# ACTION OF HYDROCORTISONE ON DNA SYNTHESIS IN THE EPITHELIAL CELLS OF THE URINARY BLADDER AND RENAL CORTEX AND ON THE BLOOD GLUCOCORTICOID LEVEL

P. A. Dyban and N. S. Sapronov

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A single dose of hydrocortisone (10 mg/100 g body weight) leads to an initial increase, followed by a significant decrease, in the peripheral blood plasma glucocorticoid concentration in rats. Elevation of the glucocorticoid level is accompanied by a decrease in the number of DNA-synthesizing epithelial cells in the urinary bladder, leading to a subsequent decrease in the number of mitoses, whereas a fall in the level of the hormone leads to an increase in the number of cells changing from the $G_1$ into the S period. Meanwhile inhibition of proliferative processes in the epithelium of the kidney continues throughout the experiment (48 h). The possible causes of the unequal sensitivity of epithelial tissues to hydrocortisone are discussed.

KEY WORDS: hydrocortisone; DNA synthesis; epithelium of the urinary bladder and kidney; blood glucocorticoid concentration.

Glucocorticoids, with an important role in the regulation of tissue homeostasis, must be classed as universal inhibitors of cell division which inhibit mitotic activity and DNA synthesis in various tissues [6].

The object of this investigation was to compare the quantitative indices of proliferative activity of the epithelium in different parts of the urinary tract (the route by which corticosteroids are excreted from the body) and the blood glucocorticoid concentration after a single dose of hydrocortisone.

## EXPERIMENTAL METHOD

Experiments were carried out on 160 male albino rats weighing 150-180 g. At 8 a.m. 88 experimental animals received an intraperitoneal injection of hydrocortisone (Richter) in a dose of 10 mg/100 g body weight. The rats were decapitated in groups of four to six (experimental) and four to five (control) at a time in each of the three series of experiments, 4, 8, 12, 16, 24, and 48 h after injection of the hormone. In series I (66 animals) the concentration of glucocorticoids was determined fluorometrically [3] in the peripheral blood plasma, in series II (50 rats) and III (44 rats) proliferative processes in the epithelium of the renal cortex and of the urinary bladder, respectively, were studied. The animals of series II and III, 1 h before sacrifice, received an injection of thymidine-$^3$H (specific activity 20.4 Ci/mmole) in a dose of 0.5 $\mu$Ci/kg body weight. The organs for study were fixed by Bouin's method. Paraffin sections 7 $\mu$ thick were coated with type M emulsion, exposed for 65 days, and stained with hematoxylin–eosin. Nuclei above which there were at least five grains of reduced silver were counted on the autoradiographs. The number of mitoses (mitotic coefficient, MC) in DNA-synthesizing cells was counted in 30,000 cells of the renal cortex and 3000 cells of the stratum basale of the urinary bladder. The intensity of turnover of thymidine-$^3$H was investigated in 30 nuclei from each animal. The results were subjected to statistical analysis with the use of the Fisher–Student and Wilcoxon–Mann–Whitney criteria.

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TABLE 1. Comparison of Proliferative Processes in Epithelium of Kidney and Urinary Bladder with Peripheral Blood Plasma Glucocorticoid Concentration after a Single Dose of Hydrocortisone (M ± m)

