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1 Gomez-Pan, A., and Rodriguez-Arnao, M. D., Clin. Endocr. Metab. *12* (1983) 469.
2 Baldissera, F. G. A., and Nordio, E. C., Riv. Biol. *78* (1985) 123.
3 Strosser, M.-T., Harvey, S., Foltzer, C., and Mialhe, P., Gen. comp. Endocr. *56* (1984) 269.
4 Hasegawa, Y., Miyamoto, K., Nomora, M., Igwashi, M., Kanagawa, K., and Matsuo, H., Endocrinology *115* (1984) 433.
5 Harvey, S., and Hall, T. R., Gen. comp. Endocr. *65* (1987).
6 Hall, T. R., Harvey, S., and Chadwick, A., Neuroendocrinology *39* (1984) 206.
7 Hall, T. R., Chadwick, A., and Harvey, S., Comp. Biochem. Physiol. *76C* (1983) 151.
8 Hall, T. R., Harvey, S., and Chadwick, A., Acta endocr. *105* (1984) 455.
9 Rabbi, J., Buonomo, F., and Scanes, G. C., J. Endocr. *90* (1981) 355.
10 Scanes, C. G., Rabbi, J., and Buonomo, F. C., in: Aspects of Avian Endocrinology: Practical and Theoretical Applications, p. 13. Eds C. G. Scanes, M. A. Ottinger, A. D. Kenny, J. Balthazart, J. Cronshaw and I. Chester. Jones Grad. Studies Texas Tech. Univ., Lubbock, Texas 1982.
11 Richardson, S. B., Hollander, C. S., D'Eletto, R., Greenleaf, R. W., and Thaw, C., Endocrinology *107* (1980) 122.
12 Hall, T. R., Harvey, S., Spencer, S. G., and Chadwick, A., Neuroendocrinology (1987) in press.
13 Harvey, S., Lam, S. K., and Hall, T. R., J. Endocr. *111* (1986) 91.
14 Harvey, S., in: Progress in Non-mammalian Brain Research, vol. 3, p. 207. Eds G. Nistico and L. Bolis. CRC Press, Boca Raton, Florida 1983.
15 Bakhit, C., Koda, L., Benoit, R., Morrison, J. H., and Bloom, F. E., J. Neurosci. *4* (1984) 411.

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Potentiating effect of aldosterone on the diuretic action of atrial extract

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Summary. The typical stimulatory effect of a rat heart atrial extract on urinary water, sodium, potassium and kallikrein excretion is significantly increased by a previous administration of aldosterone (0.5 µg/100 g b. wt) in the rat.
Key words. Atrial natriuretic factor; aldosterone; urinary kallikrein; natriuresis; kidney.

