

The Renin-Angiotensin System in Uninephrectomized Adrenalectomized Rats

The present investigation was undertaken to determine if, in adrenalectomized rats, in which there is a stimulation of the renin-angiotensin system¹⁻⁵, unilateral nephrectomy would modify such stimulation.

Material and methods. The experiment was performed on female rats (220–230 g) of the Royal Victoria Hospital strain. They were fed with Purina Laboratory Chow and given free access to tap water. The Royal Victoria Hospital strain was chosen because we have observed in our laboratory that these rats survive the adrenalectomy longer than the Sprague-Dawley or Wistar rats.

The animals were divided into 2 groups of 10 animals each. One group served as control and was unilaterally nephrectomized (R-xp). The other group was uninephrectomized and bilaterally adrenalectomized (R-xp + Adr-x). The operations were done by the lumbar route under light ether anesthesia. The experiment lasted 12 days. Body weight was measured on the 1st and 6th days and at the end of the experiment to assess the completeness of adrenalectomy. This was further checked by inspection at the autopsy. The remaining kidney was sectioned in 3 parts for renin determinations and histological and histochemical studies.

Renin determination. At the end of the experiment and under light sodium pentobarbital anesthesia (Nembutal® 6 mg/100 g body weight), blood samples were withdrawn from the jugular vein (0.3 ml). Renin concentration in the plasma (PRC) was measured by the micromethod of BOUCHER et al.⁶. The same micromethod was used for the measurement of the renal renin content (RRC). Results

are expressed as ng of generated angiotensin per ml of plasma or as µg of generated angiotensin per g of renal tissue per h of incubation.

Morphological procedure. One part of the sectioned kidney was processed as previously described⁷ in order to stain the granular juxtaglomerular cells. The other part of the sectioned kidney was used for the histochemical study of Glucose-6-phosphate dehydrogenase (G6PD) activity by the Nitro-Bt-Co-Ba method⁸, in which the incubation medium had the following composition: D-Glucose-6-phosphate disodium salt (0.2 M) 0.1 ml; NADP (5 mg/ml) 0.1 ml; nitro-BT (1 mg/ml) 0.25 ml; sodium azide (6.5 mg/ml) 0.1 ml; Tris (0.2 M, pH 7.6) 0.25 ml; barium chloride (adjusted to pH 7.2, 0.5 M) 0.05 ml; cobaltous chloride (adjusted to pH 7.2, 0.5 M) 0.05 ml; sodium fluoride (0.01 M) 0.05 ml; distilled water to make 1 ml; polyvinylpyrrolidone (M.W. 11,000) 75 mg. The final pH was adjusted to 7.2 and the frozen sections were incubated at 37°C during 30 min.

The granularity of juxtaglomerular cells was measured semi-quantitatively as the granulation index (JGI) devised by HARTROFT and HARTROFT⁹. A macula densa index (MDI) was used for the G6PD activity in the macula segment⁸. Over 500 glomeruli were counted for each index and per kidney.

Results. The adrenalectomized animals developed a gradual asthenia and loss of body weight which reached approximately 15% of the initial values at the end of the experiment.

Juxtaglomerular apparatus (Figure 1): Adrenalectomized uninephrectomized rats showed a marked decrease in JGI ($p < 0.001$) associated with a significant increase in MDI (G6PD) ($p < 0.001$). No G6PD activity was visualized in the media of the afferent arterioles at its entrance to the glomeruli.

Renal renin content (RRC) and plasma renin concentration (PRC) (Figure 2): There was no significant changes in RRC of the adrenalectomized uninephrectomized rats as compared to the uninephrectomized control rats. However, the PRC values were highly elevated ($p < 0.001$).

Discussion. It has been reported that the uninephrectomized rat had lower values of PRC than the intact animals^{10, 11}; when the uninephrectomized rats received a sodium-deficient diet, the PRC values increase only to the values of the intact rats¹¹. In our experiment, the approximately 15-fold increase in PRC in adrenalectomized uninephrectomized rats as compared to uninephrectomized rats indicated that, in severe adrenal insufficiency in

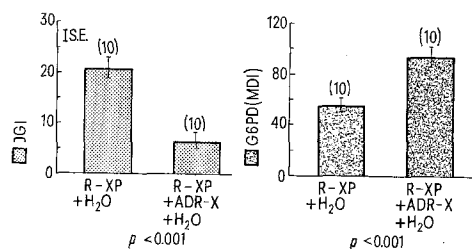


Fig. 1. Juxtaglomerular granulation index (JGI). Macula densa index (MDI) of Glucose-6-phosphate dehydrogenase activity (G6PD). Uninephrectomized rats (R-xp). Uninephrectomized adrenalectomized rats (R-xp + Adr-x). Number of rats studied in parenthesis. All the animals were fed with Purina Laboratory Chow and tap water ad libitum.

