EXPERIMENTAL STUDY

Effects of water deprivation on atrial natriuretic peptide secretion and density of binding sites in adrenal glands and kidneys of maternal and fetal rats in late gestation

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Abstract

The effects of water deprivation for 3 days were studied in pregnant rats and their fetuses on day 21 of gestation. Maternal water deprivation induced a significant decrease of the body weight in both maternal and fetal rats. This weight loss was accompanied by significant increases in plasma osmolality and haematocrit in both maternal and fetal rats. Similarly, dehydration significantly decreased plasma atrial natriuretic peptide (ANP) concentrations and increased plasma aldosterone concentrations in maternal and fetal rats. Water-deprived maternal rats presented a significant increase in total ANP receptor density in isolated renal glomeruli and adrenal zona glomerulosa membranes. This increase was due to a significant increase in ANP_C receptor density in both renal glomeruli and adrenal zona glomerulosa. The densities of total ANP, ANP_B and ANP_C receptors in fetal kidneys and adrenal glands were not affected by maternal dehydration. These results suggest that the dehydrated maternal rat is able to up-regulate the number of its ANP receptors in its kidneys and adrenal glands, in response to a decrease in plasma ANP concentrations. In contrast, the fetal rat does not seem to be able to regulate its own ANP receptors in response to maternal dehydration, in spite of a decrease in plasma ANP concentrations.

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Introduction

Rat pregnancy is characterized by a marked expansion of plasma volume and a retention of sodium (1). This implies that several hormonal factors, including arginine vasopressin (AVP), renin, angiotensin II, aldosterone and atrial natriuretic peptide (ANP), might be involved in the gestational volume expansion and sodium accumulation. Among these factors, ANP could play an important part. Indeed, ANP is a cardiac hormone that is stored in atrial secretory granules (2) and released into blood circulation in response to atrial distension (3). ANP stimulates natriuresis and diuresis by enhancing glomerular filtration rate and excretion of sodium and water (4) and inhibiting the secretion of aldosterone induced by angiotensin II (5), renin (6) and AVP (7). The biological activity of ANP is mediated through specific receptors, mainly located in renal glomeruli and adrenal zona glomerulosa of the adult rat (8).

In the fetal rat at term, immunoreactive plasma concentrations of ANP are higher than the maternal concentrations and increase in response to volume expansion (9, 10). Autoradiographic studies have shown the presence of ANP receptors in the kidney and the

adrenal gland on day 16 of gestation (11). Alterations of water intake were performed in the adult rat in order to study the role of ANP in the regulation of body water. All the experiments have shown that water deprivation decreases plasma ANP concentrations (12–16) and increases the total number of ANP receptors in the kidney and the adrenal gland (13–15, 17).

In maternal and fetal rats, there is no information about the effects of water deprivation on the regulation of ANP secretion and receptor density. The aim of the present study was to evaluate the effects of 3-day water deprivation on plasma ANP and aldosterone concentrations of maternal and fetal rats in late gestation. In addition, in those experimental rats, the density and affinity of the different classes of ANP receptors were determined in adrenal glands and kidneys.

Materials and methods

Animals

Experiments were performed on Wistar rats bred in the laboratory. They were housed in a light-controlled room

(light period 0700 to 1900 h). Females were mated with a male for 1 night. The following day was taken as day 0 of pregnancy if spermatozoa were found in vaginal smears. Pregnant females were divided into two groups: control group and dehydrated group. In the control group, the females had free access to tap water and standard rat chow. In the dehydrated group, the females were deprived of drinking water for 3 days from day 18 of gestation, but had free access to standard rat chow. All females were killed on day 21 of gestation by decapitation and trunkal blood was collected between 0800 and 1000 h. Their fetuses were rapidly delivered by caesarean section and bled at the trunk level in less than 4 min in order to avoid fetal stress.

Blood collection

Blood samples from fetal and pregnant females were collected in chilled plastic tubes containing 5% EDTA (20 μ l/1 ml blood) and aprotinin (500 U/1 ml blood). The blood samples were centrifuged at 5000 *g* for 10 min at 4 °C and plasma samples were stored at –80 °C. To obtain one fetal plasma sample, it was necessary to pool blood from eight to 10 fetuses.

