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RAPID COMMUNICATION

INVOLVEMENT OF THE ADRENAL GLANDS IN THE ACTION OF THE ATRIAL NATRIURETIC FACTOR

WALDEMAR DEBINSKI, OTTO KUCHEL, NGUYEN T. BUU, RAUL GARCIA, MARC CANTIN AND JACQUES GENEST

Clinical Research Institute of Montreal
110 Pine Avenue West, Montreal, Quebec, Canada H2W 1R7

Abstract. Adrenalectomized, medullectomized and sham operated rats were treated with either a chronic infusion or a bolus injection of the synthetic atrial natriuretic factor (ANF). ANF did not enhance natriuresis and diuresis in sham operated conscious animals during chronic infusion, but it had a potent action when injected as a bolus into anesthetized rats. The absence of the whole adrenal glands, but not adrenal medulla profoundly modified the renal response to ANF: a) following chronic administration of ANF, the baseline natriuresis paradoxically decreased in adrenalectomized rats, and b) in response to a bolus injection of ANF the natriuretic and diuretic actions of the peptide were attenuated in these animals. The medullectomy-induced decreased natriuresis and dopamine excretion were corrected by ANF infusion. Furthermore, ANF suppressed the compensatory increase of norepinephrine excretion secondary to adrenalectomy. The data suggest that the presence of the adrenal cortex is necessary for the natriuretic and diuretic actions of ANF. The decrease in urinary DA excretion may reflect diminished dopaminergic activity and contribute to the post-medullectomy antinatriuresis, a phenomenon which can be corrected by ANF infusion. ANF may also have a depressing activity on the increased sympathetic tone.
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Introduction. The atrial natriuretic factor (ANF) is a circulating peptide(s) with a natriuretic, diuretic and vasodilating action suggestive of its involvement in the volume homeostasis (1-3). It has been recently demonstrated that peripheral chemical sympathectomy resulted in an attenuation of the natriuretic response to both atrial extract injection and blood volume expansion in rat (4). A possible interaction of ANF with the peripheral sympathetic nervous activity has been also suggested by a report indicating that the renal action of ANF is blunted in animals pretreated with antagonists of dopaminergic receptors (5). In addition, adrenal steroids, such as aldosterone could also be involved in the natriuretic action of ANF by being negatively modulated by ANF (6-8); alternatively, glucocorticoids could be factors conditioning the action of ANF in the kidney at the glomerular or tubular level (9). The ANF induced natriuresis and diuresis may thus depend on the integrity of the adrenocortical and adrenomedullary functions which are, independently of ANF, involved in the adjustment of the sodium and volume balance. It was thus of interest to explore how the presence or absence of the adrenal cortex, adrenal medulla respectively, modifies the natriuretic and diuretic actions of ANF in rats. Since it is not known, whether ANF may affect the catecholaminergic activity in rats, and adrenalectomy induces a compensatory activation of the peripheral sympathetic nervous sys-

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tem (10) we measured also urinary catecholamine excretion in animals chronically infused with ANF.

Materials and Methods. Chronic infusion of ANF: Male Sprague-Dawley rats (250-310 g) underwent adrenalectomy (ADREX), medullectomy (MEDX) and sham operation (SHAM). The rats were kept in separate metabolic cages on regular rat chow and 0.9% sodium chloride for drinking ad libitum for 48 hr prior to the study. Bilateral adrenalectomy was performed 2 days before and medullectomy (Charles River) at least one week (to avoid early adrenal insufficiency following demedullation procedure) before the insertion of the miniosmotic pumps (Alza 2001, Palo Alto, CA) filled with synthetic ANF (Arg 101-Tyr 126, Merck Sharp & Dohme) to release 1 μg/hr of the peptide. A control group received an infusion of the vehicle. The pumps were implanted in the animals subcutaneously at the back of the neck, and connected via catheter (PE-60, Intramedic) with the jugular vein under light ether anesthesia. The urine was collected daily, and free catecholamines and sodium excretions were determined on the second day of continuous infusion of ANF. Mean blood pressure (MBP) was measured directly in the tail artery by the method of Chiueh and Kopin (11) before and after ANF infusion into ADREX rats. Each group was comprised of 8-10 rats.

