Effect of Cerebellin on the Pituitary-Adrenocortical Function in Adult Rats and the Proliferative Activity of Immature and Regenerating Rat Adrenal Cortex

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ABSTRACT

We have investigated the effects of cerebellin on the pituitary adrenal axis and the proliferative activity of immature and regenerating adrenal cortex in the rat. The acute bolus administration of cerebellin did not alter ACTH plasma concentration, but at its higher dose (15 nmol/kg) the peptide enhanced the blood levels of both aldosterone and corticosterone. Three injections of cerebellin (10 nmol/kg) markedly increased the mitotic index (% of metaphase-arrested cells) in the zona glomerulosa of immature rat adrenals. This treatment did not alter either aldosterone or corticosterone plasma concentrations in rats bearing regenerating adrenals, but did it decrease the proliferative activity at day 8 of adrenal regeneration. We conclude that cerebellin exerts its adrenocortical secretagogue and proliferogenic actions acting directly on the adrenal gland of adult and immature rats, independently of any effect on the pituitary ACTH release. The lack of secretagogue effects and the clearcut antiproliferogenic action observed in rats with regenerating adrenals may be ascribed to the effects of cerebellin on the various mechanism involved in this process.

Cerebellin, a 16-amino acid peptide originally isolated from rat cerebellum (14), has been subsequently found to be widely distributed in the central nervous system, where it seems to exert neuromodulatory functions (3, 9).

The presence of cerebellin in the peripheral tissues is controversial (15). However, cerebellin protein and mRNA has been detected in human adrenal medulla (13). Accordingly, it has been demonstrated that cerebellin enhances catecholamine release by human and rat adrenomedullary fragments (1, 8). These investi-

gators also provided evidence that cerebellin stimulates steroid hormone secretion by adrenal slices, but not by dispersed adrenocortical cells.

All the above summarized studies were carried out *in vitro*. Hence, it seemed worth while to investigate the possible effects cerebellin *in vivo* on the pituitary-adrenocortical axis (PAA) of adult rats and the proliferative activity of immature and regenerating rat adrenals.

MATERIALS AND METHODS

Adult female Wistar rats (2-month-old, bred in our laboratory) and their offspring (20-day-old) were kept under 12 : 12 h light-dark cycle (illumination onset at 8:00 a.m.) at 23°C, and maintained on a standard diet (Rat-Mouse Chow, Zoofarm, Padua, Italy) and tap water *ad libitum*.

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The protocol of the experiment was approved by the local Ethical Committee for Animal Studies.

Adult rats were given subcutaneous (*s.c.*) injections of 0.2 mL isotonic saline for two weeks in order to dampen the response of their PAA to the handling stress. At day 15, they were divided in three groups, two of which were given a *s.c.* injection of 0.2 mL isotonic saline, the vehicle where 5 or 15 mmol/kg cerebellin (Bachem AG, Bubendorf, Switzerland) were dissolved. Control groups received a *s.c.* injection of the saline vehicle. All the injections were made at 9:00 a.m., and rats were decapitated 60 or 120 min after the injection (8 rats for each time point).

Under ether anaesthesia, the left adrenal gland was enucleated in a number of rats, and the control atelal gland removed. Operated rats were given 0.9% NaCl to drink, and were killed 5 or 8 days after surgery. Groups of immature and operated rats ($n=8$) were given three *s.c.* injections (28, 16 and 4 h before the sacrifice) of 0.2 mL isotonic saline where 10 nmol/kg cerebellin were dissolved. Control groups received *s.c.* injections of the saline vehicle. Animals were given an intraperitoneal injection of 0.1 mg/100 g vincristin (Gedeon-Richter, Budapest, Hungary) 180 min before the autopsy.

Rats were decapitated at 11:00 a.m., and the trunk blood was collected in the presence of EDTA (1 mg/mL). Plasma was separated and stored at -30°C until radioimmune assay (RIA). Adrenal glands were promptly removed and freed of pericapsular fat.

ACTH was extracted from plasma (2), and its concentration measured by RIA: ACTH Double Antibody kit (Diagnostic Products Company, Los Angeles, CA, U. S. A.). Sensitivity: 8 pg/mL. Cross-reactivity: ACTH(1-24), 100%; (α -MSH, 0.3%; other hormones, less than 0.001%. Intra- and interassay variations: 6.2% and 9.1%, respectively. Aldosterone and corticosterone were extracted from plasma (10), and their concentrations measured by RIA, as described previously (7). Intra- and interassay variations were: aldosterone, 5.2% and 7.5%; corticosterone, 7.3% and 8.9%, respectively.

