Hydrocortisone Inhibits the Basal and Angiotensin II-Stimulated Proliferation of Rat Adrenocortical Cells in Organ Culture

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With 3 Figures

Summary. The present study has been to test the effect of hydrocortisone (HC) on the mitotic incidence in adrenocortical cells of organ-cultured rat adrenal explants during a 24-hour incubation with or without angiotensin II (ANG). It was shown that HC significantly decreased the mean mitotic activity rate (MMAR) of adrenocortical cells in organ culture. That decrease concerned all the three cortical zones vs respective controls. In turn, ANG markedly increased the MMAR of the zona glomerulosa cells, when compared to the values recorded in controls, while there were no changes of the MMAR of the zona glomerulosa in the adrenal explants incubated with joint exposure to HC and ANG. The obtained results indicate that HC exhibits an inhibitory effect on the adrenocortical cell proliferation, which may suggest that arachidonic acid metabolites play an important role in the process of adrenocortical hyperplasia, their participation in the ANG-induced zona glomerulosa cell proliferation being strongly assumed.

Key words: Angiotensin II — Hydrocortisone — Mitotic activity — Adrenal cortex — Organ culture

Introduction

Considerable evidence was accumulated indicating that prostaglandins (PGs) could affect cell proliferation in the endocrine glands (for review see: Pawlikowski, 1983). It was found at our laboratory that indomethacin (IND) and aspirin, PGs synthetase (cyclooxygenase) inhibitors, suppressed the mitogenic effect of certain hormones. For example, IND was shown to inhibit the estradiol-induced DNA synthesis in the rat anterior pituitary gland (Pawlikowski et al., 1981). Moreover, the treatment with IND and aspirin decreased the mitotic activity of the anterior pituitary cells in male rats (Pawlikowski, 1981). In another study, PGs synthesis inhibitors suppressed the proliferative response of the rat thyroid to thyrotropin (Sluszkiewicz and Pawlikowski, 1980). Furthermore, prostacyclin (PGI₂) and arachidonic acid increased the mitotic incidence in organ-cultured rat thyroid slices (Pawlikowski et al., 1982).

In earlier reports we demonstrated that PGE₂ and PGI₂, but not PGF₂α, stimulated the mitotic activity of the adrenal cortex in hypophysectomized rats (Szkudlinski and Lewinski, 1985; Szkudlinski et al., 1988). At the same time, there is a bulk of evidence
indicating that PGs, as well as lipoxygenase metabolites play an important role in angiotensin II (ANG) action on the adrenocortical secretion (Hindawi et al., 1985; Kojijima et al., 1985; Perroteau et al., 1984). Accordingly, ANG stimulated the synthesis of PGs in adrenocortical cells (Campbell and Gomez-Sanchez, 1985). It is worth recalling that ANG is a potent mitogenic factor for adrenocortical cells (Gill et al., 1977).

Glucocorticoids, which suppress the PGs synthesis by inhibition of phospholipase A, are believed to exert an antiproliferative effect on different tissues (Guzek, 1968; Svec, 1984). However, in opinion of other authors, glucocorticoids may also stimulate, or have no effect on cell growth, depending on the cell type, donor, species, or culture conditions used (Finlay and Cristofalo, 1987).

To our knowledge, there were no reports on the effect of glucocorticoids on the proliferogenic action of ANG.

Therefore, the aim of the present study has been to examine the effects of hydrocortisone (HC), ANG, as well as interactions among HO and ANG in their action on the rat adrenocortical cells in organ culture.

Materials and Methods

Male Wistar rats, weighing 220 ± 10 g each, were used as donors of adrenals. The animals were killed by decapitation. The left adrenals collected from all the animals under sterile conditions were divided into two parts and immediately placed in a culture vessel on the stainless grid in such a way that the fluid under the grid moistened the adrenal explant resting on it. The culture medium was composed of RPMI-1640 medium (Gibco) (85%), supplemented with 15% fetal calf serum, 10 nM Hepes buffer, penicillin (200 U/ml) (Polfa) and streptomycin (10 μg/ml) (Polfa). The adrenal explants were cultured under the atmosphere of O₂ : CO₂ (95:5, v/v) in the presence of the following compounds:

Group I – controls, n = 6;
Group II – HC (Hydrocortisonum hemisuccinatum, Polfa), 10 μg/ml, n = 5;
Group III – ANG (Angiotensin, Germed), 1 μg/ml, n = 5;
Group IV – HC, 10 μg/ml + ANG, 1 μg/ml, n = 5.

