Neuroscience and Behavioral Physiology, Vol. 28, No. 1, 1998

EFFECTS OF CONTROLLABLE AND UNCONTROLLABLE STRESSES ON THE RECEPTOR BINDING OF DEXAMETHASONE IN THE HYPOPHYSIS AND HIPPOCAMPUS OF RATS WITH DIFFERENT BEHAVIOR STRATEGIES

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The effects of controllable and uncontrollable stress on the receptor binding of dexamethasone in the hypophysis and hippocampus were studied in KHA and KLA rats, lines selected for the ability to development of active escape. Presentation of the controllable stimulus led to a significant reduction in receptor binding of dexamethasone in the hippocampus with significant changes in the plasma corticosterone concentration and receptor binding in the hypophysis. KLA rats were sensitive both to the controllable and the uncontrollable stresses, with increases in plasma corticosterone and receptor binding of dexamethasone in the hypophysis. It is concluded that receptor binding of dexamethasone in the hippocampus and hypophysis depend not only on the behavioral strategy of the animal, but also on the possibility of controlling the situation.

Key words: *Behavioral genetics, corticosterone, receptors, hypophysis, hippocampus.*

The endocrine and behavioral responses of the body depend on the general strategy of the subject's behavior [6]. Rats of two lines, named Koltushi low avoidance (KLA) and Koltushi high avoidance (KHA), selected at the I. P. Pavlov Institute of Physiology, Russian Academy of Sciences, have different behavioral strategies in terms of the rate of developing conditioned reflex escape behavior [5]: KHA rats develop active, and KLA rats develop passive strategies [18]. Responses to changes in the environments of these rat lines depend on whether the animal can control the situation. Pathology affecting active behavior [3] and lesions in the feedback inhibition of corticosterone secretion [19] occur only in rats with active behavioral strategies and only after uncontrollable stress, and not after controllable stress.

The numerous CNS effects of corticosteroid hormones are mediated by intracellular receptors, whose localization and affinity determine the specificity of the hormonal signals responsible for maintaining homeostasis and forming endocrine and behavioral responses to environmental changes [12]. In the present work we report studies of the effects of controllable and uncontrollable stresses on the numbers of corticosteroid receptors in the hypophysis and hippocampus of KHA and KLA rats.

Some of the results obtained here have already been published [20].

METHODS

Studies were carried out on male KHA and KLA rats; these were selected and reared in the I. P. Pavlov Institute of Physiology, Russian Academy of Sciences. Animals were kept in standard animal house conditions with free access to food and water.

The apparatus used for imposing stress consisted of two chambers with a conductive floor of 13 × 16 × 26 cm for the uncontrollable and 41 × 21 × 13 cm for the controllable stress. An electric current (1 mA, 50 Hz, maximum duration 15 sec) was applied simultaneously to both chambers 60 times over the course of 1 h. Intervals between presentations of the

Laboratory of Endocrine System Physiology and Pathology, I. P. Pavlov Institute of Physiology, Russian Academy of Sciences, 199034 St. Petersburg. Translated from Fiziologicheskii Zhurnal imeni I. M. Sechenova, Vol. 82, No. 2, pp. 50-54, February, 1996. Original article submitted March 6, 1995.

 0097-0549/98/2801-0022$20.00

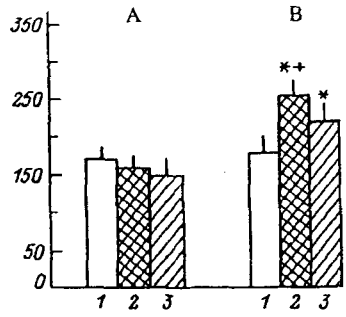

Fig. 1

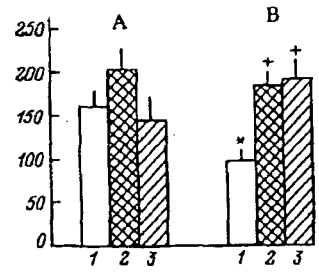

Fig. 2

Fig. 1. Plasma corticosterone levels in KHA (*A*) and KLA (*B*) rats. *1*) Intact rats; *2*) uncontrollable stress; *3*) controllable stress. The vertical scale shows the corticosterone concentration, ng/ml. *Statistically significant differences between lines; +statistically significant differences from intact animals.

Fig. 2. Binding of ^{3}H-dexamethasone in the hypophysis of KHA and KLA rats. The vertical scale indicates binding, fmol/mg protein. For further details see caption to Fig. 1.

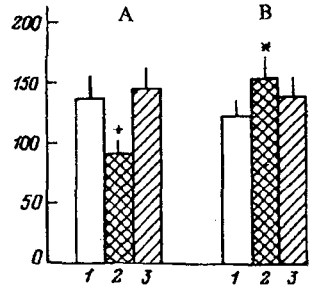

Fig. 3. Binding of ^{3}H-dexamethasone in the hippocampus of KHA and KLA rats. For further details see caption to Fig. 2.

current varied from 15 to 45 sec. In the chamber used for controllable stress, rats could move to the safe sector, while in the chamber used for uncontrollable stress, there was no possibility for the rats to avoid the electric current. An important point regarding the electrical design of the apparatus was that when a rat from the controllable stress group entered the safe sector, the electric current fed to the uncontrollable stress group was switched off, so that rats of both experimental groups received electrical stimulation of the same strength and duration, and the only difference between the controllable and uncontrollable stress groups was the possibility of controlling the situation.