Preparation of tissue samples

Adrenal glands and kidneys of fetuses and pregnant females were rapidly dissected and defatted. Adrenals from mothers were decapsuled in order to separate the capsule with zona glomerulosa from the inner tissue. All the tissues were frozen in liquid nitrogen and stored at –80 °C. For each experiment, six to eight maternal adrenals, four maternal kidneys, 180–200 fetal adrenals and 40–50 fetal kidneys were pooled to prepare tissue samples for one binding assay.

Fetal adrenals and kidneys with medulla and maternal capsules with zona glomerulosa were ground with a Teflon homogenizer in Tris–HCl buffer (50 mmol/l, pH 7.4) containing MnCl_2 (5 mmol/l), NaCl (120 mmol/l), aprotinin (1 μ mol/l) and bacitracin (0.1%). Homogenates were centrifuged at 1000 *g* for 20 min at 4 °C and the supernatants were centrifuged at 20 000 *g* for 30 min at 4 °C. The pellets containing the membranes were rinsed with Tris–HCl buffer, centrifuged again at 20 000 *g* for 30 min at 4 °C and then diluted with Tris–HCl buffer. An aliquot was taken for protein determination and the remainder frozen in liquid nitrogen and stored at –80 °C until required for the binding assay. Maternal glomeruli were prepared according to the technique previously described by Sraer *et al.* (18). The kidneys excised from the renal capsule were cut longitudinally in order to remove the inner tissue. The minced cortical tissue was passed successively through a 106 μ m metal sieve, which excluded the tubules and blood vessels, and a 53 μ m metal sieve, which retained the glomeruli. The glomerular suspensions were rinsed three times with ice-cold

Tris–HCl buffer and centrifuged at 1000 *g* for 5 min at 4 °C and then diluted with Tris–HCl buffer. The purity of the preparation was verified by light microscopy and tubular fragments were always less than 2% of the total number of glomeruli. An aliquot was taken for protein determination and the remainder frozen in liquid nitrogen and stored at –80 °C until required for binding.

Binding assay

The fetal and maternal membrane preparations at concentrations of 100 μ g/tube were incubated in a final volume of 250 μ l Tris–HCl buffer containing 0.5% bovine serum albumin (BSA), 25 pmol/l ^{125}I -rANP_(1–28) (Amersham International, Amersham, Bucks, UK) and unlabelled rANP_(1–28) (Peninsula Laboratories, Belmont, CA, USA) ranging from 10^{-11} to 10^{-8} mol/l, for the determination of the total population of ANP receptors. The density of ANP_B receptors was assessed with rANP_(1–28) in the presence of an excess of cANP_(4–23) (0.1 μ mol/l) (Peninsula), a truncated ANP peptide which binds selectively to ANP_C receptors. The density of ANP_C receptors was obtained by subtracting the number of ANP_B receptors from the total receptor population. Binding studies were performed in duplicate at room temperature for 90 min. At the end of incubation, free ^{125}I -rANP_(1–28) was separated from that bound to membranes, by filtration through 0.1% polyethylenimine-treated Whatman GF/C filters (Prolabo, Marcq-en-Barœul, France), washed with 0.9% NaCl and counted in an LKB gamma-counter. Non-specific binding was measured in the presence of an excess of rANP_(1–28) (0.1 μ mol/l) and cANP_(4–23) (0.1 μ mol/l). Specific binding was determined by subtracting non-specific binding from total binding.

