INFLUENCE OF PLASMA ON ACTH STIMULATED CORTICOSTERONE PRODUCTION OF ISOLATED ADRENAL CELLS*

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1. Introduction

Recently we have described a plasmatic 'ACTH binding factor', which prevents adsorption of ACTH to silicates and binding of ACTH to a specific antibody [1]. As the activity of this factor differed from one plasma to the other, valid radioimmunology measurement was impossible in presence of plasma. Therefore we developed a simple method for extraction of ACTH from plasma using microfine precipitated silica granules (QUSO G 32), which adsorb ACTH well [6] leaving the binding factor in the supernatant [2].

The aim of the present study was to demonstrate whether or not this factor has any implication in the regulation of biological ACTH action. A model using isolated adrenal cells as described by Sayers et al. [3] seemed to be most appropriate. In the same manner as this factor competes with QUSO or an antibody for ACTH, it may compete with the receptor of the adrenal cell surface, thus inducing an inhibition of corticotrophic activity.

2. Material and methods

Isolated adrenal cells were prepared according to Sayers et al. [3]. Female rats (BW 150–180 g, strain FW 49 Kirchb. Bib. Lemgo) were used throughout. The final cell suspension contained 100 000–150 000 cells/ml and 1 mg/ml trypsin inhibitor. Incubations were carried out for 2 hr at 37°C in a Warburg metabolic shaker with 0.9 ml cell suspension, 0.1 ml ACTH solution and in some experiments an additional 0.1 ml heparin plasma or QUSO supernatant or QUSO-eluate from plasma.

2.1. *Extraction of ACTH from plasma*

1.5 ml plasma were stirred for 5 min with 1.0 ml QUSO suspension containing 50 mg QUSO in 0.125 M phosphate buffer (pH 7.4). After centrifugation the supernatant was discarded and the sediment washed briefly with 3.0 ml phosphate buffer (0.125 M, pH 7.4). The ACTH was eluted from the QUSO granules by stirring with 1.5 ml 0.1 HCl – 0.9% NaCl for 5 min.

2.2. *Estimation of 'binding activity' of plasma*

0.5 ml Plasma were diluted 1:5 in 0.02 M barbital buffer containing 400 KIU Trasylol/ml, and 0.02% mercaptoethanol to prevent incubation damage. After 1 hr incubation at room temperature with [^{125}J]α_p^{1-39}-ACTH (labelling procedure has been described elsewhere [4]), the 'free' ACTH was adsorbed to 50 mg talcum powder. It has been demonstrated previously that under these conditions degradation of [^{125}J]ACTH was negligible [1]. As 'control plasma' a 5% bovine serum albumin – 0.9% NaCl – solution (BSA–NaCl) was used.

Hypophysectomy of rats was carried out by a semistereotactic transauricular method as described recently [5].

3. Results

Addition of heparin plasma to the suspension of

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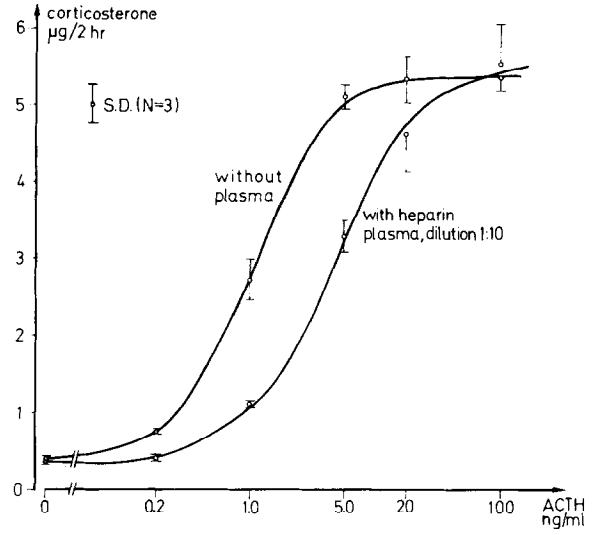

Fig. 1. Influence of heparin plasma on ACTH-stimulated corticosterone production of isolated adrenal cells. The samples without plasma contained a 5% BSA–0.9% NaCl-solution instead.

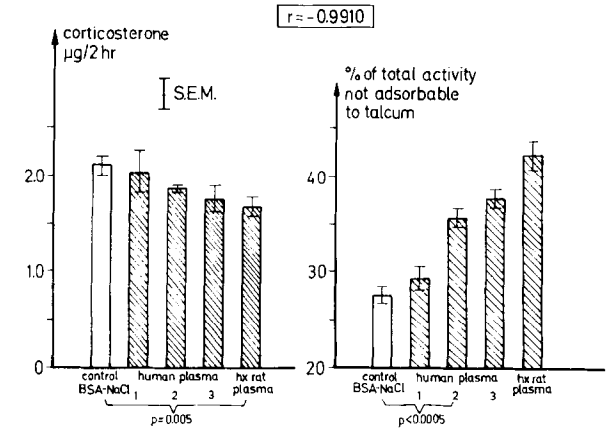

Fig. 3. Left part: inhibition of the steroidogenic activity of 2 ng/ml ACTH by 3 different human plasma samples and plasma from hypophysectomized rats. Each plasma sample was shown to contain no ACTH. Right part: inhibition of adsorption of $[^{125}J]\beta^{1-24}$-ACTH to silicates by the same plasma samples as in the keft part. There is a linear correlation between inhibition of steroidogenic activity and inhibition of adsorption ($r = -0.9910$).