Three days after stress, rats were decapitated and the brain was extracted. Plasma was collected after decapitation for assay of corticosterone using a direct (no extraction) method based on competitive protein binding. The hypophysis and hippocampus were removed from the brain on ice. Structures from 2-4 animals were homogenized in cold 10 mM tris pH 7.4 containing 10 mM Na_2MoO_4, 1 mM dithiothreitol, and 1 mM EDTA. Cytosol was prepared by centrifuging the homogenate at 60,000 *g* for 1 h. Receptor binding was determined using [1,2,4,6,7-^{3}H]-dexamethasone (specific activity 2700 TBq/mol, obtained from Izotop, Russia). Cytosol was placed in pairs of tubes, one containing 20 nM labeled dexamethasone and the other containing the same concentration of labeled hormone with a 500-fold excess of unlabeled ligand. Tubes were incubated for 18 h at 0°C. Published data [16] indicate that 18 h of incubation is sufficient for ^{3}H-dexamethasone to bind with types I and II corticosteroid receptors. Receptor-bound and unbound dexamethasone was separated on dextran-charcoal suspension (5% charcoal, 0.5% dextran). Radioactivity was counted using an Ultrabeta counter from LKB (Sweden). The level of specific receptor binding was taken as the difference in radioactivity of the samples incubated with and without excess unlabeled hormone. Specific binding was expressed in fmol steroid per mg protein in the sample.

Results were analyzed statistically using Student's test. A level of $p < 0.05$ was taken as significant.

RESULTS

Studies of the plasma corticosterone content in intact animals revealed no significant differences in this measure between KHA and KLA rats (Fig. 1). Three days after both types of stress, KHA rats showed no significant changes in the plasma corticosterone level, unlike KLA rats, where there were increases in the hormone level after both uncontrollable and controllable stress.

In the hypophysis, receptor binding of dexamethasone in KLA rats was lower than the level in KHA rats (Fig. 2). Binding in KLA rats increased after uncontrollable and controllable stress. There were no changes in receptor binding in KHA rats.

In the hippocampus, dexamethasone binding in intact rats showed no difference (Fig. 3). Three days after uncontrollable stress, KHA rats had a reduction in dexamethasone binding, while controllable stress had no effect. In KLA rats, both uncontrollable and controllable stress increased dexamethasone binding; binding was significantly higher after uncontrollable stress than in the corresponding group of KHA rats.

DISCUSSION

KHA and KLA rats have different behavioral strategies. KHA rats not only have better development of conditioned reflex active escape in a shuttle box, but they also have higher levels of movement activity, lower anxiety, and lesser ability to develop passive avoidance, as compared with KLA rats [1, 2]. Thus, the behavioral response of KHA rats to environmental changes may be determined as an active strategy, while KLA rats develop a passive strategy. Additionally, KHA and KLA rats have different sensitivities to the type of stress. Only controllable stress leads to a deficiency of escapes in the shuttle box and only in KHA rats. The different stress sensitivities of these two rat lines are supported by the fact that only KHA rats show dysfunction of the hypophyseal-adrenocortical system (HAS), and only after uncontrollable stress. While the plasma corticosterone level remains unchanged at rest, the decrease in corticosterone after administration of dexamethasone is significantly weakened [18, 19].

Our proposal was thus of interest, i.e., that only KHA rats subjected to uncontrollable stress would show a reduction in the number of corticosteroid receptors in the hippocampus -- a structure involved in regulating both the HAS and a variety of forms of behavior [8, 9, 10, 13]. Experiments using lesioning and electrical stimulation of the hippocampus have demonstrated that this structure can have inhibitory influences on the activity of the HAS. Additionally, acting by means of mineral- and glucocorticoid receptors, the hippocampus coordinates the effects of corticosteroid hormones on the adaptive component of animal behavior [11]. Reductions in the number of corticosteroid receptors in the rat hippocampus, resulting in pathological changes in adaptive behavior after one or another type of stress, have been reported by many authors [4, 11].

On the other hand, only KLA (and not KHA) rats showed increases in plasma corticosterone three days after both uncontrollable and controllable stress. In addition, these animals showed an increase in receptor binding of dexamethasone in the hypophysis in response to both types of stress. We suggest that these changes in KLA rats at the level of plasma corticosterone and hypophyseal receptor numbers occurring three days after stress are associated with a later post-stress normalization of HAS function. This process may be connected with the reduction in the initial number of dexamethasone receptors in the hypophysis of this line of rats. Corticosteroid receptors regulate HAS function by a feedback mechanism, and define the time at which stress responses are completed [7, 9]. When the numbers of corticosteroid receptors are reduced in structures responsible for controlling the HAS, there is not only an increase in the stress reactivity of this system, but also a later post-stress normalization of its functions, as has particularly been observed in sick and old animals [14, 15, 17]. Our observations of an increase in receptor binding in the recovery period after stress (at three days in the current experiments) would appear to be compensatory in nature, directed to eliminating the consequences of stress.

Thus, comparison of the effects of uncontrollable and controllable stress in rats of two lines with different behavioral strategies demonstrated significant differences in the effects of these two types of stress. The uncontrollable stress situation led to a significant reduction in the number of dexamethasone receptors in the hippocampus of KHA rats, with no significant change in the plasma corticosterone concentration or receptor binding of dexamethasone in the hypophysis. Conversely, KLA rats were sensitive to both uncontrollable and controllable stress stimulation, with increases in plasma corticosterone and the number of dexamethasone receptors in the hypophysis.

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