Bolus injection of ANF: ADREX, MEDX and SHAM rats (250-400 g) with the same interval between surgery and the experiment as in the chronic infusion protocol were kept on 0.9% saline for drinking for 2 days prior to the study. The rats were anesthetized with intraperitoneal sodium pentobarbital (MTC, Canada). The catheter (PE-60) was placed in the jugular vein for the injection of ANF and volume replacement by isotonic saline, and urine was collected through the bladder catheter (PE-50). The preparation was allowed to stabilize for 30 minutes and then urine samples were collected for a control period of 20 minutes. ANF was injected intravenously at a dose of 4 μg/kg. Urinary volume and sodium excretion were measured at five minute intervals for 20 minutes following injection. This experiment was repeated in another group of ADREX and SHAM rats, which had an additional catheter (PE-50) in the carotid artery for the MBP measurement (Gross-Polygraph 7PCPB). Each group was comprised of 5-7 rats.

Urinary catecholamines were determined radioenzymatically (12), urinary sodium by flame photometry and creatinine colorimetrically.

Results are expressed as mean ± SEM. Single comparisons were done by the Student's t-test. One way analysis of variance, repeated measure design and the Dunnet's test were used for multiple comparisons.

Results. As seen in Table I, there was a significantly lower baseline sodium excretion in MEDX and ADREX rats than in SHAM animals. Surprisingly, chronic ANF infusion did not change urinary volume and sodium excretion in SHAM rats. It produced however a further decrease in urinary volume and sodium excretion in ADREX rats; the ANF-induced decrease in Na^+ excretion was not significant when expressed in mmol Na^+/mg creatinine (ANF treated 0.29 ± 0.1 and vehicle treated 0.35 ± 0.2). The ANF infusion led to a disappearance of the difference in urinary Na^+ excretion found between the vehicle-infused MEDX and SHAM rats, although treatment with ANF did not increase significantly the natriuresis and diuresis in MEDX rats. Administration of ANF for 48 hr had no effect on the MBP in ADREX rats (in control group = 73 ± 3 mmHg, and ANF infused group = 71 ± 4 mmHg).

Completeness of medullectomy was confirmed by very low values of urinary epinephrine (E), which did not change during ANF administration. Urinary dopamine (DA) excretion was lower in MEDX than in SHAM rats, and this difference was abolished by ANF treatment, as it was the case for sodium excretion in this group of rats.

More evident were the differences in the responsiveness to ANF following a bolus injection into anesthetized rats, shown in Figure 1. Sham operated and medullectomized rats increased their sodium excretion and urinary volume to a comparable degree, whereas adrenalectomized rats exhibited an attenuated natriuretic and diuretic response to a

Table I

EFFECT OF A 48 HR ANF INFUSION ON NATRIURESIS, DIURESIS AND URINARY DOPAMINE AND EPINEPHRINE EXCRETION IN CONSCIOUS RATS

	SODIUM EXCRETION (mmol/24 hr)		URINARY VOLUME (ml/24 hr)	
	Control	ANF	Control	ANF
SHAM	5.0 ± 0.6	5.1 ± 0.9	19.9 ± 3.3	15.7 ± 2.6
MEDX	3.3 ± 0.7*	6.2 ± 1.5	12.4 ± 3.0	28.5 ± 8.5
ADREX	2.8 ± 0.4*	1.6 ± 0.2*†	18.5 ± 2.4	10.7 ± 1.4*†
	DOPAMINE (μg/24 hr)		EPINEPHRINE (μg/24 hr)	
SHAM	4.4 ± 0.6	3.3 ± 0.6	0.28 ± 0.05	0.28 ± 0.09
MEDX	2.5 ± 0.6*	3.1 ± 0.5	0.05 ± 0.01*	0.12 ± 0.03
ADREX	2.6 ± 0.4	2.3 ± 0.4	0.06 ± 0.01*	0.06 ± 0.01*

* $p < 0.05$ vs SHAM
† $p < 0.05$ between ANF and vehicle treated rats.

