whole animal. The presence of an asymmetry detectable as a left-right eye difference could mean that the position of a stimulus in space relative to the bird may influence its likelihood of eliciting a copulatory response. Alternatively, the bird may overcome the eye asymmetry by having a mechanism which ensures that it will turn its head to view any copulatory stimulus with both eyes. In either case, it may be expected to influence the pattern of courtship display behavior, such as the direction of circling during waltzing.

On the later days of testing a LES-RES asymmetry for copulatory behavior developed even in control male chicks which had not received testosterone treatment (fig. 1). The group using their LES had significantly higher scores than the group using their RES ($F_{1,22} = 11.74$, $p \leq 0.001$ for a comparison of RES and LES groups over the 4 days prior to eye-patch reversal on day 14). Indeed, the control group tested using their LES showed a significant linear increase in copulation from days 6–14 ($F_{1,11} = 6.99$, $p \leq 0.025$), while the group using their RES showed no significant change over this period ($F_{1,11} = 1.58$, $p \leq 0.05$). This could possibly be due to endogenous levels of androgen rising over the first 2 weeks of life and/or to prolonged monocular eye occlusion.

It is also possible that prolonged wearing of eyepatches may interact with testosterone treatment, since after eyepatch reversal there is no longer any difference between the testosterone-treated and control groups using their RES ($F \leq 1$). Thus, in testosterone-treated animals prior monocular experience using the eye dominant for copulation (i.e. LES) may have led to a suppression of any ability of testosterone to elevate copulation in the RES.

Asymmetry for copulation was also demonstrated in chicks which had prior binocular experience in the test situation. In the second experiment, a group of testosterone-treated chicks were tested binocularly on days 6 and 7 of life. On day 8 either the right or left eye was occluded and copulation was tested 3 h later ($n = 16$ per group). Copulation scores remained high in the LES group (the mean copulation score with SE was 5.8 ± 0.5 in the binocular test on day 7 and 5.3 ± 0.5 when using the LES on day 8) but dropped significantly in the RES group (5.6 ± 0.5 in the binocular test on day 7, which fell significantly to 2.7 ± 0.4 when using the RES on day 8).

A comparison of monocularly and binocularly tested chicks (see fig. 2) reveals that controls using their RES score at the basal levels characteristic of binocularly-tested controls; whereas testosterone-treated chicks using their LES have scores comparable to those found in testosterone-treated chicks tested binocularly. Therefore, when tested binocularly, the RES is dominant in control chicks and the LES is dominant in testosterone-treated chicks. That is, although a control chick possess the neural mechanisms which can activate copulation at least to some degree, when tested monocularly (fig. 1), a control with both eyes open does not copulate because the RES system is dominant. The action of testosterone is to reverse dominance from RES to LES thus disinhibiting, or activating, copulation. We have previously demonstrated that light experience prior to hatching can also alter brain asymmetry[3]. The final organization of brain asymmetry in the chicken must be dependent on an interaction between genetic, hormonal and environmental factors. While not underplaying the importance of hormonal influences on brain development, recognition of the interaction between these variables must caution us against explanations of brain asymmetry based solely on hormonal causes as implied by Geschwind and Behan[2] for sex differences in the human brain.

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Induction of the synthesis of thymidine kinase by aldosterone in the kidney of the immature male rat

A.R. Bukhari, H.G. Hartmann and P. Jouan*

Laboratoire de Biochimie des Hormones (U.A.-CNRS 626), Centre Régional de Recherche en Endocrinologie, Faculté Médecine-Pharmacie, 2, av. du Prof. L. Bernard, F-35043 Rennes Cedex (France), and Abteilung für Nephrologie und Dialyse, Universität des Saarlands, D-6650 Homburg (Federal Republic of Germany), 12 July 1984

Summary. Thymidine kinase activity was studied in kidneys from immature male rats after administration of aldosterone. Kinetic studies showed that the enzyme activity reached its maximum level 24 h after aldosterone injection. That increase was specific for aldosterone and could be related to the synthesis of new molecules of enzyme.
Key words. Aldosterone; kidney; thymidine kinase activity.

