Atrial natriuretic factor release during pregnancy in rats

Yunlong Zhang, Kelli Novak and Susan Kaufman *

Department of Medicine, 475 Heritage Medical Research Centre, University of Alberta, Edmonton, Alberta, Canada T6G 2S2

1. We investigated the control of atrial natriuretic factor (ANF) secretion during pregnancy.

2. Plasma ANF levels were measured in conscious virgin female rats under basal conditions, and after atrial distension with an indwelling balloon catheter. The rats were then mated, and the measurements repeated at 7, 14 and 21 days of pregnancy, and at 1 week postpartum. Plasma ANF levels were also measured in ovariectomized rats injected with progesterone, oestradiol, or oestradiol plus progesterone.

3. Basal plasma ANF levels were elevated at 7 and 14 days of pregnancy, but returned to pre-pregnant levels by 21 days. At 1 week postpartum, they were again elevated.

4. In response to atrial stretch, plasma ANF increased significantly in virgin rats (from 100 ± 10 to 148 ± 13 pg ml^{-1}, $P < 0{\cdot}001$, $n = 20$). In contrast, there was no such secretory response observed in the pregnant and postpartum animals i.e. stretch-induced secretion of ANF was markedly attenuated.

5. Treatment with exogenous oestradiol caused a significant increase in plasma ANF levels in acyclic rats. However, neither progesterone nor a combination of oestradiol plus progesterone had any effect.

6. It is concluded that basal and stretch-induced ANF secretion are differentially influenced by pregnancy; oestradiol is identified as a potential stimulatory factor.

Atrial natriuretic factor (ANF) is a peptide which is synthesized by, and stored in, the atrial myocytes and released in response to atrial stretch (de Bold, Borenstein, Veress & Sonnenberg, 1981; Lang, Unger & Ganten, 1987). This hormone exhibits potent natriuretic, diuretic and vascular smooth muscle relaxant properties (Lang *et al.* 1987; Goetz, 1988). Many attempts have been made to implicate ANF in the changes in fluid and electrolyte balance and blood pressure regulation that are observed during pregnancy. However, little is known about the factors that influence secretion under these circumstances. Indeed, despite the fact that blood volume is increased (Lindheimer & Katz, 1985; Barron, 1987), there is still no consensus as to how, in humans, plasma ANF levels change during pregnancy (Jackson, Hodsman, Allen & Johnston, 1988; Hirai, Yanaihara, Nakayama, Ishibashi & Yamaji, 1988; Fournier *et al.* 1991).

In rats, a clearer picture seems to be emerging. Plasma ANF levels seem to be normal or reduced close to term (Nadel, Ballermann, Anderson & Brenner, 1988; Castro, Arora, Parvez, Parvez, Valenzuela & Hobel, 1989; Jansakul, King, Boura, Brennecke & Handberg, 1989; St Louis & Sicotte, 1992). At mid-pregnancy, levels are reported to be either elevated or very variable (Castro *et al.* 1989; Jansakul *et al.* 1989; St Louis & Sicotte, 1992). There have been no measures of plasma ANF in early pregnancy.

Since clearance rates of ANF do not change during pregnancy, at least not at term (Castro, Arora, Krakow & Allen, 1994), attention has been focused on secretion. We have recently shown that, although atrial distension provokes an increase in ANF secretion from isolated perfused atria derived from virgin and early-pregnant rats, there is no such response with atria from mid- and late-pregnant animals (Kaufman, Deng & Thai, 1994). We wished to determine whether our *in vitro* findings could be confirmed *in vivo*, i.e. whether plasma ANF levels are depressed, and whether the secretory response to atrial stretch is attenuated in mid- to late-pregnancy. We also wished to determine whether attenuation of the renal response to atrial stretch that we observe during pregnancy (Kaufman & Deng, 1993) might be due, at least in part, to deficient ANF secretion. Rats were thus implanted with right atrial balloons, and the effect of pregnancy on stretch-induced secretion of ANF was measured.

We have also demonstrated that, if rats are pretreated with oestradiol, ANF secretion from their isolated perfused atria

* To whom correspondence should be addressed.

is increased (Deng & Kaufman, 1993). We therefore proposed that oestradiol should increase plasma ANF levels *in vivo*. Since not only oestradiol but also progesterone increases during pregnancy, we investigated the effects of administration of oestradiol, progesterone and progesterone plus oestradiol on basal plasma ANF levels.