Analytical methods

Plasma rANP concentrations were measured by RIA according to the method described by Deloof & Chatelain (9). Briefly, rANP was extracted from plasma samples with C₁₈ Sep-Pak cartridges (Waters, St-Quentin en Yvelines, France) with acetonitrile and trifluoroacetic acid in distilled water (60:1:39 vol). The evaporated eluates were incubated overnight at room temperature with rANP antibody (Bioproducts, Nanterre, France) and ^{125}I -rANP_(1–28) (Amersham). Free and bound fractions were separated by the double antibody method. Recovery of ^{125}I -rANP_(1–28) from plasma was $81.00 \pm 1.74\%$ ($n = 10$). The intra-assay coefficient of variation was $\pm 5.95\%$ ($n = 6$) and the interassay coefficient of variation was $\pm 12.39\%$ ($n = 4$). Immuno-reactive rANP concentrations were expressed as pmol/l.

Plasma aldosterone concentrations were measured by RIA. Details have been described previously (19). Briefly, aldosterone extracted from plasma samples with ethyl acetate after delipidation with iso-octane was incubated overnight at 4 °C with aldosterone antibody

(Bioproducts) and 1,2,6,7- ^3H aldosterone (Amersham). Free and bound fractions were separated with a dextran-coated charcoal mixture. Recovery of labelled aldosterone from plasma was more than 95% ($n=11$). Intra- and interassay coefficients of variation were respectively $\pm 5.06\%$ ($n=11$) and $\pm 10.26\%$ ($n=7$). Aldosterone concentrations were expressed as nmol/l.

Haematocrit was determined by the microcapillary technique (Clay-Adams microhaematocrit). Protein content was determined by the method of Lowry *et al.* (20) using BSA as standard. Plasma osmolality was measured by depression of freezing-point (Röbling osmometer).

Statistical analysis

The results are presented as means $\pm$ S.E.M. Significance of differences between mean values was estimated by Student's *t*-test. The binding results were analysed by the EBDA/LIGAND program to determine the affinity (K_d) and density ($B_{\max}$) of ANP receptors (21).

Results

Main effects of dehydration in maternal and fetal rats in late gestation

In response to maternal dehydration, both maternal and fetal body weights decreased significantly, and plasma osmolality and haematocrit increased significantly ($P < 0.001$; Table 1). After 3 days of water deprivation, maternal and fetal plasma ANP concentrations decreased ($P < 0.001$ and $P < 0.05$ respectively; Table 1) and plasma aldosterone concentrations increased significantly ($P < 0.01$ and $P < 0.001$ respectively; Table 1). In control fetal rats, plasma ANP concentrations were 18 times higher than those in control pregnant rats ($P < 0.001$; Table 1).

Effects of water deprivation on density ($B_{\max}$) and affinity (K_d) of ANP receptor subtypes in kidneys and adrenal glands of maternal and fetal rats in late gestation

Analysis of competition curves obtained from maternal rats revealed that water deprivation significantly increased the density of total ANP receptors in isolated renal glomeruli ($P < 0.01$; Fig. 1a) and adrenal glomerulosa membranes ($P < 0.05$; Fig. 1b). Quantification of the ANP_B and ANP_C receptors showed that dehydration significantly increased the ANP_C receptor population in isolated renal glomeruli ($P < 0.01$; Fig. 1a) and adrenal zona glomerulosa membranes ($P < 0.001$; Fig. 1b). The density of ANP_B receptors in renal glomeruli and adrenal zona glomerulosa membranes was unchanged ($P > 0.05$; Fig. 1a,b).

No significant difference in the affinity of total ANP and ANP_B receptors was observed in renal glomeruli and adrenal glomerulosa membranes when dehydrated maternal rats were compared with controls ($P > 0.05$; Fig. 2a,b). Maternal dehydration did not significantly alter the density and the affinity of total ANP, ANP_B and ANP_C receptors in the adrenal glands and the kidneys of the fetal rat ($P > 0.05$; Fig. 3a,b, Fig. 4a,b).

In control fetal kidneys, the densities of the different ANP receptors were five to six times lower than those in control maternal glomeruli ($P < 0.001$; Figs 1, 3) and the K_d value of total ANP receptors was nearly 2.5 times higher than that of control maternal glomeruli ($P < 0.05$; Figs 2a, 4a). In control fetal kidneys and adrenal glands, the K_d values of ANP_B receptors were not very different from those observed in control maternal glomeruli and adrenal zona glomerulosa ($P > 0.05$; Figs 2a,b, 4a,b).

