldehyd derivatives. The separated amino acids are: Asp: aspartate, Glu: glutamate, Ser: serine, Asn: asparagine, Gln: glutamine, Gly: glycine, Thr: threonine, Ala: alanine, Arg: arginine, Tau: taurine, GABA: γ-aminobutyric acid.

propanol. Gradient: B: 0–80% in 16 min, 80% in 4 min, 80–0% in 5 min (separation was done by the same column as case of amino acid analysis).

2.5. Hormone measurements

Plasma ACTH was measured by direct radioimmunoassay (RIA) as described previously [22]. The antibody (#8514), directed against the midportion of h-ACTH$_{1-39}$ molecule, was raised in rabbit. The intra- and interassay coefficients were 4.7% and 7% respectively. Plasma corticosterone was measured by RIA without extraction. The antiserum raised in rabbit against corticosterone–carboxymethyloxime–BSA. [^{125}I]-labelled corticosterone–carboxymethyloxime–tyrosine-methyl ester was used as tracer. The interference of plasma transcortin was eliminated by inactivating transcortin at low pH. The sensitivity of the assay was 0.1 pmol. Intra- and interassay variations were 6.4% and 23.8% respectively.

2.6. Electrophysiological measurements

Separate group of rats was implanted with bipolar recording electrode attached parallel to the microdialysis probe. The distance between probe and recording electrode was approximately 300 μm. A bipolar stimulation electrode was implanted into the perforant pathway (coordinates according to [30]: A: −8.3, L: 4.5, V: −3.5, from bregma and dura). The type of the electrodes: diameter 127 μm, Stainless steel 316, (Heavy Formvar Nylon Each Conductor, Nylon Bondcoat Bifiler, California Fine Wire Company). To monitor the level of vigilance EEG electrodes (1 × 5 mm stainless steel screws 1 × 5 mm) were implanted into frontal and parietal bones. The reference electrode was placed over the cerebellum 2 mm off the midline and behind the lambdoid suture. During anaesthesia the recording and stimulating electrodes were slowly adjusted vertically to record population spike. The electrodes, connector plug and the microdialysis probe were cemented in place. A combined electric and liquid swivel was used to allow the rats to move freely. Measurements were carried out one day after the operation. Recording was done by Grass model 8B amplifiers (gain, 7 μV/mm; bandpass, 0.01–10,000 Hz) and sampled at 1 kHz with Cambridge Electronic Devices (Cambridge, UK; CED 1401) analogue to digital converter and stored in a personal computer using SIGAVAG 6.0 program. We used double pulse stimulation paradigm. The stimulus intensity was 1.5 times larger than the threshold of population spikes. The 110-ms interpulse interval was experimentally tested to get the paired pulse facilitation in each animals. The 90-μs current square wave pulse with 110-ms interpulse intervals were applied ten times with 15-s time intervals as a complete stimulation and the responses were averaged. The evoked response in CA3, population spike, was characterized by amplitude as it is described in Fig. 3A. We also calculated the paired-pulse index for the population spike which was defined as the ratio of the amplitude of the population spike evoked by the second pulse divided by that of the first pulse. Microdialysis

sampling was carried out in parallel with electrophysiological measurement. Two dialysis samples were collected before the electrical stimulation as a control to evaluate the effect of perforant path stimulation on the basal amino acid level. We applied complete stimulation twice before infusion of dexamethasone as a baseline and at 30, 60, 90, 120, 150, 180 min during the dexamethasone infusion. The baseline value of the responses is derived from the average of two baseline measures. At the end of the sampling period dexamethasone was washed out and one complete stimulation were applied on the next morning to test the recovery of response.

2.7. Statistical analysis

Amino acid levels and population spike amplitudes were expressed as percentage of the baseline because of the high individual variability of the basal values. Data are expressed as mean ± S.E.M. Repeated measures ANOVA with Tukey post-hoc test was used in the case of the amino acids and electrophysiological measurements to test the significance ($P < 0.05$).

