GENETICS

Different Effects of Glucocorticoids on the Density of β-Adrenoreceptors in the Lungs and Cerebral Cortex

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Tissue specificity of the effect of glucocorticoids on the density of β-adrenoreceptors is shown. The hormone increases the number of receptors in the lungs, but has no effect on their density in the cerebral cortex either in the norm or after down-regulation.

Key Words: *β-adrenoreceptors; glucocorticoids; adrenalectomy; desipramine; cerebral cortex*

β-Adrenoreceptors (β-AR) of the brain and peripheral tissues are involved in the regulation of various functions of the organism. The adrenoreactivity of tissue and the efficacy of adrenomimetic therapy largely depend on the density of the receptors.

However, long-term treatment with adrenomimetics reduces the density of β-AR. In clinical practice adrenoreactivity of the lungs may be successfully restored with glucocorticoids [3]. Treatment with these steroid hormones increases the number of β-AR in the lungs, heart, and cells of the vas deferens [3,6], while their effect on the brain remains unclear. The hormones are shown to prevent adrenomimetic-induced reduction of the capacity for adenylate cyclase activation [12]. At the same time, corticosterone administration during prenatal development results in a reduced number of β-AR in the cerebral cortex of one-week-old animals [1]. This effect of glucocorticoids may be due to an elevated concentration of norepinephrine in the cerebral cortex [2] and the absence of an inducing effect of the hormone on brain receptors. These facts imply tissue specificity of glucocorticoid regulation of the density of β-AR. Our study was aimed at investigating this assumption by comparing the effect of a disturbed balance of glucocorticoids on the density of β-AR in the lungs and cerebral cortex of rats.

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MATERIALS AND METHODS

The experiments were performed on mature male Wistar rats, which were caged individually under natural illumination, at 22-24°C, and with free access to food and water. In some animals the organs producing endogenous glucocorticoids, the adrenal glands, were excised under nembutal narcosis, or a sham operation was performed. The experiments were started one week after the operation. A suspension of corticosterone (Calbiochem) in 0.2% Tween-80 in 0.2 ml distilled water was injected intraperitoneally in a dose of 5 mg/100 g body weight during 7 days. An inhibitor of reuptake of catecholamines, desipramine, was injected in a dose of 1 mg/100 g body weight via the same route. Control animals received either the same volume of solvent, or nothing. The animals were decapitated and samples from the lungs and frontal cortex were isolated. β-AR were studied using ^{3}H-dehydroalprenolol (^{3}H-DHA, 75 Ci/mM,

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TABLE 1. Effect of Adrenalectomy and Corticosterone on Binding of ^{3}H−DHA in Lung and Cortex Homogenate ($M \pm m$)

Experimental conditions	Lungs		Cortex	
	B_{max}, fmol/mg protein	Kd, nmol	B_{max}, fmol/mg protein	Kd, nmol
1. Intact animals	114±2.1	2.16±0.06	93±6.5	1.85±0.20
2. Sham operation	89±8.0	1.06±0.16[1]**	119±28.7	2.12±0.73
3. Adrenalectomy	80±2.1[1]	1.10±0.04[1]	41±5.6[1,2]	1.32±0.30[1]
4. Adrenalectomy + corticosterone	123±19.8[3]	1.28±0.44[1]	41±5.6[1,2]	1.36±0.33[1]

Note. Here and in Table 2: the superscripts indicate the group number, differences between groups are significant at $p<0.05$. ** $p<0.01$.

Amersham) in concentrations ranging from 0.2 to 3.2 nM. Propranolol (10 μM, Sigma) was used as the unlabeled competitor. The method of determining and calculating the parameters of specific ligand binding was described earlier [2].

RESULTS

On day 14 after adrenalectomy the maximal binding of ^{3}H-DHA (B_{max} - the number of β-AR) with membrane preparations from homogenized lung and brain tissues dropped by 30 and 56%, respectively, in comparison with intact animals (Table 1). Replacement therapy with corticosterone during 7 days increased the density of β-AR in the lungs 1.5-fold in comparison with the adrenalectomized nontreated animals, while in the brain the reduced number of β-AR due to adrenalectomy remained practically unaffected. Evidently, the effect of adrenalectomy on the brain receptors did not result directly from hormonal insufficiency. This effect may be mediated through enhanced metabolism of norepinephrine in the brain [5], followed by down-regulation of intrinsic receptors by the transmitter. It should be noted that all the interventions used in our experiments are to some extent accompanied by altered availability of catecholamines to the receptors. This apparently caused a marked (though unreliable) reduction of the density of β-AR in the lungs of sham-operated animals. Being a stressful factor, the operation elevates the blood level of catecholamines [8] and their delivery to the lungs.

