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Changes in Beta-Adrenergic Receptors in the Neurohypophysis and Intermediate Lobe of Rat Hypophysis Exposed to Stress

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Summary

Changes in beta-adrenergic receptors in the neurohypophysis and intermediate lobe of the rat have been characterized under physiological and stress conditions. Classical immobilization stress (IMO) was also combined with the immersion of rats into water (IMO + COLD stress). Both types of stress were applied for 30, 60 or 150 min. The intensity of stress stimuli were controlled by measuring the level of plasma ACTH. Changes in the level of plasma ACTH indicate that both types of experimental protocol induced reliable and reproducible stress conditions. Binding studies dealing with beta-adrenergic receptors in the intermediate lobe and neurohypophysis were performed in saturation binding studies by using of ^{125}I-iodopindolol. Binding parameters, maximal binding capacity (Bmax) and dissociation constant (Kd) were assessed by nonlinear analysis with computer program Viewfit. In the neurohypophysis, no changes of Kd were found in the stressed animals. However, maximal binding capacity was decreased significantly with the increased exposure to the stress. In the intermediate lobe Kd values were slightly decreased and Bmax values decreased gradually with increasing duration of stress exposure. Our findings suggest that the process of receptor desensitization of beta-adrenergic receptors can also be detected under stress conditions in the neurohypophysis and intermediate lobe of the pituitary gland where it could contribute to the mechanisms involved in stress reactions.

Key words

Stress – Neurohypophysis – Intermediate lobe – ACTH – Beta-adrenergic receptors

Introduction

One of the most frequently used approaches for the study of neuroendocrine regulations is a pharmacological approach. In our previous papers we already started to solve these problems at the molecular level, by estimating the role of cyclic AMP in the regulation of pituitary function (Klenerová and Hynie 1974) and by the study of beta-adrenergic receptors in the intermediate lobe and neurohypophysis of the rat pituitary gland (Klenerová and Hynie 1987a).

Increasing attention is being paid to the relationship between stress and neurochemical changes within the brain (Van Loon *et al.* 1989). There is also abundant literature indicating the participation of adrenergic system in stress reactions (see Brown *et al.* 1991). However, only scarce information is available concerning the changes of beta-adrenergic receptors in the intermediate lobe of the hypophysis and neurohypophysis during stress.

The intensity of stress stimuli can be assessed in intact animals by measuring the plasma level of ACTH which is one of the main stress hormones (Axelrod and Reisine 1984). ACTH release during the stress appears to be controlled by central neurotransmitters (Ježová *et al.* 1984). It can be therefore expected that in a stress situation there might be changes in the responsiveness of receptors, including beta-adrenergic receptors, to these agents.

Thus, the aim of this study was to investigate the changes in beta-adrenergic receptors in the neurohypophysis and the intermediate lobe of the rat under well characterized stress conditions.

Materials and Methods

Adult male Wistar rats weighing 220–250 g which were fed *ad libitum* were used in this study. The stress was induced by two different procedures – classical immobilization stress (IMO) lasting 30, 60 or 150 min and its combination with the immersion of rats into water 25 °C (IMO + COLD) for the same time periods. This new model using the combination of two different stressors offers stress induced by two various mechanisms (Klenerová and Šída 1990, Šída and Klenerová 1990). The use of animals in this study was in accordance with the Declaration of Helsinki and the guiding principles in the Care and Use of Animals (DHEW Publication, NHI 80-23). Each group consisted of 7 rats. Rats were decapitated at the appropriate intervals.

Monitoring of plasma levels of ACTH was performed in two separate experiments. Trunk blood was collected into ice-cold heparinized tubes and centrifuged (1000 x g, 15 min, 4 °C). Plasma was stored at −20 °C until assayed. ACTH was extracted from plasma and measured by radioimmunoassay as described elsewhere (Ježová 1985).

The neural and intermediate lobe of the hypophysis were separated with the aid of a microscope. The histological examination showed 0–10 % contamination of the neural lobe by cells from the intermediate lobe (Klenerová and Hynie 1987a).

Characterization of beta-adrenergic receptors by ^{125}I-iodopindolol was performed as described earlier (Hynie 1990, Klenerová and Hynie 1987b) and binding parameters were evaluated by nonlinear regression analysis using a program Viewfit (Hynie and Bojar 1988). Saturation binding studies were performed in triplicates with eight radioligand concentrations with pooled hypophyses from all seven animals in the group. The experiments were repeated 3–4 times and the mean values are presented. The values of maximal binding capacity (Bmax) are presented in absolute values expressed in fmol per mg protein ± S.E.M. The values of dissociation constants (Kd) are presented as geometrical means and no standard deviations can be calculated.

Proteins were estimated by the method of Lowry *et al.* (1951), human albumin being used as a standard.

Where appropriate, the data were evaluated by two-tailed Student's t-test and significance was expressed for $p<0.05$.

Results

The effects of stress on plasma ACTH levels

Since our preliminary experiments indicated differences in the beta-adrenergic receptors in neurohypophysis and intermediate lobe exposed to two kinds of stress (IMO or IMO + COLD), we decided to assess the intensity of these stress conditions by monitoring plasma ACTH levels. Table 1 shows a high increase in plasma level of ACTH in Experiments 1 and 2 in which rats were exposed to both stress conditions for periods varying from 30 to 150 min. All stressed animals had a highly significant ($p<0.001$) elevation of plasma ACTH levels when compared to controls.

