is injected against the background of lidocaine injection. These data are important for choosing the strategy of the treatment of the postischemic reperfusion syndrome.

REFERENCES

1. M. L. Belen'kii, *Quantitative Evaluation of Pharmacological Effect* [in Russian], Leningrad (1963).
2. E. I. Gendenshtein, V. P. Balashov, Ya. V. Kostin, *et al.*, *Farmakol. Toksikol.*, **53**, No. 1, 30-32 (1990).
3. E. I. Gendenshtein, Ya. V. Kostin, N. N. Markelova, *et al.*, *Ibid.*, No. 4, pp. 28-30.
4. A. I. Grinevich, I. S. Chekman, P. A. Galenko-Yaroshevskii, *et al.*, *Dokl. Akad. Nauk Ukraine*, No. 5, 169-173 (1994).
5. P. F. Litvitskii, V. A. Sandrikov, and E. A. Demurov, *Adaptive and Pathogenic Effects of Reperfusion and Reoxygenation of the Myocardium* [in Russian], Moscow (1994).
6. V. B. Prozorovskii, *Farmakol. Toksikol.*, **25**, No. 1, 115-119 (1962).

Autostimulation of Prolactin Receptors in Adrenal Cortex of Guinea Pigs

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Prolactin injected to male guinea pigs for 7 days considerably enhanced binding of ^{125}I-prolactin by adrenal cortex microsomes. Scatchard analysis showed that this rise is due to an increase in the receptor binding capacity but not in their affinity.

Key Words: *prolactin; prolactin receptors; adrenal cortex; autoregulation of receptors*

Adrenal cortex contains a considerable number of prolactin receptors (PRL). In some animal species (for example, rabbits) binding of PRL to the adrenal cortex membranes is higher than its binding to mammary glands and ovaries [10]. After this phenomenon had been described, the high affinity of PRL binding and abundance of PRL receptors in the adrenal gland were repeatedly confirmed [1,7,8,14]. Intense expression of the long form of PRL receptor in the adrenal cortex in rats has been recently demonstrated [9]. The biological role of abundant PRL receptors in adrenocorticocytes remains unclear.

The study of the prolactin postreceptory signal transduction system in the adrenal cortex provides an insight into the role of this hormone in cellular regulation in steroidogenic tissues. This system includes phosphatidylcholine hydrolysis to diacylglycerols and phosphorylcholine and activation of protein kinase C in different cell fractions [2,11,12]. This mechanisms of signal transduction is most typical for proliferative stimuli.

Prolactin receptors in standard target organs (mammary gland, gonads, and liver) are the subject of hormone regulation, and first of all, self-regulation [4,6]. However, hormone regulation and self-regulation of PRL in the adrenal cortex has been poorly investigated [3,5].

In the present study we explore the effect of long-term PRL treatment *in vivo* on PRL binding in microsomes from the adrenal cortex of male guinea pigs.

MATERIALS AND METHODS

Experiments were carried out on adult male guinea pigs weighing 300-400 g. Bovine PRL in a dose of 2 U/100 g (Kaunas endocrine plant) in 500 μl physiological saline was subcutaneously injected to the experimental animals for 7 days. Control animals received physiological solution. The purity of PRL

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was verified by disk-electrophoresis by the method of Laemmli and by corticotropin radioimmunoassay. This analysis showed that the preparation contains no adrenocorticotropin, somatotropin, and gonadotropins. After termination of PRL injections, a microsomal fraction was isolated from the adrenal cortex by differential centrifugation (100,000*g*), suspended in 25 mM Tris-HCl buffer (pH 7.4) containing 10 mM $MgCl_2$ and 0.1% bovine serum albumin, and stored at −40°C before use.

Prolactin was iodinated by the chloramine method. ^{125}I-PRL was purified by gel filtration, and its nativity was tested as described elsewhere [13,14]. Specific activity of ^{125}I-PRL (1900 Ci/mmol) was determined as previously [13].