3. Results

3.1. Effect of intrahippocampal and intrastriatal dexamethasone on extracellular level of amino acids

At the time of the microdialysis the animals were recovered from surgical stress as revealed by the baseline levels of stress hormones. During the whole experiment plasma ACTH and corticosterone levels were in unstressed baseline range (ACTH: 10–17 pM, corticosterone: 20–50 nM). The baseline amino acid levels in the hippocampal dialysates were expressed in μM: Glu: 1.24 ± 0.6, Asp: 0.34 ± 0.17, Asn: 0.44 ± 0.18, Ser: 3.15 ± 1.92, Gln: 9.25 ± 4.5, Gly: 1.3 ± 0.58, Thr: 6.65 ± 3.12, Arg: 1.23 ± 0.49,

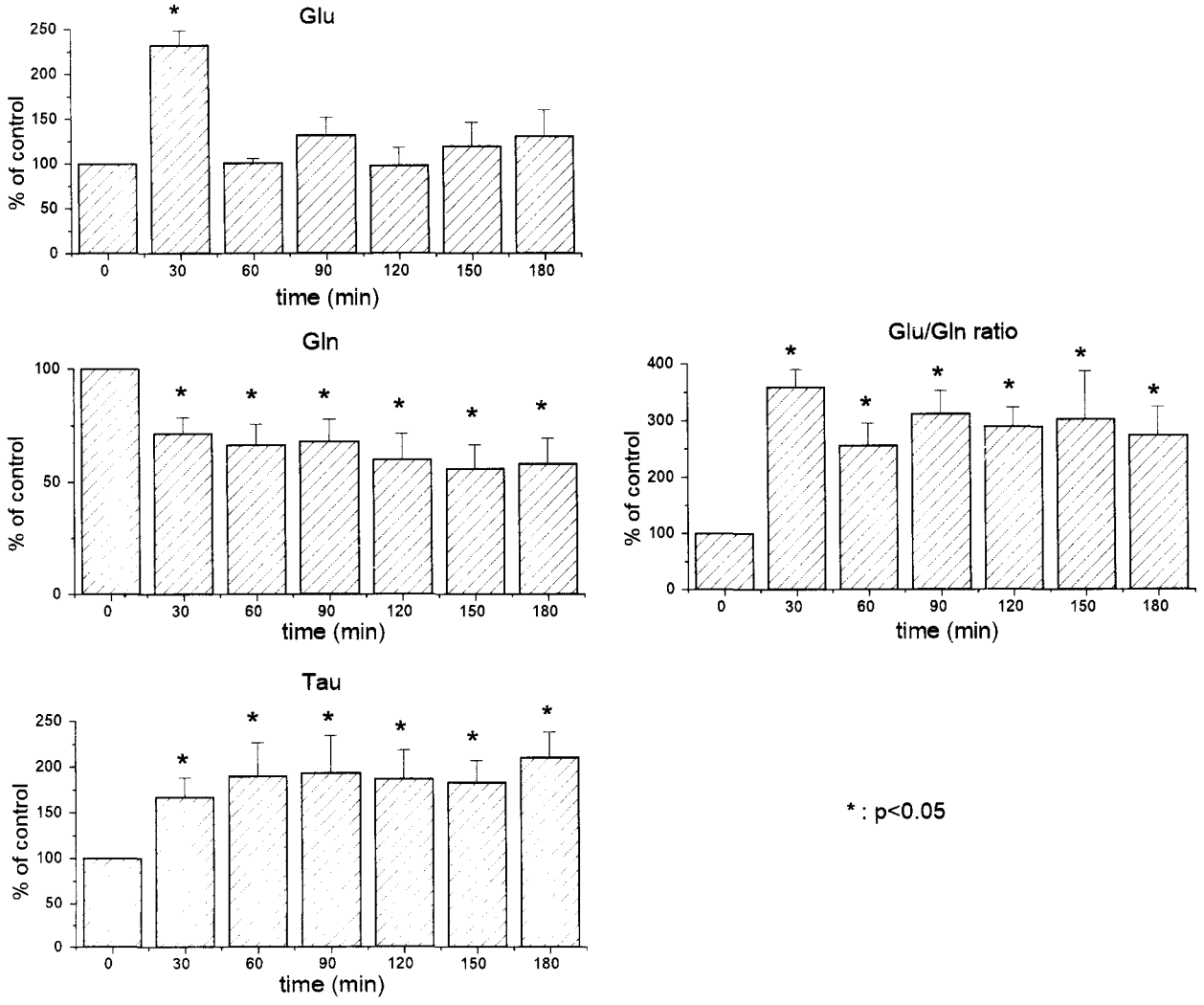

Fig. 2. Effect of intrahippocampal dexamethasone on extracellular level of Glu, Gln, Glu/Gln ratio and Tau ($n = 6$). The perfusion media (ACSF) contained dexamethasone after 0 min. The extracellular amino acid concentration expressed as percentage of the baseline. The data are expressed mean and S.E.M. Statistical analysis were assessed by using repeated measures ANOVA with Tukey post-hoc test (* $P < 0.05$).

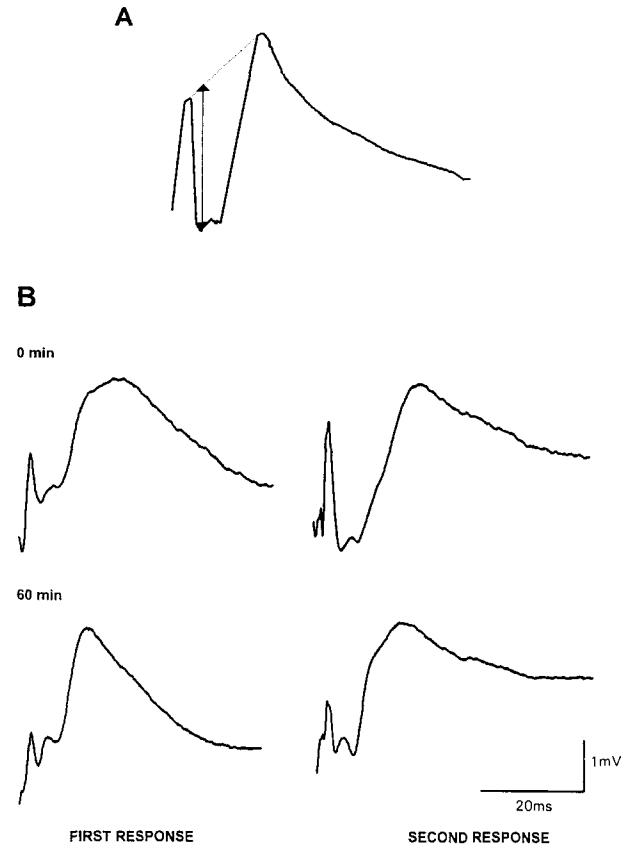

Fig. 3. A representative electrophysiological recording of averaged population spike. Double pulse stimulation paradigm was used. A 90-μs current square wave pulse with 110-ms interpulse intervals were applied ten times, then the responses were averaged. A: a typical averaged evoked response illustrating the measurement of the population spike. The double head arrow indicates the amplitude of population spike as the target of the measurement. The first dip was used for calculation of population spike amplitude as indicated by arrow. The calculation was performed from the first evoked response of the paired pulse response. B: representative paired-pulse response during ACSF infusion at 0 min and during dexamethasone infusion at 60 min (example from CA3, ten responses were averaged). Positive is up.

Ala: 0.81 ± 0.34, Tau: 4.02 ± 2.95, GABA: 0.28 ± 0.19. In some animals extracellular levels of aspartate and GABA were below the integration levels and therefore were not considered in the calculation. The Asp and GABA did not change in those animals where the Asp and GABA were measurable during the dexamethasone infusion into hippocampus.