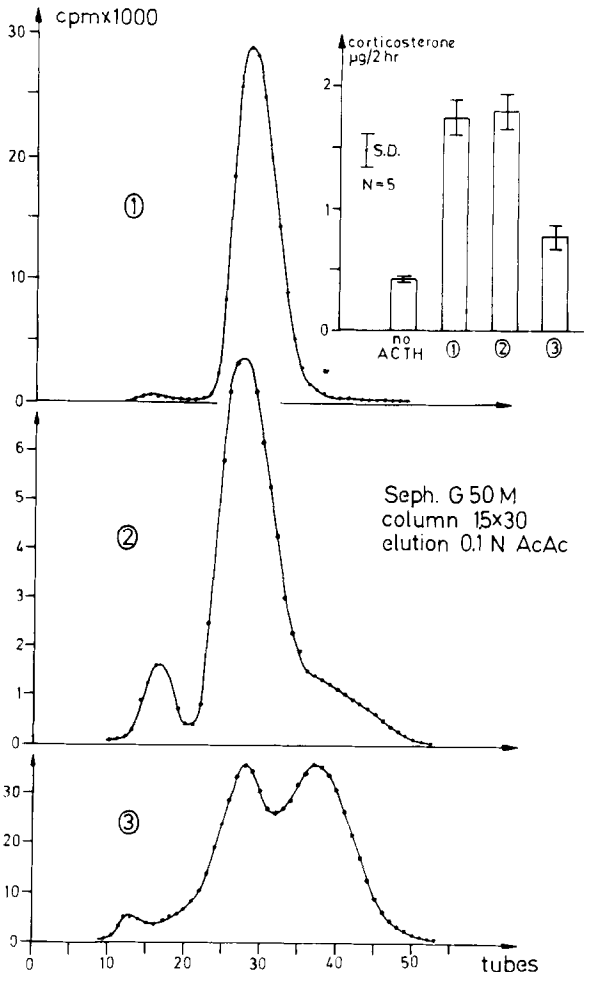

Fig. 2. Radiochromatography of $[^{125}I]\alpha_p^{1-39}$-ACTH incubated 2 hr in: (1) BSA–NaCl, diluted 1:10 in KRB buffer; (2) Heparin plasma, diluted 1:10 KRB buffer with 1 mg/ml trypsin-inhibitor; (3) Heparin plasma, diluted 1:10 in KRB buffer without trypsin-inhibitor. Right upper part: 2 ng/ml unlabelled ACTH were incubated under the same conditions. 0.1 ml of solution (1) or 0.1 ml of QUSO-eluate from solution (2) and (3) were added to suspension of adrenal cells to assess the biologic activity.

adrenal cells induced a remarkable inhibition of the steroidogenic activity of ACTH (fig. 1). Heparin in concentrations as they occur in these experiments was shown to be without effect.

To estimate the influence of degradation of ACTH by proteolytic enzymes, $[^{125}J]\alpha_p^{1-39}$-ACTH was incubated in heparin plasma, diluted 1:10 in KRB-buffer with 1 mg/ml trypsin-inhibitor: radiochromato-

Table 1
Behavior of ACTH and 'inhibition factor' during treatment of 1.5 ml plasma with 50 mg QUSO G-32.

	Corticosterone µg/2 hr	$\bar{X}$	±S.D.
Control with ACTH 2 ng/vial	2.44 2.19 2.62 2.42	2.42	0.17
+0.1 ml hx Rat plasma	1.46 1.54 1.46	1.49	0.04
+0.1 ml QUSO supernatant from hx rat plasma	1.62 1.58 1;47	1.56	0.10
+0.1 QUSO eluate from hx rat plasma	2.58 2.57 2.42	2.52	0.10
Control without ACTH	0.43 0.42 0.41 0.43	0.42	0.01
+0.1 ml QUSO eluate from hx rat plasma containing 20 ng/ml ACTH	2.25 2.03 2.10 2.09	2.12	0.10

graphy revealed that only a negligible amount of ACTH was degraded in the presence of trypsin-inhibitor as compared to the control (incubation in a 5% BSA–0.9% NaCl solution diluted 1:10). On the other hand most of the ACTH was destroyed in the absence of trypsin-inhibitor (fig. 2). Furthermore, 2 ng/ml unlabelled ACTH were incubated under the same conditions and the biologic activity on isolated adrenal cells was assessed: 0.1 ml of the control incubate were as potent as 0.1 ml of the QUSO eluate derived from the incubate with plasma and trypsin-inhibitor, whereas most of the steroidogenic activity was destroyed by plasma in absence of trypsin-inhibitor (right upper part of fig. 2).