Table 1 Effect of water deprivation (3 days) on maternal and fetal body weights, plasma osmolality, haematocrit, and plasma concentrations of ANP and aldosterone in late gestation. Values are means $\pm$ S.E.M.; numbers of experiments in parentheses.

	Maternal rats		Fetal rats	
	Control	Dehydration	Control	Dehydration
Body weight (g)	341 $\pm$ 6 (8)	284 $\pm$ 7*** (8)	5 $\pm$ 0.07 (20)	4 $\pm$ 0.07*** (19)
Osmolality (mosm/kg H ₂ O)	282 $\pm$ 0.7 (6)	299 $\pm$ 1.9*** (6)	279 $\pm$ 1.0 (14)	299 $\pm$ 1.0*** (14)
Haematocrit (%)	35 $\pm$ 0.8 (8)	46 $\pm$ 0.9*** (8)	31 $\pm$ 0.6 (9)	36 $\pm$ 1.0*** (9)
Plasma ANP (pmol/l)	49 $\pm$ 2 (10)	29 $\pm$ 3*** (7)	878 $\pm$ 168 (16)	297 $\pm$ 64* (12)
Plasma aldosterone (nmol/l)	4 $\pm$ 0.5 (9)	12 $\pm$ 1.9** (9)	2.8 $\pm$ 0.2 (14)	5.6 $\pm$ 0.7*** (14)

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared with control values.

Figure 1 Effect of water deprivation (3 days) on the density ($B_{\max}$) of total ANP, ANP_B and ANP_C receptors in (a) isolated renal glomeruli and (b) adrenal zona glomerulosa membranes from control (open bars) and dehydrated (solid bars) maternal rats. Inserts represent one example of Scatchard transformation of binding data: bound/free (B/F) versus bound ANP (fmol/mg protein) in (a) isolated glomeruli and (b) adrenal zona glomerulosa membranes. ○, ●, Curves of total ANP receptors in maternal control and dehydrated tissues respectively; □, ■, curves of ANP_B receptors in maternal control and dehydrated tissues respectively. Results are means $\pm$ S.E.M.; the numbers of experiments are given in parentheses under the columns. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared with control.

Discussion

In our present study, maternal water deprivation for 3 days led to a parallel decrease in both maternal and fetal body weights, and an increase in plasma osmolality and haematocrit. Several previous reports showed that water deprivation led to a weight loss that increased with the duration of dehydration in the dog (22) and the adult rat (13, 14, 16, 23, 24), and an increase in plasma osmolality and haematocrit in the dog (22), the adult rat (13, 14, 16, 23, 24), the child and the human adult (25, 26). In the pregnant ewe, water deprivation results in a significant increase in both maternal and

fetal plasma osmolality (27–29). However, whereas the haematocrit remains unchanged in the dehydrated ewe, it increases significantly in the ovine fetus (27, 28). In the dehydrated pregnant ewe, the decrease in the ruminal volume could explain the absence of a significant change of haematocrit and delay the effects of dehydration on plasma volume (27, 28).

Previous studies demonstrated the existence of at least two distinct types of ANP-binding sites in renal glomeruli and adrenal glomerulosa cells (30, 31). One is the biological receptor coupled with guanylate cyclase (ANP_B receptor), which is more abundant in the adrenal zona glomerulosa cells than in renal glomeruli; the

Figure 2 Effect of water deprivation (3 days) on the affinity (K_d) of total ANP and ANP_B receptors in (a) isolated renal glomeruli and (b) adrenal zona glomerulosa membranes from control (open bars) and dehydrated (solid bars) maternal rats. Values are means $\pm$ S.E.M.; the numbers of experiments are given in parentheses under the columns.