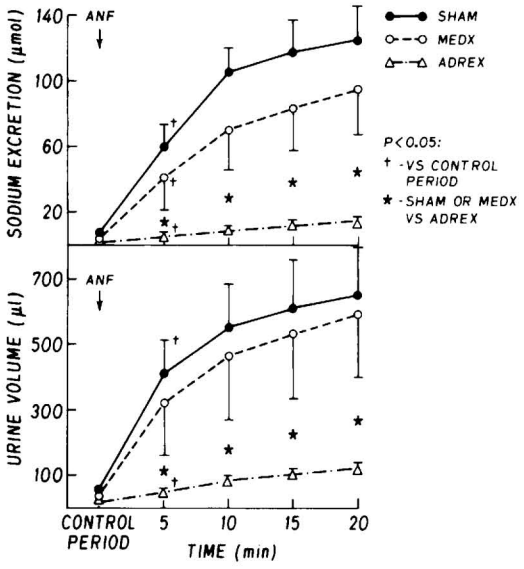

FIGURE 1: Natriuretic and diuretic response to an ANF bolus in anesthetized rats. The control period represents a 5 minute equivalent of the baseline urinary collection.

bolus of synthetic ANF. During the first five minutes after the injection, sodium excretion was significantly higher in all three groups of rats when compared with that of the control period. There was no difference in sodium excretion between MEDX and SHAM rats for 20 minutes following the injection of ANF, however the natriuresis in both SHAM and MEDX groups of animals was significantly higher than in ADREX rats during this period of time. The changes in urinary volume in response to a bolus injection of ANF had a similar pattern to that of the sodium excretion. The same experiment repeated with concomittant measurement of the arterial blood pressure in ADREX and SHAM rats (Table II) showed no difference between both groups of rats in the baseline MBP. The injection of the peptide caused a comparable decrease in blood pressure in the two groups of rats. The initial weight loss after surgery was also similar.

As seen in Figure 2, the excretion of norepinephrine (NE) in ADREX rats

Table II

EFFECT OF ANF BOLUS INJECTION ON MBP IN ADREX AND SHAM RATS

	BASELINE MBP (mmHg)	MBP DECREASE (mmHg)	WEIGHT LOSS 48 hr after surgery (g)
ADREX	92.9 ± 5.4	- 15.7 ± 3.7	- 15
SHAM	99.2 ± 4.0	- 20.5 ± 5.5	- 17

increased several times when compared with that observed in SHAM and MEDX rats. Chronic infusion of ANF caused a significant decrease in the NE excretion in adrenalectomized animals but this remained still higher than in the other two groups. When the values were corrected for creatinine, the difference between ANF and vehicle treated rats persisted suggesting that such a decrease was not due to an effect of ANF on the renal excretory mechanism of NE.

Discussion. The absence of the adrenal glands results in distinct changes in blood volume, cardiac performance and state of hydration (13, 14). Against this background has to be discussed the adrenalectomy-induced modification of the renal response to ANF. Though the diuretic and natriuretic responses to bolus administration of ANF were clearly attenuated in ADREX rats, the chronic infusion caused lowering of sodium excretion and urinary volume from control values, thus a paradoxical response to ANF. The attenuated response of ADREX rats to the ANF bolus injection appears not to be due to any of the changes associated with adrenalectomy: the animals were adrenalectomized 48 hr before the experiment, kept on a 0.9% NaCl for drinking after surgery and did not respond to ANF with excessive hypotension. Weight loss in ADREX rats was comparable with that of SHAM animals excluding rather marked changes in the state of hydration of these rats. Interestingly, adrenalectomized rats retain the ability to respond to