The inhibitory activity differed from one plasma sample to the other, as did the binding activity (fig. 3). There was a linear correlation between these two activities: with increasing the binding activity also the inhibitory activity increased.

Index of correlation in this experiment was $r = -0.9910$.

In contrast to ACTH the plasmatic inhibitory factor was not adsorbed to QUSO and remained in the supernatant (table 1). The QUSO eluate had no inhibitory activity. The stereoiodogenic activity of 0.1 ml of the QUSO-eluate from hx rat plasma to which 20 ng/ml ACTH had been added, was assessed correctly. The somewhat lower response was due to losses during extraction which have been estimated by tracer studies to amount constantly to 35%.

4. Discussion

A factor has been demonstrated in plasma which inhibits ACTH-mediated corticosterone production of isolated adrenal cells. This is in agreement with the findings of Sayers et al. [3] and Giordano and Sayers [7], whereas Kitabchi et al. [17] did not note interference of ACTH action with plasma factors. The phenomena described by us might be the consequence of the degradation of ACTH by plasmatic proteolytic enzymes, as ACTH is destroyed rapidly in plasma [8]. Moreover, only slight alterations of the ACTH molecule as e.g. deamidation are followed by losses in biologic activity [9]. However, our results indicate that the trypsin-inhibitor preserved the chromatographic pattern of labelled ACTH as well as the biologic activity of unlabelled ACTH under the conditions employed. Accordingly degradation as the responsible cause could be excluded.

The activity of the inhibitory factor differed from one plasma to the other. Thus valid measurement of plasma ACTH levels was impossible in the bioassay system using isolated adrenal cells. However, as the inhibitory factor in contrast to ACTH is not adsorbed to QUSO, QUSO-extraction of ACTH from plasma successfully circumvents this difficulty.

From the studies presented, it might be presumed that binding and inhibitory activity are two aspects of the same plasma factor. This hypothesis is strongly supported by the fact that these two activities correlated well. Of course, no actual proof can be offered unless the factor is isolates.

There are many studies on the influence of plasma on different aspects of ACTH action [10–16]. It seems feasible to assume that at least some of them pertain to the same phenomenon as that described by us. There is a definite likelihood that the plasmatic ACTH-binding or inhibitory factor has important

implications on the function of the adrenal cortex, both physiologically and pathophysiologically. At this time, however, nothing definite can be cited to the physiological significance of this factor.

References

[1] Fehm, H.L., Voigt, K.H. and Pfeiffer, E.T. (1972) Hormone Metab. Res. 4, 477.
[2] Voigt, K.H., Fehm, H.L., Reck, R. and Pfeiffer, E.F. J. Clin. Endocrinol. Metab. (in press).
[3] Sayers, G., Swallow, R.L. and Giordano, N.D. (1971) Endocrinology 88, 1063.
[4] Voigt, K.H., Fehm, H.L. and Pfeiffer, E.F. (1971) Horm. Metab. Res. 3, 313.
[5] Baetzner, P., Fehm, H.L., Voigt, K.H., Schleyer, M. and Pfeiffer, E.F. (1972) Acta Endocrinol. 70, 231.
[6] Rosselin, G., Assan, R., Yalow, R.S. and Berson, S.A. (1966) Nature 212, 355.
[7] Giordano, N.D. and Sayers, G. (1971) Proc. Soc. Exptl. Biol. Med. 136, 623.
[8] Meakin, J.W., Tingey, W.H. Jr. and Nelson, D.H. (1960) Endocrinology 66, 59.
[9] Graf, L., Hajós, G., Patthy, A. and Cseh, G. (1973) Horm. Metab. Res. 5, 142.
[10] Segal, B.M. and Christy, N.P. (1968) J. Clin. Endocrinol. 28, 1465.
[11] Upton, G.V., Hollingsworth, D.R., Lande, S., Lerner, A.B. and Amatruda, Th.T. (1970) J. Clin. Endocrinol. 30, 190.
[12] Doerner, G., Poppe, J. and Stahl, F. (1968) Acta Biol. Med. Germ. 21, 405.
[13] Benuzzi-Badoni, M., Felber, J.P. and Vanotti, A. (1968) in: Pharmacology of Hormonal Polypeptides and Proteins (Back, Martini and Paoletti, eds.), p. 138, Plenum Press, New York.
[14] Landau, B. (1970) J. Endocrinol. 47, 389.
[15] Werder, K., Schwarz, K. and Scriba, P.C. (1968) Klin. Wochenschr. 46, 940.
[16] Werder, K., Kluge, F., Schwarz, K. and Scriba, P.C. (1968) Klin. Wochenschr. 46, 1028.
[17] Kitabchi, A.E., Sharma, R.K. and West, W.H. (1971) Hormone and Metab. Res. 3, 133.