Delayed effect of chronic administration of corticoids on the taste aversion learning

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Abstract

BACKGROUND: Long term permanent changes of eating behavior and concomitant structural changes in the CNS are matter of debade in literature. Often there is not enough distinction beween acute and chronic exposure to corticoids in evaluating its effect on behavior and/or brain structural changes. For behavioral evaluation we used well established conditioned taste aversion (CTA) paradigm and coronal Nissl-stained brain sections for evaluation of neuroanatomical changes. The CTA is part of complex adaptive behavioral processes controling food intake. It is well established methodological tool for study of biological substrates of learning and memory.

AIM: Our hypothesis was that long term changes in laboratory rat behavior induced by exogenous corticosterone are not accompanyied by neurohistological changes in the rat brain, previously described in literature.

RESULTS: Firstly, our results support CTA paradigm as promising tool for testing chronic influence of stress hormones on eating behavior and memory. The results support fact that previous long term elevated corticosterone levels disrupt normal eating behavior and it could also lead to structural changes, which could be biological substrates of behavioral changes. The fact we have not found significant morphological changes in brain strengthen the notion of possible subcellular impairment taking place instead of simple neuronal loss.

INTRODUCTION

Neurotoxicity of corticoids belongs to actually debated problems. Animal models shows, that exposition to high and/or long-term functioning doses of corticoids or intensive stress leads to structural and behavioral changes and often to hippocampal neuronal death. Also patients suffering from diseases connected with high levels of corticoids at the onset of disease (post traumatic stress disorder) or during illness course (afective disorders, Alzheimer´s dementia and also group of eating disorders, such as anorexia nervosa or bulimia (Gluck, 2006), showed statistically important shrinkage of hippocampus and worse results in tests focused on short term memory evaluation in comparison with control groups (Alfarez *et al.* 2006; Brunner *et al.* 2005).

In the literature often appears to be discrepancies in description of categories of corticoids administration as for the timing. There exists at least following possibilities concerning timing of corticoids administration to the laboratory rat: acute effect and chronic effect (Skórzewska *et al.* 2006). Acute effect of corticoids manifests primarily on non-genomic level of changes, on the contraty chronic effect of corticoids administration targets primarily genomic level, which include also onset of permanent degeneration changes (de Vries *et al.* 2002). In the acute administration there is vital dosing of the corticoids. Corticoids in a given range dose are inevitable for learning and memory formation (Payne & Nadel 2004).

Chronic corticoids administration to artificially high levels induces apical dendritic retraction and debranching (20%) in rat CA3c pyramidal neurons (Watanabe *et al.* 1992b; Woolley *et al.* 1990), while longer durations of corticoids administration result in more substantial hippocampal damage, such as neuronal death, gliosis, and atrophied perikarya in principal layers, most notably in the CA3c region (Sapolsky *et al.* 1985). Repeated stress exerts similar effects as corticoids on dendritic remodeling in CA3. One key feature of prolonged stress is the alteration in dendritic spine number and morphology in the hippocampal formation. These changes could be neurobiological substrate for behavioral changes and stress induced reactivity.

The aim of our experimental work was to find out whether long term behavioral changes induced by chronic corticosterone treatment are followed by significant gross neurohistological changes in the selected structures – hippocampus, amygdala, prefrontal cortex and parabrachial nucleus of the brain stem.

MATERIALS AND METHODS

Characteristics of experimental animals

The experiments were performed in 3-month-old male hooded rats of the Long-Evans strain. In total 21 animals were used in the study. The animals were obtained from the breeding colony of the Institute and housed in a room with constant temperature (20–22 °C) and 12 h/12 h light/dark cycle with the light switched on at 7 AM. While standard food was freely available, access to water was limited as described later. The experiments were performed in agreement with the Animal Protection Law of the Czech Republic and corresponded fully to the European Communities Council recommendations for the use of laboratory animals (directive 86/609/ECC).