Administration of corticosterone during 7 days to intact animals increased the density of β-AR in the lungs 2-fold but did not affect it in the cerebral cortex (Table 2). Desipramine, an inhibitor of reuptake of catecholamines, inducing the accumulation of the transmitter in the synaptic gap, reduced the density of β-AR in the cortex 2-fold, but did not affect it in the lungs. These different effects of desipramine are due to different origins of the ligand for the receptors in these tissues: blood for pulmonary receptors and a presynaptic terminal for brain receptors; the latter is affected by desipramine. Combined administration of desipramine and corticosterone increased the density of the receptors in the lungs 2-fold, while the number β-AR brain receptors reduced with the inhibitor of transmitter reuptake remained unaffected.

In the lungs all experimental interventions lowered the dissociation constant (Kd) of the ligand-receptor complex in comparison with the intact animals, while in the brain Kd decreased after adrenalectomy or desipramine administration (Tables 1 and 2). The affinity of the receptors increased as the accessibility of catecholamines rose. In the lungs this was induced by either type of stress: operation or injection, while in the cortex this resulted from the blockade of reuptake of the transmitter, or, as was discussed above, from its enhanced metabolism due to adrenalectomy [8]. However, neither in the cortex nor in the brain did corticosterone influence the affinity of the receptors for the ligand.

One possible cause of the revealed peculiarities of the effect of the hormone on the density of β-

TABLE 2. Effect of Corticosterone and Desipramine on Binding of ^{3}H−DHA in Lung and Cortex Homogenate ($M \pm m$)

Experimental conditions	Lungs		Cortex	
	B_{max}, fmol/mg protein	Kd, nmol	B_{max}, fmol/mg protein	Kd, nmol
1. Solvent	99±10.2	1.04±0.17	106±8.9	2.04±0.23
2. Corticosterone	199±11.6[1]	1.28±0.12	95±20.0	1.60±0.48
3. Desipramine	102±6.0[2]	1.34±0.12	46±4.8[1,2]	1.01±0.16[1]
4. Desipramine + corticosterone	201±9.3[1,3]	0.92±0.07	50±5.4[1,2]	1.06±0.20[1]

AR in the lungs and cortex is an unequal abundance of β_1 and β_2 subtypes in these tissues. In the lungs the β_2 subtype predominates [4]. The regulatory region of the β_2-AR gene has been found to possess a glucocorticoid-dependent site [7] which is responsible for glucocorticoid-dependent induction of these receptors [9]. In the cortex β_1-AR are prevalent [11]. No glucocorticoid-dependent site has yet been found in the β_1-AR gene. Another possible reason is the absence of glucocorticoid receptors in β-AR-bearing cortical cells. There are glucocorticoid receptors in the cortex [10], but whether or not norepinephrine-reactive neurons possess these receptors remains unclear. Elucidation of which of the above two mechanisms does underlie the difference in the hormonal effects requires further investigation.

On the whole, the data obtained suggest the tissue specificity of the regulatory effect of glucocorticoids on the density of β-AR, a fact which may be of practical importance, since it proves that it is safe to use of glucocorticoids for the correction of adrenoreactivity of the lungs without affecting the density of β-AR of the brain. It should also be emphasized that the hormone does not interfere with down-regulation of the density of β-AR by desipramine, and therefore this antidepressant remains effective in combined administration with glucocorticoids.

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REFERENCES

1. N. N. Dygalo, A. A. Milova, and G. T. Shishkina, *Ontogenez*, **22**, 606 (1991).
2. N. N. Dygalo, G. T. Shishkina, and A. A. Milova, *Ibid*, **24**, 93 (1993).
3. A. O. Davies and R. L. Lefkowitz, *Ann. Rev. Physiol.*, **46**, 119 (1984).
4. C. M. Fraser and J. C. Venter, *Biochem. Biophys. Res. Commun.*, **109**, 21 (1982).
5. M. Jhanwar-Uvial, K. J. Renner, M. T. Bailo, *et al.*, *Brain. Res.*, **500**, 247 (1989).
6. J. R. Hadcock, H. Wang, and C. C. Malbon, *J. Biol. Chem.*, **264**, 19928 (1989).
7. B. K. Kobilka, T. Frielle, H. G. Dohlman, *et al.*, *Ibid.*, **262**, 7321 (1987).
8. M. Konarska, R. E. Stewart, and R. McCarty, *Physiol. Behav.*, **45**, 255 (1989).
9. C. C. Malbon and J. R. Hadcock, *Biochem. Biophys. Res. Commun.*, **154**, 676 (1988).
10. B. S. McEwen, E. R. De Kloet, and W. Rostene, *Physiol. Rev.*, **66**, 1121 (1986).
11. K. P. Minneman, M. D. Dibner, B. B. Wolf, and P. B. Molinoff, *Science*, **204**, 866 (1979).
12. E. A. Stone, M. Egawa, and C. N. Colbiornsen, *Life Sci.*, **44**, 209 (1989).