Changes of amino acid levels were expressed as a percentage of the baseline. Unilateral infusion of dexamethasone into CA1–CA3 fields of the hippocampus resulted in a peak of extracellular glutamate level (280%) at 30 min ($P < 0.05$) (Fig. 2). Then the glutamate level decreased to the baseline in spite of the continuous dexamethasone infusion. Glutamine decreased (Fig. 2) after administration of dexamethasone at 30 min ($P < 0.05$) and remained decreased during dexamethasone infusion. Glu/Gln ratio increased at 30 min and it was elevated until the end of sampling period ($P < 0.05$) (Fig. 2). Taurine significantly ($P < 0.05$) increased (Fig. 2), while alanine, arginine, asparagine, threonine, serine, glycine did not change. Amino acid levels in samples dialyzed from the striatum do not change during dexamethasone infusion ($n = 6$, Glu: 108%, Gln: 105%, Tau: 98%). Infusion of steroid precursor, cholesterol has no effect on the extracellular level of hippocampal amino acids ($n = 5$; Glu: 103%, Gln: 97%, Tau: 110%).

3.2. *Effect of intrahippocampal dexamethasone on hippocampal excitability and amino acids levels*

Electrical stimulation of perforant path evoked a field potential consisting population spike superimposed on a positive excitatory postsynaptic potentials (Fig. 3) in CA3 region. Infusion of dexamethasone reduced population spike (50%) starting after 30 min. ($P < 0.05$) (Figs. 3 and 4). The effects of dexamethasone on population spikes was reversible: one day (24 h) after the washing out dexamethasone there was not significant change in population spikes compared to baseline conditions (data not shown).

The perforant path stimulation failed to effect the basal and dexamethasone induced amino acid changes. Population spike amplitude did not change but the glutamate significantly increased comparing to the baseline conditions at 30 min (Fig. 4). After 30–180 min the population spike significantly decreased below the baseline level while the glutamate decreased to the initial conditions (Fig. 4).

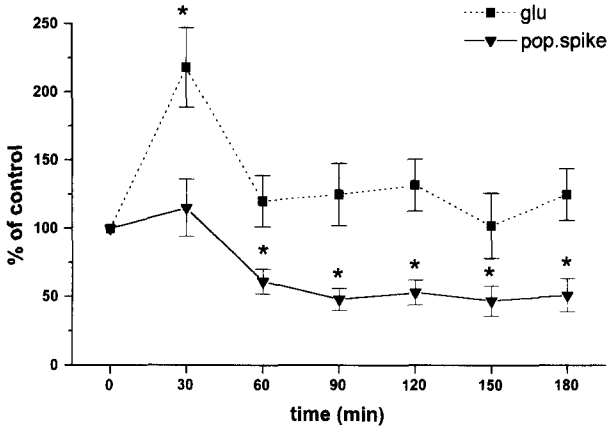

Fig. 4. Effect of intrahippocampal dexamethasone on the population spikes (pop. spike) and extracellular level of Glu in hippocampus. Double pulse stimulation paradigm was used. The complete stimulation was: 90-μs current square wave pulse with 110-ms interpulse intervals applied ten times with 15-s intervals and the responses were averaged. Complete stimulation was applied twice before infusion of dexamethasone and the responses were averaged as a baseline and at 30, 60, 90, 120, 150, 180 min during the dexamethasone infusion. The calculation of the population spikes amplitude was performed from the first evoked response of the paired pulse response. The microdialysis was parallel to the electrophysiological measurement in the same region. The amplitudes and Glu levels were expressed as a percentage of the baseline (repeated measures ANOVA, Tukey post-hoc test; * $P < 0.05$).

4. Discussion

The present study demonstrates that intrahippocampal infusion of dexamethasone increases glutamate and taurine levels while glutamine decreased. Simultaneous in vivo electrophysiological recordings showed that population spike decreased during dexamethasone infusion. The intrastriatal dexamethasone and intrahippocampal cholesterol failed to effect extracellular levels of amino acids.