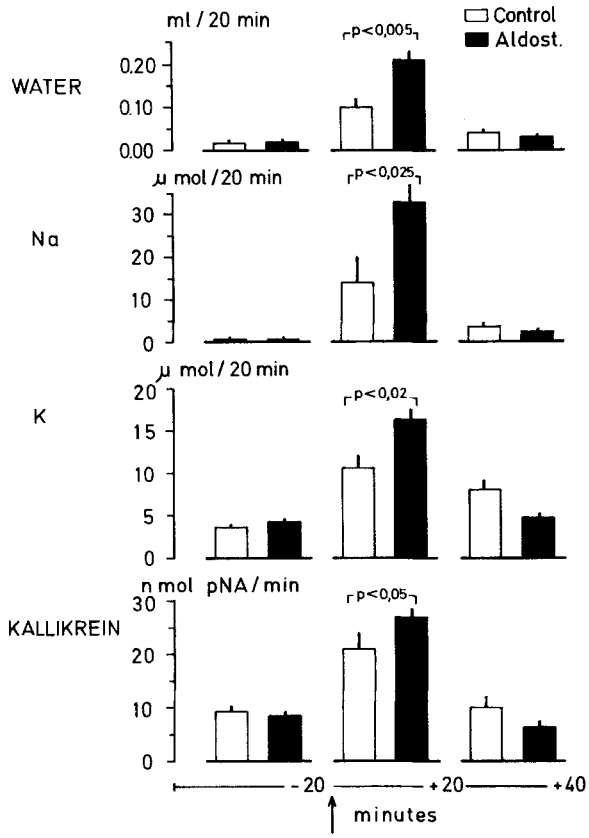
Crude extracts of mammalian cardiac atria have powerful natriuretic and vascular muscle relaxant activity[1,2]. These effects are mediated by peptides identified as atrial natriuretic factors (ANF). All contain the same core sequence of 17 amino acids but differ in the lengths of their amino and carboxyl termini[3]. Circulating ANF have been detected in the blood by radioimmunoassay, suggesting that they have an endocrine role in fluid and electrolyte homeostasis[4-7]. One striking feature of ANF is its ability to inhibit the secretion of aldosterone, the most potent physiological factor promoting sodium reabsorption by the kidneys. ANF reduces basal secretion of aldosterone in vivo[8] and the secretion induced by angiotensin stimulation of the adrenal cortex in vitro[9,10]. Suppression of aldosterone secretion in vivo is amplified by the concurrent inhibition of renin secretion, the enzyme which liberates angiotensin[11]. The accumulated evidence leads to the cogent assumption that a rise of aldosterone concentration in the blood by exogenous administration may hamper ANF-induced natriuresis. But contrary to this expectation, our results show that natriuresis is significantly enhanced by administration of a moderate dose of aldosterone 4 h before an i.v. bolus of a standard dose of ANF.

In the present experiments, the urinary excretory rates of water, sodium, potassium and kallikrein were compared in two groups of rats (8 in each). Both groups were given the same dose (0.1 ml) of rat cardiac atria extract containing semi-purified ANF. One group, 200–220 min before the administration of ANF, received 0.5 µg/100 g b. wt i.p. of d-aldosterone (Sigma) diluted in isotonic glucose solution (0.1 ml); the other one only the vehicle. According to previous reports the effects of exogenous aldosterone can be registered within a few hours (2–4 h). The dose of this mineralocorticoid employed here is close to the physiological range[12].

Female Sprague-Dawley rats (200–220 g) were used. They were fed with the normal laboratory diet, which contains 180 mg of Na and 750 mg of K/100 g, and had tap water ad libitum. 12 h before the experiment no food was given. The animals were kept at constant temperature with a 12-h alternated rhythm of light and darkness, they were gently manipulated and any stressing factor (noise, seclusion, etc.) was prevented. According to our experience, prevention of any type of stress during the manipulation of the rat significantly reduces the variability in the bioassay, which is particularly important for the assessment of the natriuretic activity[13]. Both groups, those injected with aldosterone and the controls injected with the vehicle, were anesthetized 1 h later by i.p. injection of sodium penthotal (4 mg/100 g b.wt). The rats were then returned to the cages, and (while deeply anesthetized) transported to the place where the surgical procedure was carried out. Blood pressure was continuously recorded through a cannula placed in the left carotid artery; a catheter introduced in the bladder through the urethra allowed continuous collection of urine.

Urine volume, the amounts of sodium and potassium, and kallikrein activity, excreted every 20 min, were measured for a period of 200 min. Throughout the experiment the rats were infused with 0.60 ml/100 g b.wt/60 min of isotonic glucose solution into the jugular vein. The amounts of sodium and potassium excreted, measured by a flame photometer (Eppendorf) were expressed in µmol per 100 g b.wt excreted in 20 min. Kallikrein activity was determined by the amidase method[14] and expressed as nmol of p-nitroaniline generated per min at 37 °C. The urinary excretion of kallikrein was calculated by the urine volume and referred to 100 g b.wt. The determination of kallikrein was included in this study because several lines of investigation suggest a role for the renal kallikrein-kinin system in the regulation of renal hemodynamics, fluid and electrolyte homeostasis[15].