Corticosterone administration

Corticosterone was administered by means of subcutaneous pellets. Pellets were inserted in the regio interscapularis. Each pellet contained 200 mg of corticosterone, release time 28 days (pellets purchased from Innovative Research of America Ltd.). Control animals received equivalent pellets containing cholesterole (pellets purchased from Innovative Research of America Ltd.)

CTA procedure

Water-deprived rats were trained for 2 days to drink their daily supply of water during a 15-min interval in the drinking box ($40 \times 30 \times 20\,cm^3$). It was equipped with an array of ten calibrated pipettes (2 ml each) arranged along the shorter wall of the box. Rats were placed into the drinking box individually and the fluid consumed from all ten pipettes was measured for each animal. On day 3, the rats were offered 0.1% solution of sodium saccharin in all pipettes. Thirty minutes after drinking the rats received i.p. injection of lithium chloride (LiCl; 0.15 M; 2% b.w.). On day 4 the animals were again offered water in the drinking box for 15 min. For the retrieval test on day 5, the pipettes were alternately filled with water and saccharin solution and the individual preference scores for each rat were expressed as the percentage of saccharin intake from the total fluid consumption.

Time schedule of experiment

First, to the animals were implanted subcutaneous pellets containing corticosterone (resp. cholesterole in control group). Animals were allowed spontaneous activity during this phase. Substances from pellets were released for 28 days, (Figure 1). Following three weeks were animals left without any behavioral manipulations. Next week followed behavioral procedure in order to reveal delayed effect of corticosterone on CTA formation. The animals were for last two weeks again left without any behavioral manipulations. Animals were enabled spontaneous activity during this phase. At this point were animals ready for histological analysis.

Histology

Animals were perfused with 4% paraformaldehyde under deep pentobarbital anesthesia. Brains were removed from the skull and embedded in paraplast. Serial sections (in coronal plane) of whole brains were performed (20 µ) on table microtome (Leica). All brain sections were then mounted on microscope slides and
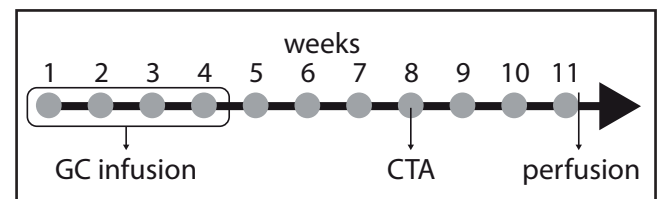

Fig. 1. Time schedule of experiment. The animals were implanted with subcutaneous pellets containing corticosterone (GC infusion) (resp. cholesterole in control group). Substances from pellets were released for 28 days. Following three weeks were animals left without any behavioral manipulations. Next week followed behavioral procedure in order to reveal delayed effect of corticosterone on CTA formation.

stained according to Nissl. Brain sections were then viewed on Leica light microscope attached to the standard PC computer with Leica IM 1000 stereology/database software (Leica Microsystems, GmbH, Wetzlar, Germany).

Neurohistology and stereology

Brain regions (prefrontal cortex (PFC) – it´s infralimbic, prelimbic and cingulate portion, CA3 of hippocampus, central nucleus of the amygdala and ncl. parabrachialis – its medial and lateral groups of subnuclei) were distinguished using Paxinos atlas of the rat brain (Paxinos & Watson 2005). We have started always with the cell numbers measurement in the middle of the selected structure of the interest of the rat brain (except for PFC) (West *et al.* 1991). Since all crossections were obtained in the frontal plane, optimal shape of the structure was approximated as ovoid (except for PFC, where we have selected lateral part of PFC according to Paxinos atlas without estimating ovoid shape). All stereology procedures were performed according to (Cerqueira *et al.* 2005). Average cell numbers were estimated using the optical fractionator method, as described previously (West *et al.* 1991). Briefly, for analysis were selected always 5 sections depending on the region being analyzed. Beginning at the central area of the structure of interest (except for PFC), a grid of virtual three-dimensional boxes (30x30x15 µm) that were equally spaced were used and neurons were counted whenever their nucleus came into focus within the counting box. Neurons were differentiated from other cells on the basis of nuclear size (larger in neurons than in glial cells), a prominent nucleolus, and the shape

of their perikarya attributable to dendritic emergence (Peinado *et al.* 1997).