Steroid hormones interact in their target organs after binding to cytoplasmic receptors. These are translocated into the nucleus and nuclear receptors interact on the acceptor sites of the chromatin. It is established that the activation of genes regulated by steroids is followed by the induction of the synthesis of specific proteins. Such a mechanism of action is probably applicable to aldosterone. In fact publications have shown that this steroid binds in the kidney, having cytoplasmic and nuclear receptors[5,6,7,13,15]. More recently, it was shown by autographic study that in the kidney, the receptors of aldosterone are exclusively localized in the nucleus[2,8,9]. These results suggest that this mineralocorticoid could regulate the activity of certain genes and induce the synthesis of specific proteins. Recently in our laboratory it has been shown that estrogen receptors specifically induce the synthesis of thymidine kinase in the uterus[1,10] and that the androgens produce same effect in the prostate (in preparation). These conclusions led us to investigate whether aldosterone might effect the induction of thymidine kinase in the kidney. The results obtained show that this hormone specifically induces the synthesis of this enzyme in the kidney.

Materials and methods. 4-Pregnan-11β, 21-diol-3, 18, 20-trione (aldosterone) was furnished by Steraloids (USA) and [2^{14}C]-thymidine (sp. act. 54–58 mCi/mmole) by Amersham (UK). Antibiotics (cycloheximide, actinomycin) were purchased from Sigma (USA).

Determination of thymidine kinase activity was carried out in the kidney cytosol from immature male rats (22–23 days), bought from Elevage Janvier, Laval (France).

The different hormones were injected s.c. in 100 µl alcohol-DMSO (2:1, v/v).

The techniques of preparation of cytosols, enzyme determination, protein assay and radioactivity measurement have been described elsewhere[1, 10]. The activity of thymidine kinase was expressed as pmoles of d-TMP synthesized per mg of protein per min.

Results. Kinetics of the induction of the activity of thymidine kinase. The injection of 10 µg of aldosterone in immature male rats provoked a significant induction of the activity of thymidine kinase in the kidney (fig. 1). This increase was 4–6 times higher than control respectively 18–24 h after injection of aldosterone. The maximal activity was reached 24 h after the administration of aldosterone. Then it gradually decreased and returned to the basal level within 72 h.

Study of the hormonal specificity of thymidine kinase induction in the kidney. The activity of thymidine kinase was measured after administration of different steroid hormones in the target organ kidney. Aldosterone and estradiol very significantly increased the enzyme activity (table). 5α-Dihydrotestosterone (100 µg), 11-deoxycorticosterone (10 µg) and corticosterone (10 µg) did not induce enzyme activity.

Induction of the synthesis of thymidine kinase by aldosterone. The administration of actinomycin (25 µg/100 g b.wt) and cycloheximide (100 µg/g b.wt) totally inhibited the effect of aldosterone on thymidine kinase activity (fig. 2). The inhibition of the transcription by actinomycin and translation by cycloheximide allowed us to conclude that aldosterone could induce the synthesis of this enzyme rather than the increase in its activity.

Discussion. The presence of binding sites for aldosterone in the kidney is well established[5, 6, 7, 13, 15]. In the classic scheme of the mechanism of action of steroid hormones, the nuclear receptors of aldosterone interact very probably with specific genes, and induce the synthesis of specific proteins. It is important to know the nature of these proteins, the synthesis of which is stimulated by aldosterone. Our results show that this hormone is able to stimulate the synthesis of thymidine kinase in the kidney of the immature male rat. Following the injection of 10 µg of aldosterone, a rapid increase of the activity of thymidine kinase was observed. It is significant 1 h after hormone administration. It rises regularly, and reaches a maximal level 24 h after the injection of aldosterone. At this time, the activity of thymidine kinase is 6 times higher than that in the control. The effect is specific for aldosterone, since corticosterone and 11 deoxycorticosterone at the same dose, and 5α-dihydrotestosterone, are without effect. On the other hand, 17β-estradiol administration (0.1 µg) increases the activity of thymidine kinase in the kidney of the immature male rat. This effect is not surprising, since it has been shown that estrogens are able to induce the synthesis of this enzyme in their target organs and the presence of the receptors of estradiol in the kidney is also well established[3, 11, 14, 16].