According to previous reports [24,42] constant amino acids levels were detected in the extracellular space of hippocampus indicating a controlled release and uptake of Glu, Gln, Asn, Arg, Ala, Tau, Ser, Gly, Thr, GABA, Asp. In the present study, detected concentration of amino acids were in the same range as it was reported by other labs [24,42,43].

Amino acid measurements indicated that local infusion of dexamethasone rapidly and transiently elevated extracellular concentration of glutamate within the first 30 min after starting dexamethasone infusion. Comparing the timing of changes in extracellular levels of amino acids and neural excitability, reveals that the first effect of steroids in the hippocampus is a change in amino acid concentrations in the extracellular space. There are several lines of evidence suggesting that adrenal corticosteroids affect glutamate levels in the hippocampus. In adrenalectomized rats, marked attenuation of basal- and stress-induced extracellular glutamate concentration was observed [24]. Stein-Behrens et al. [43] found augmented extracellular level of glutamate in adrenalectomized rats systemically supplemented with high levels of corticosterone. Our results are consistent with these findings and suggest that type-II receptor agonist dexamethasone targets directly the hippocampus and controls extracellular glutamate.

Extracellular glutamate detected by microdialysis is not only reflecting to the synaptic overflow but it also contains metabolic glutamate in high extent [25]. Extracellular concentrations of glutamate is affected by the release and removal mechanisms. Glutamate increase at 30 min after dexamethasone infusion may reflect stimulation of glutamate release and/or inhibition of glutamate uptake [47]. It is known that in the central nervous system astrocytes exclusively contain glutamine synthase perform glutamate–glutamine conversion [36,40]. Astrocytic glutamine is presumably exported to neurons as a precursor for glutamate synthesis [48]. This 'glutamate–glutamine shuttle' is completed when glutamine, following conversion to glutamate via glutaminase [51] released as a newly synthesized glutamate at neuronal synapses. Glu/Gln ratio increased after infusion of dexamethasone suggesting that astrocytic glutamate uptake is inhibited. This is supported by the fact that glucocorticoids inhibit glutamate uptake in hippocampal astrocyte culture [47]. Glucocorticoids have a fast effect on the cell surface [26,27,50]. The rapid onset of the dexamethasone induced glutamate increase suggest a direct steroid effect on the cell membrane although the effect of dexamethasone on the astrocyte membrane is not established. On the other hand, the possibility of genomic effect in rapid action of steroids can not be excluded because glucocorticoids can induce new protein synthesis within 30 min [3]. Another possibility of elevated glutamate levels would be induction by glutamine synthase [9] however, this enzyme induction required 4 h, fairly more than the changes in glutamate and glutamine levels were found in the present study. The reduced glutamine level also makes this assumption unlikely.

Glucocorticoids increase vulnerability of hippocampal neurons [38,39], in turn, glutamate release may be involved, at least in part in neurotoxic effect of glucocorticoids via hippocampal glutamate excitotoxicity. Taurine — an inhibitory neurotransmitter — also increased in response to dexamethasone infusion. This is in agreement with previous findings of Lehman [23] and Stein-Behrens [43] and thought to be a compensatory protective response to the release of excitatory amino acid transmitters. Another possibility for elevated taurine could be hypoosmolarity induced taurine release [5,12] due to the microdialysis. The fact that dexamethasone induced taurine release was observed in the hippocampus but not in the striatum rules out this possibility. The mechanism of dexamethasone-induced extracellular elevation of taurine is unknown but astrocytic origin can be hypothesized because taurine is the amino acid found in highest concentration in astrocytes [5,11]. The delayed decrease of glutamate after the initial increase and changes in Glu/Gln ratio accompanied also with taurine increase may indicate an activation of some neuroprotective process.