The cultures were maintained for 24 h at 37°C. Colchicine (Colchicinum purum, FLUKA) was added to all the culture media in concentration of 0.1 μg/ml, 5 h prior to the end of the incubation. At the termination of incubation, the explants were fixed in Bouin’s fluid. Paraffin sections (6 μm thick) were stained with hematoxylin and eosin.

In the microscopic preparations the mitotic activity rates of the adrenal cortex were calculated according to the method of Michat and Nouët (1975).

The data were analyzed statistically using a one-way analysis of variance (ANOVA). The statistical significance of differences of mean mitotic activity rates (MMARs), observed between the individual groups, was subsequently determined by Newman-Keuls’ test (Hinkle et al., 1979).

Results

It was found that HC (Group II) exerted an inhibitory effect on the adrenocortical cell proliferation. This decrease included all the three zones analyzed collectively (a total MMAR) and with regards to the zona glomerulosa and the zona fasciculata analyzed separately, when compared to the respective controls (Group I) (Fig. 1).

As expected, ANG (Group III) significantly increased the mitotic activity of the zona glomerulosa cells, but did not change the total MMAR (all the three zones analyzed together), as well as the MMAR of the zona fasciculata and zona reticularis (Fig. 2).

There was a significant decrease of the MMAR of the zona glomerulosa cells exposed to ANG and HC jointly, when compared to ANG-treated group (Group IV) (Fig. 3).
Fig. 1 Mean mitotic activity rates of adrenocortical cells in the adrenal explants incubated with exposure to HC, when compared to controls (C); bars represent means ± SEM; p - level of significance.

Fig. 2 Mean mitotic activity rates of adrenocortical cells in the adrenal explants incubated with ANG; C - controls.
Discussion

The data presented above indicate that HC reveals an inhibitory effect on the basal and ANG-stimulated adrenocortical cell proliferation, what may suggest that PGs and/or lipoxygenase pathway products play a substantial role in the processes of adrenocortical hyperplasia. Particularly, PGs seem to participate in the ANG-induced zona glomerulosa cell proliferation. A similar role has recently been proposed for PGs in mediating ANG-induced aldosterone secretion by the rat zona glomerulosa cells.

The present data are concordant with our earlier reports demonstrating the stimulatory effect of PGE$_2$ and PGI$_2$ on the mitotic activity of the adrenal cortex in hypophysectomized rats (Szkudlinski and Lewinski, 1985; Szkudlinski et al., 1988). It is tempting to speculate that the proliferogenic effect of PGE$_2$ and PGI$_2$ might, however, be mediated by the renin-angiotensin system. It is well known that PGI$_2$ and PGE$_2$ enhance the release of both renal and adrenal renin (Baba et al., 1985; Keeton and Campbell, 1980). In turn, renin was demonstrated to stimulate the mitotic activity of the zona glomerulosa cells in rats (Payet and Isler, 1976).

The present results are in compliance with previous studies reporting the mitogenic effect of ANG on the adrenocortical cells in cell culture (Gill et al., 1977). Furthermore, ANG was reported to stimulate the PGs and lipoxygenase metabolite synthesis in adrenocortical cells (Campbell et al., 1986). Similarly, numerous hormones which are known to promote mitogenesis in their target organs, enhance the PGs synthesis (Pawlikowski, 1983). Recently, we have found that IND inhibits the proliferative response of the adrenal cortex to ANG in rats (Szkudlinski and Lewinski, 1988). In the present paper we have demonstrated that HC reduces mitogenic response of zona glomerulosa...
cells to ANG in vitro. In accordance with the above, it was found at our laboratory that HC inhibited the proliferative response of the thyroid to thyrotropin (Sluszkiewicz and Pawlikowski, 1980).

It is possible that HC could directly suppress adrenocortical cell divisions, but the causal relation between phospholipase-inhibiting and antimitotic effects of HC remains obscure. The effect of HC may ensue through the inhibition of both cyclo- and lipoxigenase metabolite pathways. Since IND has also been shown to inhibit the adrenal cortex mitotic activity in vivo and in vitro, it is suggested that PGs, above all, and, perhaps other arachidonic acid derivatives participate in the regulation of adrenocortical cell proliferation.

Henderson and Loeb (1974) showed that glucocorticoids rapidly decreased the rat liver DNA polymerase activity. Dexamethasone, a synthetic glucocorticoid, inhibited the cell division and 3H-thymidine incorporation into DNA in the rat adrenal glands (Saez et al., 1977). It has been suggested that glucocorticoids inhibit the activity of thymidine kinase, which has regulatory function in the control of DNA synthesis (Sanfacon et al., 1977). Nevertheless, the exact mechanisms of glucocorticoid action on adrenocortical DNA synthesis and cell divisions require elucidation in further studies.

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References


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