other, not coupled with guanylate cyclase, is the clearance receptor (ANP_C receptor), which is more abundant in renal glomeruli than in adrenal zona glomerulosa cells and can remove ANP from blood circulation (32). Both types of ANP receptor are also present in the kidneys and the adrenal glands of the fetal rat at term, in proportions similar to those observed in the adult rat (33). In the control fetal kidney, the weak density of ANP_C receptors compared with that of control maternal glomeruli might contribute to the increase in fetal plasma ANP concentrations, which are greater than those of pregnant rats. Indeed, the ANP_C receptors are responsible for the degradation of plasma ANP, and, when they are blocked with cANP₍₄₋₂₃₎, the plasma ANP concentrations are much greater (34). In contrast, in the control fetal kidney, the high concentrations of fetal plasma ANP could explain the lower affinity of total ANP receptors. Indeed, these ANP receptors are probably occupied by large amounts of fetal plasma ANP and, in those conditions, the endogenous peptide still present in membrane preparations could compete with the radioligand, thus increasing the apparent K_d . Unfortunately, we have not determined the K_d values of ANP_C receptors in fetal and maternal kidneys, but ANP_C receptors may mainly be occupied by endogenous fetal ANP, as the K_d values of ANP_B receptors are not

significantly different in kidneys and adrenal glands of control fetal rats and those of control maternal rats.

Our results show that maternal water deprivation significantly increases the total population of ANP receptors in isolated renal glomeruli and adrenal zona glomerulosa membrane suspensions of the pregnant rat. Our results are in agreement with those of different investigators who demonstrated that water deprivation significantly increased the total number of ANP receptors in renal glomeruli of the adult rat (13–15, 17) and the pregnant ewe (29) and in the adrenal zona glomerulosa cells of the adult rat (17). The use of the truncated ANP peptide (cANP₄₋₂₃) allowed us to demonstrate that the population of ANP_C receptors increases in the renal glomeruli of the pregnant rat, whereas the ANP_B receptor density remains unchanged. These results are in agreement with those of Kollenda *et al.* (15), who demonstrated that water deprivation increased the population of ANP_C receptors, without alteration of the ANP_B receptor density, in renal glomeruli of the adult rat. Water deprivation also increases the population of ANP_C receptors without modifying the density of ANP_B receptors in the adrenal zona glomerulosa of the pregnant rat. This increase in ANP_C receptor density in the adrenal glands and the kidneys of the pregnant rat confirms that observed in the renal glomeruli of the

Figure 3 Effect of maternal dehydration on the density (B_{max}) of total ANP, ANP_B and ANP_C receptors in (a) renal and (b) adrenal membranes from control (open bars) and dehydrated (solid bars) fetal rats. Inserts represent one example of Scatchard transformation of binding data: bound/free (B/F) versus bound ANP (fmol/mg protein) in (a) renal and (b) adrenal membranes. $\circ$, $\bullet$, curves of total ANP receptors in fetal control and dehydrated tissues respectively; $\square$, $\blacksquare$, curves of ANP_B receptors in fetal control and dehydrated tissues respectively. Results are means $\pm$ S.E.M.; the numbers of experiments are given in parentheses under the columns.

adult rat after dehydration. In fetal kidneys and adrenal glands, the densities of total ANP, ANP_B and ANP_C receptors remain unchanged in response to maternal water deprivation, as, in the ovine fetal renal glomeruli, the density of total ANP receptors was not affected by maternal dehydration (29).

Our results show that water deprivation of pregnant rats is associated with a significant decrease in immunoreactive ANP levels both in maternal and fetal plasmas. This result is in agreement with those of several investigators who showed that dehydration significantly decreased the release of ANP in the adult rat (12–15, 23, 35), the pregnant ewe (29, 36), the child and the human adult (25, 26). In our dehydrated

pregnant rats, the decrease in both maternal and fetal blood volumes estimated by the percentage of change in haematocrit could lead to a decrease in the release of ANP from cardiac tissue. Indeed, several studies have shown that the blood volume expansion induced by intravenous injection of saline or blood transfusion released ANP into the circulation by increasing atrial distension, in the adult rat (37, 38), sheep (39), fetal rats (9) and human fetuses (40). In contrast, in the adult rat, a hemorrhage, which reduces the intravascular volume and blood pressure, decreases the secretion of ANP in the circulation (24).