Neuron number estimation and statistics

Section sampling fraction and total neuronal quantity of hippocampus, amygdala, PFC and PBN were calculated according to formula by (West *et al.* 1991). CE (coefficient of error) was calculated separately for each structure and CV, coefficient of variation was estimated as CV=SD/mean as described in (West *et al.* 1991).

A repeated measures two-way ANOVA (lateralization – right, left) as the within-subject factor and group (control and corticosterone) as the between-subject factor compared neuronal numbers in the right and left hippocampus, amygdala, PFC and PBN. Newman-Keuls test was used for posthoc analysis of significant main effects and interactions. For statistical analysis of the laterality differences, independent samples t-tests were used to examine group differences.

RESULTS

Behavioral results

Twenty one male rats were randomly assigned to two groups. First group was implanted with corticosterone pellets (n=9). Second group was control (n=12). All groups did not differ in total amount of liquid consumption as shown on Figure 2. This was confirmed by two-way ANOVA with repeated measures ($F_{1,19}=0.66$, $p>0.05$). The mean saccharin preference scores during the retrieval test performed on day 5 are shown on Figure 3. T-test (two-sample assuming unequal variances) indicated significance between groups ($t_{17}=2.94$,
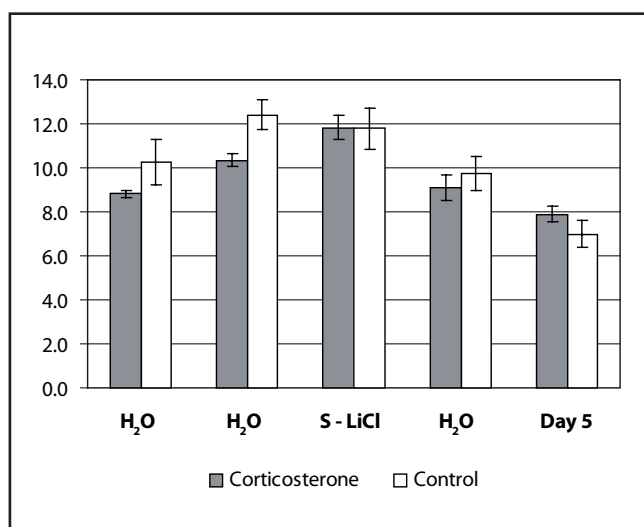

Fig. 2. Liquid consumption during experiment. The groups did not differ in total amount of liquid consumption. Each column represents the mean (±SEM) of total amount of fluid consumption in each days. Y-axis data are in ml. On the X-axis - 1st and 2nd day rats received only water, on day 3 received 0.1% solution of sodium saccharin with LiCl, 0.15 M; 2% b.w.
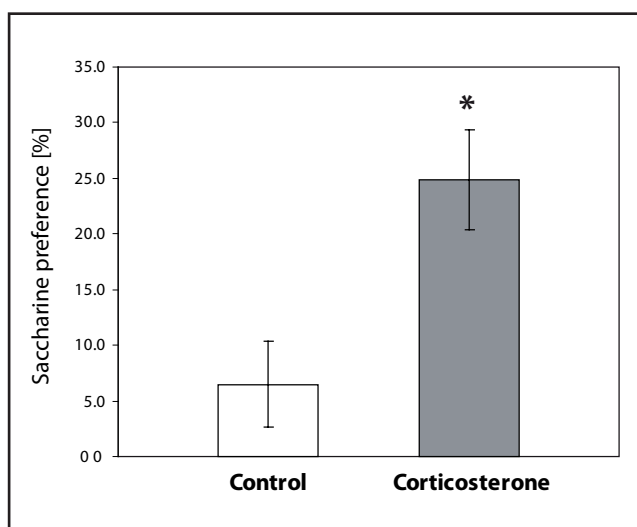

Fig. 3. The delayed effect of previous chronic corticosterone administration on CTA. Data show that corticosterone group differed from control group. Each column represents the mean (±SEM) percentage of saccharine intake from total fluid intake during the retrieval test. Y-axis shows saccharine preference in %. X-axis shows two groups – control and corticosterone.

p<0.01). These findings show delayed effect of previous chronic corticosterone administration on CTA.