There was no change in extracellular level of Asp, Ala, Arg, Asn, Thr, Ser, Gly, GABA during intrahippocampal dexamethasone administration. In agreement with our findings, intraperitoneal injection of dexamethasone failed to show effect on GABA, Gly, Asp but Glu transiently increased however Tau was not measured in hippocampus [41]. In contrast to our data immobilization and swimming stress increased hippocampal Asp level [29]. The dexamethasone insensitivity of hippocampal Ala, Arg, Asn, Thr, Ser levels is a novel finding.

Effect of corticosteroid on amino acid transmitter levels is site specific, since dexamethasone infusion into the striatum did not affect extracellular levels of amino acids. In contrast, Moghaddam [29] found elevated glutamate levels in the striatum after acute stress: this effect may not be a direct local effect of stress-induced corticosteroids rather an trans-neuronal action as it was indicated by its tetrodotoxin sensitivity. The dexamethasone induced amino acid changes are also glucocorticoid specific because cholesterol failed to affect extracellular levels of hippocampal amino acids.

Unexpectedly, neural excitability was not changed in parallel with the glutamate increase. Increased glucocorticoid levels reduced excitatory amino acid receptor mediated responses in the hippocampus (review in [17]). This

observation was supported by our findings because there was no increase of electrical activity in spite of extracellular glutamate elevation during first 30 min of glucocorticoid administration.

Evoked potentials of CA3 region are based on multisynaptic excitatory input. The first link is formed by perforant path fibres to dentate granule cells, whose axons, the mossy fibres excite CA3 pyramidal cells and result population spikes which are summation of activity of many neighbouring synchronously discharging pyramidal cells. Dexamethasone infusion resulted in a reversible reduction of population spikes in CA3. Inhibitory effect of corticosteroids on neural excitability was revealed as early as 1971, when Pfaff et al. [31] showed reduced single unit activity in the hippocampus following peripheral cortisol injection. In the early 1970s Michal [28] showed that intracerebral injection of dexamethasone reduced the multiunit activity in the hippocampus of urethane-anaesthetized rats. More recently, in vitro studies on hippocampal slices showed that low level of corticosteroids — occupying mineralocorticoid (MR) receptors — enhance neural excitability, while glucocorticoid receptors, which became occupied at higher levels of corticosterone, mediate suppression of temporarily-induced neural activity [13]. Our results confirm these data in freely moving animals. One hour after infusion of glucocorticoid receptor agonist dexamethasone to the hippocampus decreased population spike amplitude induced by perforant path stimulation. The late onset of steroid effect on neural excitability may suggest genomic action of dexamethasone. It is supported by the finding that the glucocorticoid receptor-mediated hippocampal electrophysiological effects were not detected in the presence of protein synthesis inhibitor [19]. However membrane effects also can not be excluded [26,27,50]. The major finding in our study is that dexamethasone first induces a neurochemical reaction which is followed by decreases in synaptic excitability.

High corticosterone concentration has been reported to increase the amplitude of afterhyperpolarization and accommodation [14], both attenuate transmission of excitatory signals. Considering that afterhyperpolarization is dependent on intracellular Ca^{2+} concentration [2], the high level of glucocorticoid may increase Ca^{2+} influx in hippocampus as it was shown by Joëls et al. (review in [17]). The overexposure of glucocorticoid induces intracellular Ca^{2+} which increases the probability of neurodegenerative processes in hippocampal cells [20]. Although this phenomena was detected in CA1 region it can be assumed that it is present in CA3. In our case, the population spike reduction was reversible and neuronal cell death was not detected after intrahippocampal dexamethasone application. The above mentioned compensatory neuroprotective processes and/or the relatively short administration of dexamethasone may give possible interpretation, however it is rather speculative.

The present findings provide evidences that glucocorticoids locally affect extracellular amino acid pattern and neural excitability in the hippocampus. Neurochemical reaction precedes changes in evoked responses. This effects may be implicated in learning and neurotoxic effects of stress.

Acknowledgements

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