Binding assay was carried out as described in our previous report [1]. Samples (250 μl) containing microsome suspension (75-150 μg protein), ^{125}I-PRL (100,000 cpm), and varying concentration of unlabeled PRL (4-400 ng/ml) were incubated for 16-18 h at 20°C. Specific binding was calculated as the difference between binding in the presence and absence of an excess of unlabeled PRL (5 μg/ml). The data were transformed for Scatchard analysis and approximated to a linear regression used for determination of association constant (K_a) and maximum binding capacity (B_{max}).

The significance of the differences was evaluated using nonparametric Wilcoxon—Mann—Whitney *U* and Student's *t* tests.

RESULTS

The study was performed on the microsomal fraction, since cytoplasmic membranes contain the most physiologically important pool of PRL receptors.

The binding assay revealed a single type of binding sites with K_a=0.1-0.2×10^9 M^{-1} and B_{max}=1-2×10^{-10} M (Fig. 1). Hence, PRL binding sites can be assigned to high-affinity low-capacity receptors. Similar characteristics were obtained by us for microsomes and adrenocorticocytes from guinea pig and man [1]. The above results are consistent with the parameters obtained by other investigators for different animal species [7,8,14]. The question of the true value of K_a is still discussed. In the study of H. G. Klemcke *et al.* [7] performed on porcine adrenal cortex, K_a of PRL receptors for ^{125}I-labeled sheep and porcine PRL were 2-4×10^{10} M^{-1} and 5×10^7 M^{-1}, respectively. The authors suggest that the true K_a for PRL receptors lies between these values. The K_a obtained in our experiments is very close to the predicted true K_a [7].

A 7-day course of prolactin led to an approximately 3-fold increase in ^{125}I-PRL binding. Scatchard analysis showed that this is due to an increase in the number of PRL binding sites, while the receptor affinity for the hormone remains practically unchanged (Fig. 1 and 2).

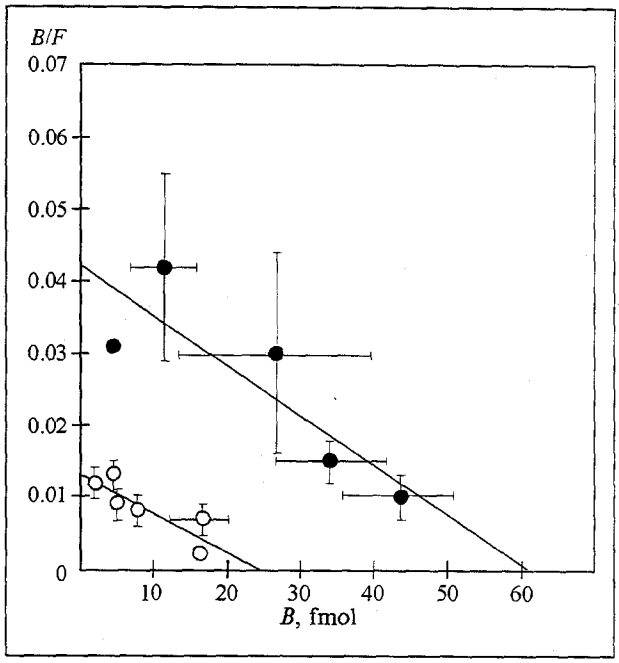

Fig. 1. Scatchard plots of binding of ^{125}I-prolactin to the microsomal fraction of guinea pig adrenal cortex. Open and dark circles correspond to control and prolactin-treated animals (2 U/100 g body weight, 7 days), respectively. *B±m* and *B/F±m* for 3 independent experiments are shown. The calculated K_a and B_{max} are: K_a= 0.12×10^9 M^{-1} and B_{max}=0.96×10^{-10} M in the control and K_a= 0.18×10^9 M^{-1} and B_{max}=2.40×10^{-10} M in experimental samples.