Neurohistology and stereology results

In the PFC in the layers II and III there was not significant reduction of the numbers of neurons of experimental animals compared to controls. In experimental group there were only rare neurons with clear marks of degeneration (pyknosis and swelling of cytoplasm). In all subsets of neurons within CA3 field (CA3a-c) there were also solitary neurons with clear marks of degeneration compared to control group. In the central nucleus of the amygdala (in the central region, containing more densely packed cells) we have observed no neurons with clear marks of degeneration in the experimental animals compared to controls. In the lateral part of parabrachial nucleus there was small reduction (less than 2% neurons totally) of the numbers of neurons of experimental animals compared to controls and there was no difference in the numbers of neurons in the medial part of the parabrachial nucleus of experimental animals compared to controls. It is necessary to mention, that due to relatively small numbers of neurons in the parabrachial nucleus, approx. hundreds of neurons (Petrovicky & Kolesarova 1989) even small changes in the total number could be potentially functionally important.

In the first three structures (PFC, hippocampus, central nucleus of amygdalar complex) there are only rare cases of neuronal degeneration in the experimental group compared to control group. On the contrary in the parabrachial nucleus there are couple of neurons with marks of neuronal degeneration compared to control group. Summary of these results are in Table 1. We have not found significant effect of laterality on neuronal numbers in all structures.

DISSCUSION

Our results document impairment of CTA formation due to previous chronic administration of corticosterone (Figure 3). These results point to long term changes of animal inability to adequately react on different cognitive tasks, which could be associated with structural changes of the brain.

Conditioned taste aversion is well established behavioral paradigm of learning and memory and represents good tool for studying neuronal plasticity (advantages of this paradigm are mainly well described anatomical structures and physiological cellular mechanisms and easy and precise measurability) (Bureš 1998). Taste aversion is integral part of eating behavior. It is biologically important phenomenon because survival of all organisms depends on their capabilities efficiently acquire and use food and avoid poisoned one. Eating behavior and long term weight regulation and food intake is arranged by complex interactions of CNS and peripheral organs (leptin, glucocorticoid, insulin, hor-

mones of GIT) (Dostálová *et al.* 2007; Zach *et al.* 2006; Papežová *et al.* 2005). Regulation is maintained primarily by hypothalamus, prefrontal cortex, amygdala, striatum and midbrain.

Influence of stress and corticosterone on the learning and memory is in the center of scientific discussions because it participates in a number of neuropsychiatric disorders (from the point of this work especially in eating disorders). Morphological studies have shown that prolonged exposure to stress or to excess glucocorticoids results in time-dependent neuronal damage, ranging from an initial and reversible atrophy of dendritic processes (Woolley *et al.* 1990; Watanabe *et al.* 1992a; Magariños *et al.* 1995) to the irreversible loss (Landfield 1987; Sapolsky *et al.* 1985) of hippocampal pyramidal cells. This stress- or glucocorticoid-induced structural damage appears to coincide with learning and memory deficits.

CTA formation enhancement by acute corticosterone administration was shown previously (Tenk *et al.* 2006). Nevertheless, there are marked differences between acute and delayed effects of corticoids exposure on the cognitive abilities. Acute administration of corticoids has been shown to facilitate the acquisition of conditioned taste aversions to a novel-flavoured solution paired with the visceral illness inducing toxin, lithium chloride (Kent *et al.* 2002).