Data in Experiment 2 indicate that IMO+COLD stress induced changes in plasma levels of ACTH which were not higher than those due to IMO stress. Even after 60 min, plasma ACTH levels were statistically higher ($p<0.05$) in the IMO than in IMO + COLD group.

Table 1
The plasma level of ACTH in rats subjected to immobilization stress (IMO) and immobilization stress combined with immersion into water (IMO + COLD) lasting 30, 60 or 150 min

Groups	Stress interval (min)	ACTH (pg/ml)
Experiment 1		
Controls	0	75.7±11.5
IMO+Cold	30	842.5±73.2
	60	743.8±72.0
Experiment 2		
Controls	0	114.0±28.5
IMO+Cold	60	692.0±66.2
	150	560.0±38.7
IMO	60	921.6±71.7
	150	527.0±61.2

Data are means ± S.E.M., n=7

Changes of beta-adrenergic receptors in the rat pituitary gland under various stress conditions

The binding parameters of beta-adrenergic receptors in the rat neurohypophysis and intermediate lobe of the pituitary gland were estimated from saturation studies using ^{125}I-iodopindolol under various stress conditions.

Table 2
^{125}I-iodopindolol binding in neurohypophysis of rats stressed by immobilization and water immersion

Groups	Stress interval	Bmax fmol/mg protein	Kd pmol.l^{-1}
Controls	0	38.9±8.0	105.3
IMO+COLD	30	29.7±2.5	100.6
	60	15.2±0.9	100.0
	150	16.8±5.0	90.3

The Bmax and Kd values were assessed in the neurohypophysis (Table 2) of animals exposed to IMO+COLD stress for 30, 60 or 150 min. We did not find any Kd changes in animals stressed for 30 or 60 min. In rats stressed for 150 min there was a slight decrease of the Kd value in all tree experiments performed. However, under these conditions Bmax, compared to the controls, decreased significantly ($p<0.05$) in all the stressed groups. The decrease of Bmax values after 60 and 150 min was also significantly greater than after 30 min lasting stress.

Table 3
^{125}I-iodopindolol binding in the intermediate lobe in rats stressed by immobilization

Groups	Stress interval	Bmax fmol/mg protein	Kd pmol.l^{-1}
Controls	0	97.3±7.6	146.5
IMO	60	65.2±1.6	132.9
	150	39.3±3.3	129.7

We determined the binding parameters of beta-adrenergic receptors in the intermediate lobe of the hypophysis after IMO stress lasting for 60 or 150 min (Table 3). In the intermediate lobe of control animals Bmax values were at least twice as high as those found in the neurohypophysis (see Table 2). IMO stress gradually decreased Kd values with increased time of stress exposure. Both stressed groups have significantly lower ($p<0.05$) values of Bmax than the control groups and 150 min lasting exposure caused further significant decrease ($p<0.05$) of Bmax values compared to the group exposed to IMO stress for 60 min.

Discussion

One of the basic problems of the study on the biochemical parameters in neurohypophysis and the intermediate lobe of the rat pituitary gland is their very small size and the difficulties with the separation of these two organs (Klenerová and Hynie 1987a). Therefore, several authors performed the experiments on the whole neurointermediate lobe together, which led to some errors in the interpretation of the data (Racke *et al.* 1982). Thus, our paper represents a small contribution to the regulations of beta-adrenergic receptors in both studied organs under stress conditions which are sufficient to cause changes in neuroendocrine regulations.

Changes in the levels of plasma ACTH indicate that both "classical" IMO stress and its combination with immersion in water, IMO+COLD stress, are producing sufficient and well reproducible stress. We also followed up, under the same stress conditions, the levels of costicosterone and oxytocin (Klenerová and Ježová 1994, unpublished data). The most interesting findings concern the relatively small oxytocin changes in IMO stress and several times higher hormone release in IMO+COLD stress. These findings indicate that both employed types of stress differ and deserve independent study.

Our findings dealing with binding studies of beta-adrenergic receptors in the neurohypophysis and the intermediate lobe clearly demonstrated the changes of Bmax, which indicate the alterations of beta-adrenergic receptor content in both tissues under stress conditions. If we accept the idea that the number of receptors is usually changed by homospecific stimuli, then we must expect that the catecholamines released during the stress reactions might participate in the reduction of Bmax of beta-adrenergic receptors in both lobe of the rat hypophysis. These findings suggest that the process of beta-adrenergic receptor desensitization could also take place in these tissues under stress conditions. This is in good agreement with other studies which describe the desensitization of beta-adrenergic receptors in various other brain regions due to stress stimuli (Stone and Platt 1982, Yamanaka *et al.* 1987, Stanford and Salmon 1989).

Our findings indicate that the evaluation of beta-adrenergic receptors in the neurohypophysis and the intermediate lobe of the rat hypophysis under stress conditions might be useful for disclosing some neuroendocrinological regulatory mechanisms of the pituitary gland.

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Reprint Requests

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