Immature adrenals and regenerating glands were fixed in Bouin's solution for 24 h, embedded in paraffin and sectioned at 5–6 μm . Sections were stained with hematoxylin and eosin. The mitotic index (% of metaphase-arrested cells) was calculated at 400 $\times$, by counting 5,000 cells in the zona glomerulosa of each immature gland and in

the regenerating adrenal parenchyma.

Data are expressed as means $\pm$ SEM ($n=8$), and their statistical comparison was done by ANOVA, followed by the Multiple Range Test of Duncan. P values <0.05 were considered significant.

RESULTS

The acute administration of cerebellin did not significantly affect the plasma concentration of ACTH (Fig. 1, upper panel). In contrast, the higher dose of cerebellin induced an about 2.5-fold increase in aldosterone blood level at both 60 and 120 min (Fig. 1, middle panel), and a 4.0-fold rise in corticosterone plasma concentration at 60 min (Fig. 1, lower panel). Cerebellin did not significantly alter either aldosterone or corticosterone plasma concentration in rats bearing regenerating adrenals (Fig. 2).

Cerebellin markedly raised (70%) mitotic index in the zona glomerulosa of immature rat adrenals (Fig. 3). As expected proliferative activity was higher at day 5 than at day 8 of adrenal regeneration. Cerebellin did not alter mitotic index at day 5, but reduced it by about 50% at day 8 of regeneration (Fig. 4).

DISCUSSION

It has been previously found that cerebellin stimulates steroid secretion by human and rat adrenal quarters, and our present results confirm *in vivo* these findings. They also show that this peptide markedly enhances the proliferative activity of the zona glomerulosa, the "cambium layer" involved in the maintenance of adrenal growth (11). Cerebellin does not affect plasma ACTH concentration, which makes unlikely the possibility that its stimulating effect on adrenals may be dependent on any action on the pituitary ACTH release.

Adrenal regeneration after enucleation is an experimental model where remnant subcapsular zona glomerulosa cells rapidly dedifferentiate and start to proliferate restoring a normal adrenocortical morphology in about 30–60 days. Adrenocortical cell dedifferentiation is coupled with their impaired ability to secrete aldosterone and corticosterone (for review, see 11), which explains the rather low plasma concentrations of these two hormones in operated rats as compared to adult intact animals (Figs. 1 and 2).

We have previously demonstrated that cerebel-

Fig. 1 Acute effect of cerebellin on ACTH (upper panel), aldosterone (middle panel) and corticosterone (lower panel) plasma concentrations in the rat. A, control; B, cerebellin 5 nmol/kg; C, cerebellin 15 nmol/kg. Bars are means $\pm$ SEM ($n=8$). * $P<0.01$ from the respective control value.

Fig. 2 Lack of effect of cerebellin on aldosterone (upper panels) and corticosterone plasma concentrations (lower panels) in rats bearing regenerating adrenals. Bars are means $\pm$ SEM ($n=8$).

lin stimulates steroid secretion indirectly, by eliciting catecholamine release that in turn activate adrenocortical cells. In fact, the *in vitro* adrenocortical secretagogue action of cerebellin is blocked by the antagonists of β -adrenoceptors (1, 8). Enucleation eliminates medullary tissue, and consequently in regenerating adrenals chromaffin cells are lacking (11). This consideration may easily explain why cerebellin does not affect aldosterone and corticosterone secretion from regenerating rat adrenals.

Although cerebellin enhances proliferative activity of zona glomerulosa of immature rat

Fig. 3 Effect of cerebellin on the proliferative activity of the zona glomerulosa of immature rat adrenal cortex. Bars are means $\pm$ SEM (n=8). * $P < 0.01$ from the respective control value.

Fig. 4 Effect of cerebellin on the proliferative activity of regenerating rat adrenal glands. Bars are means $\pm$ SEM (n=8). * $P < 0.01$ from the respective control value.

adrenals, our findings indicate that the peptide decreases mitotic index at day 8 of regeneration. At present, we are not able to provide a possible explanation of these conflicting results. However, we want to stress that they are in keeping with the contention that different mechanisms are involved in the normal growth and regeneration of adrenal glands. In contrast with normal adrenal growth, adrenal regeneration involves complex neural mechanism (4-6), which could be negatively modulated by cerebellin.

In conclusion, our study provides the first *in vivo* evidence that cerebellin, acting directly on rat adrenal gland, exerts various secretory and growth effects, which depend on the experimental model employed. Hence our findings allow us to include cerebellin in that group of peptides which modulate adrenal secretion and growth acting in

a paracrine manner (12).

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