Sex differences in adrenocortical structure and function. XI.

Autoradiographic studies on cell proliferation and turnover in the adrenal cortex of the male and female rat and its dependence on testosterone and estradiol

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Summary. Adrenal glands from orchectomized and ovariectomized rats, with and without replacement therapy, and also from intact controls of both sexes, were examined by autoradiography with $^3$H-thymidine. The labelling index after 1 or 2 nucleoside injections was higher in the zona glomerulosa of females than in male rats, while no differences were found in the fascicular and reticular zones. Orchietectomy increased the labelling index in the fascicular and reticular zones, an effect prevented by testosterone. Ovariectomy did not change the labelling index, while estradiol lowered it in the zona glomerulosa. Duration of the S phase was longer in the zona fasciculata cells of males than in females. Both orchietectomy and testosterone shortened this phase in cells of the zona fasciculata and zona reticularis. Ovariectomy prolonged the S phase in the zona fasciculata and shortened this time in the reticular zone, an effect reversed by estradiol.

In the glomerular and fascicular zones, cell cycle time was longer in males than in females. Orchietectomy shortened this time in all adrenocortical zones, an effect reversed by testosterone. Ovariectomy shortened cell cycle time in the glomerular and reticular zones and prolonged it in the zona fasciculata; these effects were reversed by estradiol. Turnover rate in adrenocortical cells was markedly higher in females than in males, a difference due to testosterone which markedly decreased turnover rate.

Key words: Adrenal cortex (rat) – Sex differences – Gonadal hormones – Cell proliferation – Cell cycle

Adrenal glands of adult female rats are larger than those of males and this difference depends mainly on the inhibitory action of testosterone on the hypothalamo-hypophysio-adrenocortical axis. Estrogen, on the other hand, is rather stimulatory. Orchietectomy results in an increase in adrenal weight, an effect reversed or prevented by testosterone replacement, whereas ovariectomy either has no effect or, usually

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after more than 10 weeks post surgery, decreases the weight of the gland. Estrogen therapy in ovariectomized rats either has no effect or increases adrenal weight (Kitay 1968; Coyne and Kitay 1969, 1971; Malendowicz 1974a, b, 1976).

Stereologic studies have revealed that in both sexes rat adrenal glands contain a similar number of parenchymal cells, but fasciculata cells are greater in females (Malendowicz 1974 b). These data are in agreement with autoradiographic studies on adrenal growth in which no differences in the rate of proliferation of adrenocortical cells have been found in male and female rats (Pappritz et al. 1972). In both sexes neither gonadectomy nor testosterone or estradiol replacement have an effect on the number of adrenocortical cells (Malendowicz 1974b). However, Stöcker et al (1965) have shown a marked increase in the index of ³H-thymidine labelled rat adrenocortical cells, estimated 1 h after the nucleoside administration, as well as in the mitotic index in orchiectomized animals.

In light of these apparent discrepancies, the aim of the present study was to investigate, by means of autoradiography with the use of ³H-thymidine, sex differences in the proliferation and turnover of adrenocortical cells in male and female rats, and determine whether they are depend on gonadal hormones.

Materials and methods

Sixty adult rats of Wistar strain were employed in the study. Animals were maintained under standardized conditions of light (12L:12D) and temperature (22 ± 1°C), with access to laboratory pellets and tap water ad libitum.

Gonadectomy was performed under ether anaesthesia. Six weeks after surgery some gonadectomized rats were injected with either a single dose of testosterone cypionate (5 mg/100 g bw) or estradiol cypionate (100 μg/100 g bw). Others received no injections were kept as controls. Eight weeks after gonadectomy animals were injected ip with 0.5 gCi [6-³H]-thymidyne/g body weight (Radiochemical Centre, Amersham, specific activity 5.0 Ci/mmol), diluted with isotonic saline. After 60 min one half of the number of rats received a second injection of the same dose of tritiated thymidine. All animals were sacrificed by decapitation between 4.20-5.20 h, 60 min after the final injection in each group. In controls the state of estrus was not considered. Special care was taken to minimalize the effects of the diurnal rhythm of adrenocortical cell proliferation by sacrificing the specimens all at approximately the same time of day (Dobrokhotov and Nikanorova 1962; Alov 1963).

Immediately after removal, the adrenal glands were immersed in Bouin solution and after 24 h were freed of adherent adipose tissue, weighed, dehydrated in alcohol, embedded in paraffin and sectioned transversely at 5μm. Sections were mounted on glass slides and coated with Kodak NTB2 liquid emulsion by dipping them vertically into the liquid. They were stored in a refrigerator at 4°C for 21 days. After development and fixation the preparations were stained with hematoxylin.

At a magnification of × 700 the index of parenchymal cells labelled with tritiated thymidine was calculated after counting 3000–5000 cells in each adrenocortical zone and expressed in promilles. Because a sudanophobic zone is lacking in the adrenals of female rats, counting in this zone extended into the zona fasciculata and estimations were based on half the outer part of this zone. A cell was considered as labelled if at least 4 silver grains were found above its nucleus.

Results were evaluated statistically by the multiple-range test of Duncan (Bliss 1967).

Results

Body and adrenal weights, and the results of counting and subsequent calculations, are shown in Table 1.