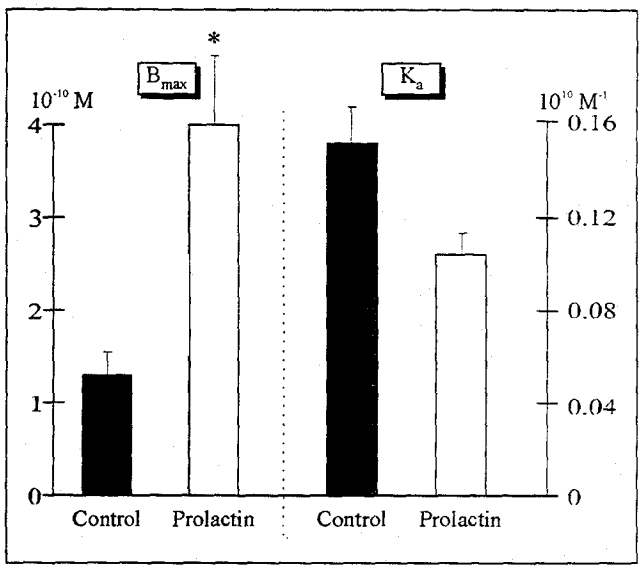

Fig. 2. Effect of prolactin (PRL) on the binding of ^{125}I-PRL to microsomes from guinea pig adrenal cortex. K_a and B_{max} are determined from individual Scatchard plots and then averaged (*n*=3); **p*<0.01 (*U* test).

Thus, our findings not only confirm the abundance of high-affinity PRL receptors in the adrenal cortex, but also demonstrate their ability to autore-

gulation. Such an ability is characteristic of hormone regulation of PRL receptors in the target tissues for PRL (mammary gland, gonads, and liver) [4,6]. Hormone regulation *in vivo* is an additional evidence for the physiological role PRL binding to adrenocorticocytes and is in an agreement with published data on PRL-dependent processes in the adrenal cortex. Autostimulation of the hormone binding provides the possibility for adaptable regulation of the function of these cells with participation of PRL.

REFERENCES

1. Yu. Yu. Sautin, N. D. Tron'ko, and A. S. Mikosha, *Byull. Eksp. Biol. Med.*, **108**, No. 8, 177-179 (1989).
2. Yu. Yu. Sautin, N. D. Tron'ko, and A. S. Mikosha, *Dokl. Akad. Nauk SSSR*, **323**, No. 3, 585-587 (1992).
3. J. C. Calvo, L. Finocchiaro, I. Luthy, *et al.*, *J. Endocrinol.*, **89**, No. 2, 317-325 (1981).
4. J. P. Hughues, H. P. Elsholtz, and H. G. Friesen, in: *Polypeptide Hormone Receptors*, B. I. Posner (Ed.), New York (1985), pp. 157-199.
5. M. Katikineni, T. F. Davies, and K. J. Catt, *Endocrinology*, **108**, 2367-2374 (1981).
6. P. A. Kelly, J. Djiane, M.-C. Postel-Vinay, and M. Edery, *Endocr. Rev.*, **12**, 235-251 (1991).
7. H. G. Klemcke, W. G. Pond, and J. A. Nienaber, *Comp. Biochem. Physiol.* [*A*], **92**, 197-206 (1989).
8. S. Ohta and K. Wakabayashi, *Endocrinol. Jpn.*, **33**, 239-249 (1986).
9. A. Ouhtit, G. Morel, and P. A. Kelly, *Endocrinology*, **133**, 135-144 (1993).
10. B. I. Posner, P. A. Kelly, R. P. C. Shiu, and H. G. Friesen, *Ibid.*, **95**, 521-531 (1974).
11. Yu. Yu. Sautin, N. D. Tronko, and A. S. Mikosha, *Biomed. Sci.*, **1**, 178-182 (1990).
12. Yu. Yu. Sautin, N. D. Tronko, and A. S. Mikosha, *Ibid.*, **2**, 198-199 (1991).
13. Y. M. L. Suard, J. P. Kraehenbuhl, and M. L. Aubert, *J. Biol. Chem.*, **254**, 10466-10475 (1979).
14. M. J. Waters, S. Lusins, and H. G. Friesen, *Endocrinology*, **114**, 1-10 (1984).