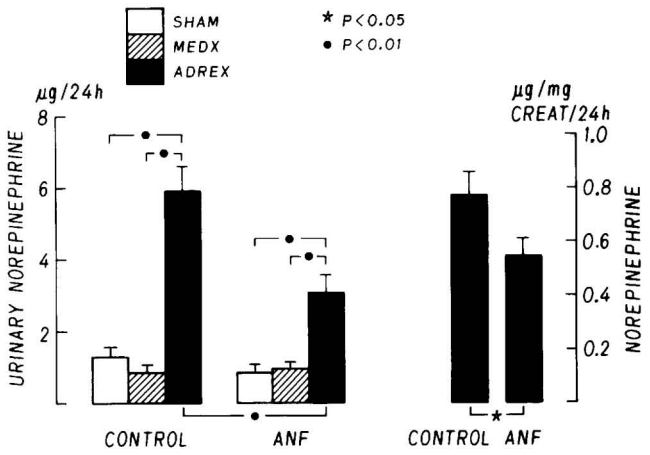

FIGURE 2: Urinary NE excretion after 48 hr of ANF chronic infusion in conscious rats.

other natriuretic agents. In an experiment with furosemide, the ADREX rats kept under the same conditions as in our protocol had even exaggerated natriuretic response when compared with that of a control group (15). The comparable MBP responses to ANF in ADREX and SHAM rats make very improbable that the decreased renal responsiveness to ANF of ADREX rats may be caused by a low output failure in which an attenuated response to ANF injected into the renal artery has been observed (16). The data support a genuine hyporesponsiveness to ANF in ADREX rats.

The medullectomy itself resulted in a decreased sodium excretion, in agreement with previous findings (17). We have demonstrated, that this altered natriuresis is associated with a decrease in urinary DA excretion, possibly related to the absence of the adrenal medulla. There is evidence of DA involvement as an endogenous natriuretic factor (18) and our finding is compatible with lower dopaminergic activity being responsible in part for the decreased natriuresis. The differences between MEDX and SHAM rats in natriuresis and urinary DA excretion were abolished by ANF treatment. Further work is required to elucidate the role that DA plays in the decreased natriuresis following medullectomy and by what mechanism ANF corrects this defect.

The difference between MEDX and ADREX rats in the bolus injection protocol stresses the role of the adrenal cortex in the renal response to ANF. The mechanisms responsible for the action of ANF are not fully elucidated. Both in vivo and in isolated rat organ preparation studies suggest an increase in the GFR (19, 20) as a contributor, at least in part, to the natriuretic and diuretic action of ANF. It is also known, that adrenal steroids, mainly glucocorticoids, are responsible for keeping the GFR at an appropriate level (9). In the present study ANF was apparently unable to exert the same action in the absence of adrenocortical steroids as in their presence. This may be due to glucocorticoids being a conditioning factor for maintaining a baseline level of GFR; such a permissive action of steroids may exist at the molecular level. Whatever the mechanism of action, the essential role of steroids is supported by a parallel study in which gluco- and mineralocorticoid substitution partially restored the ANF action in ADREX rats (21).

A long-term treatment of sham operated conscious rats with ANF did not result in an increased natriuresis and diuresis. This lack of response may be caused by contra-regulatory mechanisms to ANF, not operative after the bolus administration of the peptide but becoming effective between 2-5 hr of chronic infusion (22). Alternatively, there may be a fast degradation of the infused peptide. Against this possibility speaks however, that even at 10x lower dose than that used in our study, ANF enhanced natriuresis in one-clip, one-kidney renovascular hypertensive rats during its chronic infusion (23).

The compensatory increase in NE excretion following adrenalectomy but not medullectomy is compatible with the finding that steroids and saline substitutions prevent such a change associated with adrenalectomy (24). We have demonstrated, that chronic ANF infusion suppressed the compensatory increase in NE excretion secondary to adrenalectomy. We have also previously observed that a continuous infusion of ANF suppressed the increased sympathetic tone in 1-kidney, 1-clip renovascular hypertension (25). The mechanism through which atrial peptides may influence a function of the sympathetic nervous system remains to be elucidated.

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