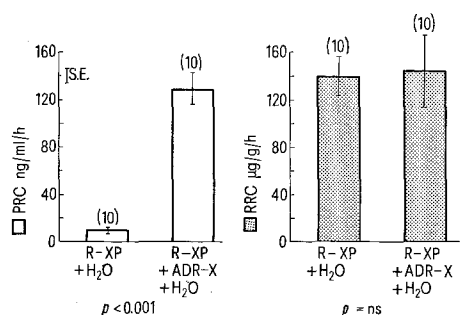


Fig. 2. Plasma renin concentration (PRC) and renal renin content (RPC) in uninephrectomized (R-xp) and uninephrectomized adrenalectomized rats (R-xp + Adr-x). Number of rats studied in parenthesis. All the animals were fed with Purina Laboratory Chow and tap water ad libitum.

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which there is a marked negative sodium balance¹², the lone kidney has an exaggerated rate of renin secretion. Similar adrenalectomy experiments in rats with both kidneys present have shown an increase in PRC that did not markedly differ from that of the present studies⁵.

In this study, in which the adrenalectomized animals were not loaded with NaCl or maintained with a substitutional therapy, the degranulation of the juxtaglomerular cells may indicate that their rate of renin synthesis and release predominate over the renin storage. In favor of such an assumption are the elevated values in PRC and the absence of changes in renal renin content of the adrenalectomized uninephrectomized rats as compared with their controls.

In experiments done on rat kidney slices, DE VITO et al.¹³ pointed out that the hexose monophosphate shunt is involved in the conversion of hypothetical prerenin to renin. The high macular G6PD activity mostly in adrenalectomized animals may well suggest that, by an unknown mechanism, the macula densa G6PD could be functionally related to the renin secreting cells. It is also possible that the macular G6PD could be functionally independent of the JG cells but could respond to variations in intracellular macula densa cells sodium balance^{7,14,15}.

Résumé. La surrénalectomie bilatérale chez des rats uninephrectomisés produit une augmentation très marquée de la concentration de rénine plasmatique, ainsi que

de l'activité de la G6PD dans la macula densa. Cependant, malgré une diminution de la granularité des cellules juxtaglomérulaires, on n'observe aucune modification du contenu en rénine du rein. Ces observations indiquent que, dans cette condition expérimentale, le rein qui reste est capable de réagir avec forte augmentation de la sécrétion de rénine.

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Brain Development in Offspring of Rats Treated with Nicotine During Pregnancy

Studies in various mammalian species have attempted to assess the effects of nicotine on the brain, particularly in terms of behavioral responses. In rats and mice, small doses of nicotine seem to facilitate elementary forms of learning and to stimulate spontaneous motor activity^{1,2}; larger doses, however, depress spontaneous motor activity³. In the sleeping cat, nicotine initially induces EEG activation and behavioral arousal, followed by a period of enhanced slow-wave sleep and activated sleep^{4,5}, whereas in rabbits, nicotine produces seizure discharges in the hippocampus^{6,7}. Most of these studies have been conducted in the adult animal; outside of the finding that nicotine administered to the pregnant female distributes rapidly throughout the body and passes into the blastocyst as well as the implanted fetus⁸ and prolongs the duration of gestation^{9,10}, we know little of the direct or indirect effects of this agent on the developing brain. That fetal development can be affected by adverse conditions imposed on the maternal organism, and result in permanent distortion of maturational patterns at a structural, functional and biochemical level, has been demonstrated^{11,12}. Furthermore, significant developmental phenomena as well as manifestations of prenatal influences can be effectively studied in the rat, for the CNS in this species is still relatively immature at birth.

The present study utilizes this species to investigate the effects of nicotine administered to the gestating animal on the development and function of the brain in the offspring. We have chosen to compare the development of seizure activity between offspring of treated and untreated animals, for such electrophysiological data provide useful information on brain maturational patterns under normal and experimental conditions. Although convulsive seizures represent fundamentally pathologic phenomena, when induced experimentally, they offer a measure of the interaction occurring between inhibitory

and excitatory systems of the CNS manifested as overt motor activity. Minimal electroshock seizure threshold (EST) and maximal electroshock seizure (MES) patterns were utilized as specific indices of subcortical and whole-brain maturation, respectively^{13,14}.

Materials and methods. From day 0–21 of gestation, pregnant Long-Evans rats were injected s.c. with 3 mg nicotine per kg body weight, twice daily, and controls were injected with saline on the same schedule. Special care was taken to handle all rats in the same manner to eliminate differences resulting from this variable¹⁵.

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