The ANF preparation was obtained as folllows: atria were excised from 300 adult rats. The tissue was rinsed with cold saline, homogenized, mixed with 5 volumes of cold 0.1 M acetic acid, and centrifuged. The supernatant was heated in a boiling water bath for 15 min. After cooling, the precipitate was removed by centrifugation and the supernatant neutralized with ammonia. To preclude the action of proteases, 1 mg of ovomucoid and 1 mg of soy-bean antitrypsin inhibitor were added. After a final centrifugation the extract was lyophilized. This material was dissolved in saline (45 ml) and stored in vials at − 40 °C. The



Effect of atrial extract on urinary volume, sodium, potassium and kallikrein excretion in the aldosterone group (black bars) and control group (white bars) in 3 successive periods of 20 min. The arrow indicates i.v. administration of atrial extract in both groups. Mean values and SE are referred to 100 g b. wt. For other details see text.

extract contained 14 mg/ml of protein, and when administered as an i.v. bolus in the rat it induced a natriuresis 40–50 times above the basal value. The atrial extract contains several active peptides, derived from the same precursor molecule, and the exact nature of the secreted forms remains to be elucidated. Further purification of the ANF was not attempted in this study. As shown in the figure, ANF produced a typical increase of all the factors analyzed, within the first 20-min period following the injection. The most remarkable finding is the significant increase of water and electrolytes excretion in the aldosterone group as compared to the control. The difference is particularly noticeable in water and sodium excretion which were more than twice the control values. In the last 20-min period the values tend to return to the basal values, except for K of the control group, which remained significantly higher.

No significant differences in Na/K ratio of the urine collected in the 3 periods of 20 min were observed. The hypotensive effect of the atria extract (duration and maximal fall) was similar in both groups (not shown). Confirming our previous report[16] kallikrein excretion is increased by ANF. The increase was greater in the aldosterone group ($p < 0.05$). The response of this enzyme, which is produced in the connecting segment[17] is an index of the participation of this part of the nephron in the diuretic-natriuretic kaliuretic action of ANF and is probably related to fluid and electrolyte load in distal tubules[18]. The effectiveness of aldosterone of the chosen dose was demonstrated in a traditional bioassay by the administration of the hormone (i.p.) to 10 non-anesthetized female rats maintained on the same standard diet. After a period of adaptation the animals were placed in metabolic cages. Food was withheld for 14 h before the study, but the rats were allowed water ad libitum. Volume and electrolyte levels were determined for the urine collected 4 h after the injection of aldosterone. This hormone induced a significant decrease in Na excretion ($p < 0.02$) as compared to the control group ($n = 10$).

It is difficult, with the available data, to suggest an explanation of the mechanism by which aldosterone facilitates the diuretic, natriuretic and kaliuretic action of ANF. However, we have described that aldosterone (5 µg) given s.c. to unanesthetized rats was also able to facilitate the stimulatory effect upon the urinary excretion of water, sodium and potassium elicited by renin[19]. The mechanism responsible for the diuretic action of renin has not been satisfactorily explained either, but it seems reasonable to suppose that ANF is involved in it. Unfortunately, the mechanism of natriuresis elicited by ANF is still a matter of controversy, nonetheless there is a growing body of evidence that one major mechanism underlying the rise in diuresis-natriuresis is the increase in glomerular filtration rate[20], although a contribution of the inhibition of Na reabsorption in the tubules has also been postulated[21]. High affinity ANF binding sites have been found over glomeruli and to a lesser extent in the arterial vasculature. Lower affinity binding sites were discovered in proximal tubules and inner medullary collecting ducts[21]. Experiments will be required to determine whether aldosterone facilitates the ANF effect on glomerular filtration rate and additional studies will be necessary to elucidate the interplay between ANF and aldosterone under the experimental conditions here described, but considering that the distal and collecting tubes are the main sites of aldosterone action, it is possible that this hormone could modulate the density (or affinity) of the ANF receptors in that tubular region. However, the invariability of Na/K ratio would point to the possibility that aldosterone can enhance the ANF renal effect both at the level of GFR and at that of the collecting tubes.

Furthermore, it is feasible to hypothesize that ANF may be involved in the 'escape phenomenon' facilitating the return of the sodium balance toward normal levels, and thus counteracting the transient sodium retention produced by the administration of mineralocorticoids.