| Animals | Duration of action of hydrocortisone, h | Renal cortex | | | Urinary bladder | | | Blood glucocorticoid concentration, μg % |
|---|---|---|---|---|---|---|---|---|
| | | % | number of grains above nucleus | MC, % | % | number of grains above nucleus | MC, % | |
| Control | — | 1,01±0,10 | 18,6±1,2 | 0,29±0,08 | 4,25±0,7 | 13,9±3,0 | 2,5±0,8 | 22,5 |
| Experimental | 4 | 0,79±0,15 | 14,2±1,4 | 0,23±0,05 | 2,0±0,6* | 9,0±1,0* | 1,3±0,5 | 44,6† |
| Control | — | 0,79±0,14 | 19,2±1,7 | 0,48±0,08 | 3,8±0,6 | 16,8±2,1 | 2,0±0,3 | 29,1 |
| Experimental | 8 | 0,38±0,08* | 11,2±1,1† | 0,46±0,10 | 1,1±0,3* | 11,2±1,3* | 0,6±0,2* | 28,9 |
| Control | — | 0,93±0,29 | 16,9±0,97 | 0,29±0,07 | 5,2±0,8 | 18,6±2,3 | 0.6±0,2 | 28,0 |
| Experimental | 12 | 0,07±0,02† | 9,0±1,2† | 0,09±0,04* | 8,5±1,5 | 18,4±1,4 | 0,1±0,1* | 25,7 |
| Control | — | 1,12±0,47 | 18,2±1,3 | 0,32±0,08 | 4,6±0,6 | 19,5±2,0 | 0,9±0,3 | 26,9 |
| Experimental | 16 | 0,09±0,03* | 11,5±2,1* | 0,08±0,04* | 8,8±1,6* | 16,3±2,0 | 0,3±0,2 | 21,8† |
| Control | — | 1,69±0,27 | 25,3±1,2 | 0,57±0,12 | 1,8±0,9 | 16,6±1,9 | 1,0±0,4 | 29,0 |
| Experimental | 24 | 0,06±0,04* | 15,7±3,1* | 0,03±0,02† | 0,9±0,5† | 20,8±6,6 | 3,5±1,5* | 20,7† |
| Experimental | 48 | 0,37±0,14* | 16,6±3,0* | 0,16±0,04* | 6,7±2,6 | 24,1±3,2 | 2,0±0,9 | 31,1 |

*$P < 0.05$ relative to control.
†$P < 0.01$ relative to control.

## EXPERIMENTAL RESULTS

The results are given in Table 1. A single dose of hydrocortisone led to inhibition of proliferative processes in the epithelium of the urinary bladder and renal cortex, which lasted for 12 and 48 h, respectively. The decrease in the number of labeled epithelial cells reflected delay in their transition from the $G_1$ into the S period, and the decrease in the number of grains of reduced silver above them reflected changes in the intensity of DNA synthesis, leading to lengthening of the S period. It must be pointed out that hydrocortisone affected DNA synthesis in the cell nuclei initially and the number of mitoses only later. After 16 h the number of cells synthesizing DNA in the epithelium of the urinary bladder was significantly greater than in the control, whereas in the epithelium of the renal cortex the inhibition of proliferation continued throughout the experiment.

The results of comparison of the dynamics of proliferative processes in the epithelium of the urinary bladder (the results of the present investigation), the ureter [1], and the sebocytes and keratocytes of the sebaceous glands [2] after a single dose of hydrocortisone are evidence both that the changes (initially inhibition, followed by significant stimulation of cell division) are common and also that there are individual differences (variability of the time of the response). In the writers' opinion, this variability can be attributed to differences in the level of accumulation of cortisol in the tissues [7], as a result of the different numbers of cell receptors for glucocorticoids [4, 8]. The inhibition of proliferative processes in the renal cortical epithelium, continuing for 48 h, could also have been due to the special character of the intensified reabsorption of glucocorticoids by that particular tissue [5].

The fact that proliferative processes depended on the blood corticosteroid level was noted: A high glucocorticoid concentration, continuing for 4 h after injection of hydrocortisone, was accompanied by a decrease in the number of DNA-synthesizing cells, leading to a subsequent decrease in the number of mitoses, whereas a fall in the level of the hormone (after 12-16 h) led to an increase in the number of proliferating cells in the epithelium of the ureter [1], the urinary bladder (the results of the present investigation), and the keratocytes and sebocytes of the sebaceous gland [2]. The earlier hypothesis [1] that the phase of stimulation of proliferative activity of the cells is connected with the fact that a single dose of hydrocortisone not only reduced the number of dividing cells (the phase of inhibition), but also blocked the secretion of endogenous glucocorticoids by the adrenals, which led to a decrease in the blood corticosteroid level (Table 1), resulting in a subsequent increase in the number of DNA-synthesizing cells, was thus confirmed.

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