The administration of actinomycin and cycloheximide inhibits the effects of aldosterone on the increase in the activity of thymidine kinase. One can conclude that this hormone provokes the

Study of the hormonal specificity of thymidine kinase. Induction in the kidney (TK = thymidine kinase)

	Dose	TK activity pmole of d-TMP/mg protein/min
Control	DMSO + ethanol	5.5 ± 0.28
Aldosterone	10 µg	28 ± 0.33
Deoxycorticosterone	10 µg	3.6 ± 0.44
Corticosterone	10 µg	4.18 ± 0.0752
Estradiol	100 µg	19.8 ± 0.386
5α-DHT	100 µg	6.1 ± 0.147

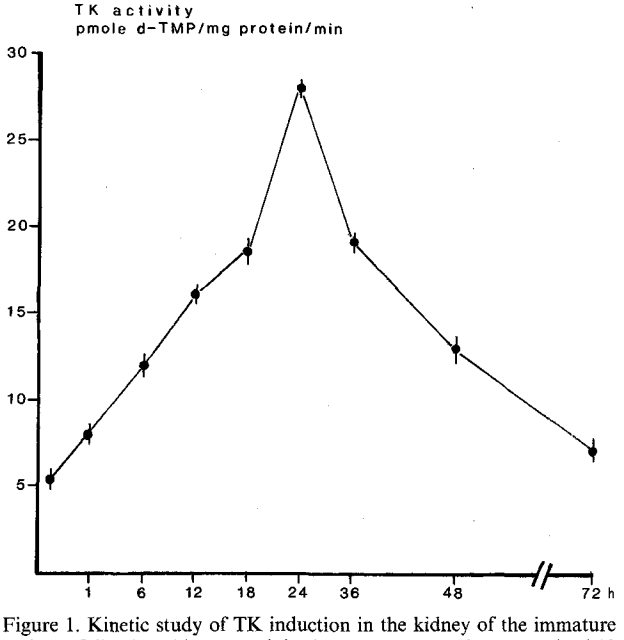

Figure 1. Kinetic study of TK induction in the kidney of the immature male rat following aldosterone injection. Immature male rats received 10 µg aldosterone s.c. Animals were sacrificed 1–72 h after the injection (TK: thymidine kinase activity).

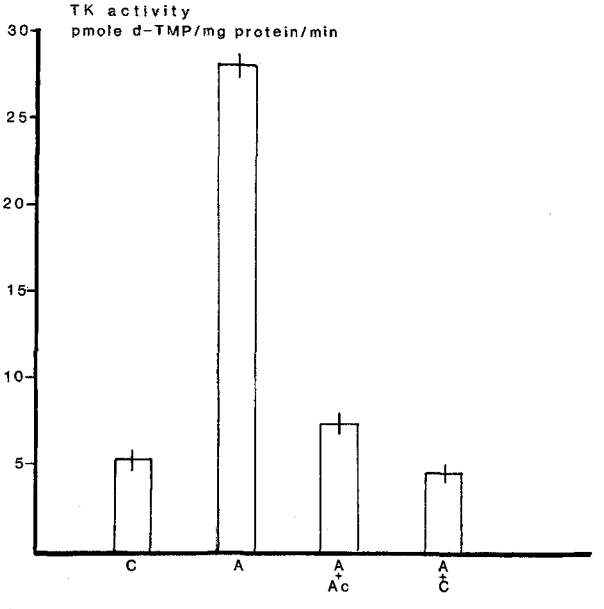

Figure 2. Variations of thymidine kinase activity in the immature male rat kidney after actinomycin D and cycloheximide (TK: thymidine kinase activity). *C* Rats receiving the vehicle alone (DMSO: ethanol (2/3). *A* Activity of TK, 24 h after injection of 10 g aldosterone. *A+Ac* Activity of TK, after simultaneous injection of aldosterone (10 µg)+actinomycin D (25 µg/100 g b.wt). *A+C* Activity of TK, after simultaneous injection of aldosterone (10 µg)+cycloheximide (100 µg/100 g b.wt).

synthesis of new molecules of the enzyme rather than an increase of its activity.

In conclusion, our data show that aldosterone is able to induce the synthesis of thymidine kinase in the kidney. This steroid possesses identical effects to those of estrogens and androgens in their target organs[1,10]. Thymidine kinase participates in the synthesis of DNA by the 'salvage pathway'. This seems to indicate that aldosterone participates in the development of the kidney, but at the present time, nothing indicates that the increase in thymidine kinase activity represents a fundamental process.