ANP is cleared from the circulation by two major pathways, including binding to specific ANP_C receptors

Figure 4 Effect of maternal dehydration on the affinity (K_d) of total ANP and ANP_B receptors in (a) renal and (b) adrenal membranes from control (open bars) and dehydrated (solid bars) fetal rats. Values are means $\pm$ S.E.M.; the numbers of experiments are given in parentheses under the columns.

and enzymatic degradation of ANP by neutral endopeptidase. In our dehydrated pregnant rats, up-regulation of the ANP_C receptors mainly decreases circulating ANP in association with neutral endopeptidase activity, as these two clearance pathways are additive (41). In the dehydrated fetuses, the decrease in plasma ANP concentrations is not due to up-regulation of the ANP_C receptors, and in those conditions we must consider other mechanisms to explain the fetal decrease in circulating ANP. First, we have previously demonstrated that ANP does not cross the placenta in the rat (10), and the decrease in plasma ANP concentrations in dehydrated fetuses cannot be mediated through the ANP_C receptor system of the mother. Another explanation for the decrease in fetal plasma ANP concentration is the presence of the placenta, which is considered to be an important site of ANP metabolism. Indeed, autoradiographic studies in the rat have shown that the placenta contains ANP receptors in abundance, located over the labyrinthine region, the decidua and the visceral yolk sac (42). Those placental receptors could be ANP_C receptors able to clear ANP from fetal and maternal blood (43, 44). Alternatively, the neutral endopeptidase that is abundant in the lung, kidney and placenta of the fetal rat in late gestation is able to cleave

ANP (45). These two clearance pathways could decrease ANP concentrations in the circulation of dehydrated fetuses, but complementary studies are necessary in order to determine if dehydration is able to up-regulate placental ANP_C receptors and increase the enzymatic activity of neutral endopeptidase.

An inverse relationship between the plasma ANP concentration and the density of ANP receptors has previously been reported in the adult rat (13–15) and the pregnant ewe (29). Thus water deprivation, which depresses plasma ANP concentrations in both maternal and fetal rats, increases the number of ANP receptors in kidneys and adrenal glands of pregnant rats, but not of fetal rats. This result seems surprising, because all the fetal changes observed after maternal dehydration are similar to those of the maternal rat. However, in the fetal rat at term, the adrenal gland and the kidney are not completely mature (46, 47) and, even if the different ANP receptor subtypes are present in those organs (33) and biologically active in the adrenal gland (19), regulation of the ANP receptors may begin after birth.

In response to maternal dehydration, plasma aldosterone concentration increases in both maternal and fetal rats. This significant increase is probably related to the decrease in ANP secretion and an increase in renin

and angiotensin II secretion. Indeed, it is well known that ANP is involved in the inhibition of the synthesis and secretion of aldosterone by adrenal glomerulosa cells in the calf (48) and the adult rat (5, 49). In the fetal rat, our previous *in vivo* and *in vitro* experiments demonstrated the role of ANP in the inhibition of aldosterone secretion (19). Moreover, adult rats dehydrated for 48 h had significantly increased renin and angiotensin II concentrations compared with control rats (50). A recent study has demonstrated that, in the adult rat, water deprivation stimulates the secretion of renin via the activation of hypothalamic histaminergic neurones (51). Indeed, dehydration stimulates the enzyme of histamine synthesis in the posterior hypothalamus (52), with the result that release of renin is stimulated (53). Unfortunately, we do not know if this histaminergic–renin system is mature in the fetal rat and can respond to maternal dehydration in order to increase aldosterone secretion.

In conclusion, our results show that, after 3-day water deprivation, the pregnant rat up-regulates its ANP receptors in adrenal glands and kidneys in response to a decrease in plasma ANP concentrations. In contrast, the fetal rat does not seem to be able to up-regulate its own ANP receptors in its kidneys and adrenal glands, in spite of a decrease in plasma ANP concentrations. The inverse relationship between plasma ANP concentrations and the number of ANP receptors is observed only in the maternal rat, and not in the fetal rat.

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