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1 De Bold, A.J., Borenstein, H.B., Veress, A.T., and Sonnenberg, H., Life Sci. *28*,(1981) 89.
2 Garcia, J., Cantin, M., Thibault, G., and Genest, J., Experientia *38* (1982) 1071.
3 Marks, M., Takayanagi, K.S., Misono, K.S., Pandey, K.N., Tibbets, C.T., and Inagami, T., Nature *309*(1984) 722.
4 Tanaka, I., Misono, K.S.T., and Inagami, T., Biochem. biophys. Res. Commun. *124*(1984) 663.
5 Gutkowska, J., Horky, K., Thibault, G., Januszwicz, P., Cantin, M., and Genest, J., Biochem. biophys. Res. Commun. *125*(1984) 315.
6 Lang, R.E., Tholken, H., Ganten, D., Luft, F.C., Ruskoaho, H., and Unger, Th., Nature *314*(1985) 264.
7 Manning, P.T., Schwartz, D., Katsube, N.C., Holmberg, S.W., and Needleman, P., Science *229*(1985) 395.
8 Chartier, L., Schiffrin, E.L., and Thibault, G., Biochem. biophys. Res. Commun. *122*(1984) 171.
9 Atarashi, K., Mulrow, P.J., Franco Saez, R., Snapjar, R., and Rapp, J., Science *224*(1984) 992.
10 Kudo, T., and Baird, A., Nature *312*(1984) 756.
11 Maak, T., Marion, D.N., Camargo, M.J.F., Kleinert, H.D., Laragh, J.H., Vaughan, E.D., and Atlas, S.A., Am. J. Med. *77*(1984) 1069.
12 Miller, D.H., Lindley, J.G., and Margolius, H.S., Proc. Soc. exp. Biol. Med. *180*(1985) 121.
13 Johnson, M.D., Am. J. Physiol. *248*(1985) F314.
14 Admundsen, E., Freiberger, P.P., Knos, M., Landsbratten, M., and Claeson, G., Advances in Experimental Medicine and Biology: Kinin II. Eds F. Moriya and T. Susuki. Plenum Press 1979.
15 Croxatto, H.R., Revta med. Chile *109*(1981) 143.
16 Croxatto, H.R., Rosas, R., Villalón, P., and Pesce, F., Nefrologia Pediatrica, p. 57. Ed. C. Saieh. Santiago, Chile 1984.

17 Vio, C.P., and Figueroa, C.D., Kidney int. *28*(1985) 36.
18 Croxatto, H.R., Corthorn, J., Roblero, J., Villalón, P., and Pérez, F., Am. J. Physiol. *250*(1986) F400.
19 Croxatto, H.R., Labarca, E., and Cofre, G., Nature *199*(1963) 182.
20 Chou-Kong, H., Lewicki, J., Johnson, L.K., and Cogan, G., J. clin. Invest. *75*(1985) 769.
21 Healy, D.P., and Fanestil, D.D., Am. J. Physiol. *250*(1986) F573.

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Increased responsiveness of the hypothalamic-pituitary axis to steroid feedback effects in ovariectomized rats treated neonatally with monosodium L-glutamate

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Summary. Chronic ovariectomized rats treated neonatally with MSG showed reduced circulating concentrations of LH coupled with elevated hypothalamic LHRH stores. Despite the apparent loss of LHRH secretion, the small pituitary glands showed an increased density of LHRH receptors and normal responsiveness to the releasing hormone. The positive feedback effects of progesterone on LH release in oestrogen-primed animals was greatly exaggerated reflecting the build-up of hypothalamic LHRH stores without loss of pituitary responsiveness to LHRH.
Key words. Monosodium L-glutamate; luteinizing hormone; luteinizing hormone-releasing hormone; ovariectomized rats; steroid feedback.

Rats treated neonatally with the neurotoxin, monosodium L-glutamate (MSG), have permanent lesions of the arcuate nucleus which cause a disruption of the neuroendocrine regulation of gonadal function and hypogonadism, despite normal serum levels of LH[1,2]. Since there appears to be no impairment in the release mechanism for LH-releasing hormone (LHRH)[3] or in the pituitary responsiveness to LHRH[4] it is likely that MSG treatment disrupts the neuronal circuits mediating steroid feedback control of LHRH secretion. Indeed, adult rats treated neonatally with MSG show an attenuated rise in LH release after ovariectomy[5,6] and a reduction of the positive feedback effects of chronic oestrogen administration to ovariectomized rats[7].