* To whom all correspondence should be addressed: Laboratoire de Biochimie des Hormones (U.A.-CNRS 626), Centre Régional de Recherche en Endocrinologie, av. du Prof. L. Bernard, F-35043 Rennes Cedex (France).

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Growth-hormone releasing factor does not antagonize somatostatin effects on pancreatic and gastric secretions

M. Chovet, M. Dubrasquet, J. Chariot and C. Rozé*

INSERM U 239 and INSERM U 10, Faculté X. Bichat, 16 Rue H. Huchard, F-75018 Paris (France), 22 October 1984

Summary. The effect of human GRF 44 upon somatostatin-inhibited pancreatic and gastric secretions was studied in rats with chronic fistulas. GRF did not show any antagonist action of somatostatin on these gastro-intestinal organs. GRF alone had a small inhibitory effect on gastric acid output.

Key words. GRF; somatostatin; pancreatic secretion; gastric secretion.

Human growth hormone releasing factors (GRF's) have been isolated and characterized from human pancreatic tumors producing acromegaly[1-3] and from the hypothalamus[4], and the corresponding peptides synthesized. Since GRF is an antagonist of somatostatin on pituitary cells, the question arose whether antagonist effects might also exist on peripheral target organs. The aim of this study was to check this hypothesis on exocrine pancreatic and gastric secretions in rats.

Materials and methods. Male Wistar rats weighing 280–320 g were obtained from R. Janvier, 53 Le Genest, France.

1. Pancreatic secretion. Subacute pancreatic fistulas were prepared under light ketamine anesthesia as described in detail elsewhere[5]. Briefly, all of the pure pancreatic juice was collected from a catheter in the lowest common bile duct, while bile was recirculated to the duodenum through the choledoco-duodenal junction, using a Silastic shunt, and another Silastic catheter was inserted in the duodenum to allow infusions in the gut. The animals were kept in Bollman cages and used for experiments 24 h after surgery (surgery = day 0). Experiments were performed daily in conscious rats from the afternoon of day 1 until day 5. The central temperature of the animals was maintained at approximately 38 °C. Water was freely available, and food was given daily ad libitum between 5 p.m. and 8 a.m. The duodenal catheter was continuously infused with 2.5 ml/h of an equivolume mixture of 100 g/l glucose and Hartmann Ringer B21 electrolyte solution, containing 8000 IU/ml of porcine trypsin (Sigma I 0134). The final pH was 5.5.

2. Gastric secretion. Under light ketamine anesthesia, a Thomas cannula was inserted into the ruminal portion of the stomach and was kept closed between experiments. Rats were free to move normally and had free access to food between experiments. No more than two experiments a week were performed, in rats fasted for 18 h.

3. Experimental protocol. Secretions were collected in 20 min samples, starting with a basal period of 20 or 40 min, and followed by a 2 h venous infusion with one of the following: GRF (human pancreatic synthetic 1–44 GRF, a gift of Prof. R. Guillemin) 2.5 nmol/kg·h, somatostatin (14 aa, a gift of Clin-Midy Laboratories) 2.5 nmol/kg·h, somatostatin+GRF, each at 2.5 nmol/kg·h, saline alone. Gastric secretion was collected for 40 min, and pancreatic secretion for 2 h, after the infusion had finished. Eight to nine animals were used in each group, with usually one, and exceptionally two experiments done in each animal.

Pancreatic and gastric juice volumes were estimated by weighing the samples in tared vials. Total protein was determined by measuring the absorbance at 280 nm after appropriate dilution. Bicarbonate was determined with an Auto-Analyzer technique[6]. Gastric acid was titrated to pH 7 with 0.01 N NaOH. The significance levels given below were obtained using Student's t-test relative to the control groups.

Results. 1. Pancreatic secretion. Pancreatic secretion did not change significantly during the experiment in the control group. GRF infused alone did not change significantly the volume of pancreatic juice (−27 ± 11%). The output of bicarbonate was slightly (−39 ± 11%, fig. 1) but not significantly reduced during the second hour of infusion, while pancreatic protein output did not change (−16 ± 15%, fig. 2).