On the contrary design of our experiment was setup to monitor long term permanent changes of eating behavior and concomitant structural changes in the CNS. It is intensively discussed in literature, that previous elevated levels of corticoids or stress events lead to delayed changes in the eating behavior and probably participate in the pathophysiology of eating disorder (Papežová *et al.* 2005).

Tab. 1. Neurohistological changes in the brain structures 4 weeks after corticosterone application termination.
We have not found significant changes in numbers of Nissl stained neurons in any of selected brain region of animals who received corticosterone, except for nc. parabrachialis compared to controls, where was . Number of severely damaged neurons observed by Nissl staining has not exceeded 5 of them in all selected brain regions in animals, who received corticosterone.

	Ctrl NN	Exp NN	Ctrl ND	Exp ND
PFC	no significant difference			rare
Hipp	no significant difference			rare
Amyg	no significant difference			none
PBN lateral medial	slightly lower in Exp no significant difference			rare none

PFC – prefrontal cortex, Hipp – hippocampus, Amyg – amygdala, PBN – parabrachial nucleus, NN – neuronal numbers, ND – neuronal damage, Ctrl – control animals, Exp – experimental animals.

Cerebral atrophy accompanying eating disorders is early detectable. Structural morphometry of the brain has shown that volume of either white and gray matter in case of acute anorexia is severely reduced. But white matter has tendency return to normal after successful realimentation, while gray matter deficit remains. This could be partially explained by permanent loss of neurons and/or glial cells, caused for example by cortisol elevation. Alternative explanation could be: loss of neurons is not effect of anorexia, but its cause and it reflects abnormal neural system development. Our results support the fact, that previously long term elevated corticosterone levels disrupt normal eating behavior.

Previous stress event plays a role in subsequent onset of eating disorders in animals as well as in humans. Most famous are examples of pig eating disorders, described as wasting pig syndrome (WPG) and thin sow syndrome (TSS), which are connected with stress in critical period of weaning and in lactation period in adult female pigs manifesting as growth slowdown and bodily weight loss and finally loss of fertility and death. Similar situation is described for humans by Schmidt *et al.* (1997) where there is always preceding stressing life event to onset of anorexia and bulimia.

We have not found significant increase/decrease in neuronal numbers and gross neuronal degeneration in PFC, amydala nor hippocampus using Nissl staining protocol in experimental animals. These findings are in a good accord with study (Tata *et al.* 2006) where mainly ultrastructural changes are described in corticoid treated animals, although previous studies (Sapolsky *et al.* 1985), (Woolley *et al.* 1990) were able to show even gross changes in neuronal bodies and dendrites. Partly this could be due to different methodologies used.

Minor loss of neurons in lateral part of the parabrachial nucleus (not exceeding 2% neurons totally) needs further verification by means of immunohistochemistry to prove it more securely. Damage of parabrachial complex neurons nucleoli could be anticipated due to its prominent position in CTA reaction neuroanatomical circuitry.

Our results show only minor damage of CNS structures participating in taste aversion formation, but significant behavioral changes. Our study offers insight into time delayed situation, where neurodegenerative and neuroreparative processes take place. We can speculate that permanent changes in the CNS are way too subtle for gross morphological methods of detection. Another explanation is that atrophy or neuronal loss after acute stress or acute corticoids application (Sapolsky *et al.* 1985) is later almost completely reversed by reparative processes.

Our results show that longer term exposure to glucocorticoids lead later to impaired ability to form taste aversion. Contemporary findings of morphological impairment of brain structures are relatively not consistent. Meanwhile some authors describe moderate atrophy or hippocampal neuronal loss (Woolley *et al.* 1990), other studies do not confirm that (Tata *et al.* 2006). Similarly, our results show only moderate permanent changes. It is possible to conclude that even significant behavioral impairment may not be accompanied by gross neuronal changes. Our model offers possibility for further study of more precise mechanisms of corticoids effect on memory and eating behavior.

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