In these experiments we have further investigated the effects of MSG-induced arcuate lesions on steroid feedback mechanisms in ovariectomized rats. The negative and positive feedback action of exogenously administered steroids has been assessed and the results have been correlated with measurements of hypothalamic LHRH content, pituitary LH responses to LHRH and pituitary LHRH receptors.

Material and methods. Female Porton Wistar pups were injected i.p. with 4g MSG/kg in 0.9% saline on days 2, 4, 6, 8 and 10 after birth; control rats received an equivalent volume of isosmotic NaCl (10% w/v). After weaning at approximately 3 weeks of age, the sexes were separated and they were housed under controlled conditions of light (lights on 06.00–18.00 h) and temperature (22 °C); food and water was available ad libitum. When they were 3 months old they were ovariectomized and three weeks later used for experimentation.

One group of animals was stunned and decapitated, trunk blood was collected for hormone measurements, and the pituitaries and hypothalami dissected out. The hypothalami measured approximately 5 × 4 × 2 mm with the rostral and caudal limits being defined by the optic chiasma and mamillary bodies respectively. Immediately after dissection, the tissue was placed in 1 ml ice cold saline containing 1mM bacitracin (Sigma), disrupted by sonication and the separated extract stored at −20 °C until assayed for LHRH.

The pituitaries were hemisected and one half was used to investigate the responses of perfused glands to LHRH and the other half for the measurement of pituitary LHRH receptors. Hemipituitaries were placed on 200-µl volume perspex chambers and perfused with Krebs ringer bicarbonate (KRB) containing 2 g glucose/l and 2.5 g bovine serum albumen/l. The perfusate was constantly gassed with 95% O_2/5% CO_2 and delivered to the tissue at a rate of 0.2 ml/min. After an initial 2 h stabilization period, 10-min fractions were collected for a further 2-h period during which time two 5-min pulses of 10 ng LHRH (Cambridge Research Biochemicals Ltd., Harston CB2 5NX)/ml KRB was delivered to the gland with an interval of 1h betwwen each pulse. Perfusate samples were stored at −20 °C.

Pituitary LHRH receptors of individual hemi-pituitaries were measured by binding of the LHRH-analogue [D-Ser(t-Bu)[6]] des-Gly[10]-N-ethylamide (buserelin, Hoechst (UK) Ltd., Middlesex House, Hounslow) using a method described previously[1]. Protein estimation of individual homogenates were determined by the method of Lowry et al.[9].

In a second group of animals, the response to exogenous steroid treatment was investigated. On day 21 at 12.00 h, a blood sample was obtained by cardiac puncture under ether anesthesia just

Effects of neonatal MSG treatment on body and pituitary weight, gonadotrophic hormone secretion, hypothalamic LHRH content and pituitary LHRH receptors in adult ovariectomized rats. Values give mean ± SEM and group numbers are given in parenthesis. †, $p < 0.005$ and * $p < 0.05$ compared with control; Student's t-test

	MSG Treated	Control
Ovarian weight at ovariectomy (mg)	43.2 ± 3.9† (10)	69.3 ± 6.2
Pituitary weight (mg)	16.8 ± 1.18† (10)	33.8 ± 1.42 (9)
Body: tail lenght ratio	0.73 ± 0.01† (10)	0.94 ± 0.02 (10)
Serum LH (µg/l)	116 ± 29† (8)	404 ± 0.02 (7)
Serum prolactin (µg/l)	53 ± 29 (8)	21 ± 3 (7)
Hypothalamic LHRH (pg)	1212 ± 87[6] (6)	362 ± 78 (6)
Pituitary LHRH receptors:		
a) fmoles/hemi-pituitary	43.5 ± 4.3 (10)	54.0 ± 6.6 (9)
b) fmoles/mg protein	65.7 ± 7* (10)	41.0 ± 6.8 (9)