METHODS

The experiments described in this paper were examined by the University of Alberta Animal Welfare Committee, and found to be in compliance with the guidelines issued by the Canada Council on Animal Care.

Animals

Long–Evans rats (female, 200–225 g) were obtained from Charles River (St Foy, Quebec, Canada). They were held for at least 1 week in a temperature-controlled room with a 12 h light regime (07.00–19.00 h). Rats were maintained on 0·3% sodium diet (Bioserv, Frenchtown, NJ, USA). The experiments were done in metabolism cages for ease of access to the cannulae.

Surgery

All rats were prepared (under sodium pentobarbitone anaesthesia, 62 mg (kg body wt)$^{-1}$) with Silastic® cannulae (0·5 mm (0·020 in) i.d., 0·9 mm (0·037 in) o.d.; Dow Corning) implanted non-occlusively into the inferior vena cava (Kaufman, 1980). The animals destined for Experiment A ($n = 29$) were implanted with small intracardiac balloon cannulae which were passed down the right jugular vein and secured to the clavicle so that the tip of the balloon lay just above the vein–atrial junction (Kaufman, 1984). The balloon was inflated with 50 μl saline, giving a diameter of about 5 mm. Visually, we have confirmed that this causes the vein–atrial junction to be gently dilated. The peculiar anatomy of the rat, whereby blood from the left jugular vein enters the *inferior* vena cava, enables one to stretch the vein–atrial junction without interfering with venous return to the heart; blood drains from the head into the left superior vena cava via cross-circulation in the head and neck. There are no accompanying changes in either central venous pressure or blood pressure when the atrial balloon is inflated (Kaufman, 1984). We have already established that distension of the vein–atrial junction by means of these implanted balloons increases plasma ANF levels in male rats (Kaufman, 1990). The animals destined for Experiment B ($n = 22$) underwent bilateral ovariectomy. After surgery, all rats were allowed to recover their pre-operative weights before the experiments began.

Experimental protocol

Effect of pregnancy (Experiment A). The rats were divided into the following groups: Group I, pregnant, balloon inflated ($n = 9$); Group II, non-pregnant, balloon inflated ($n = 11$); Group III, pregnant, balloon not inflated ($n = 9$). Seven days after surgery, the rats were placed in metabolism cages and allowed to rest overnight for acclimatization to their surroundings. A saline infusion of 3 ml h^{-1} I.V. was then started to ensure each rat had a stable, uniform state of hydration and to maintain patency of the cannulae. One hour later, blood was taken from the venous cannula (0·8 ml). The next day, after following exactly the same procedure (same time, same infusion), the intracardiac balloons in the two balloon-inflated groups were inflated with 50 μl saline (Groups I and II). Five minutes later, a second blood sample was taken. Balloon inflation was then confirmed by observing that fluid escaped when the balloon cannula was uncapped. The remaining nine pregnant rats were treated in exactly the same manner except that the balloons were not inflated (Group III).

Three days later, vaginal smears were taken from the eighteen rats that were to be mated (Groups I and III). The remaining eleven rats were not mated (Group II). The balloon inflation/blood sampling procedure described above was repeated at days 7, 14 and 20 of pregnancy. One week after delivery, during which time the pups remained with the dam, the experiment was again repeated. The rats were then anaesthetized with sodium pentobarbitone (62 mg (kg body weight)$^{-1}$), the thorax was opened, and the position of the balloon at the vein–atrial junction was confirmed visually. Only those animals in which the balloons were correctly placed are reported in this paper. The non-pregnant, balloon-inflated animals (Group II) were treated in exactly the same manner as the pregnant animals, the atrial balloons being inflated at times corresponding to the various stages of pregnancy.

Effect of hormone treatment (Experiment B). Seven days after surgery, the rats were placed overnight in metabolism cages. The first blood samples were taken to measure basal plasma ANF. Hormones were then administered daily for 10 days: oestradiol valerate (25 μg in 100 μl sunflower oil, s.c., $n = 6$); progesterone (gesterol, 500 μg in 100 μl sunflower oil, s.c., $n = 6$); or a combination of oestradiol (25 μg in 100 μl sunflower oil) plus progesterone (500 μg in 100 μl sunflower oil) ($n = 5$). A fourth group of animals was treated in exactly the same manner (handled, injected, blood sampled, etc.), except that they did not receive any hormone treatment ($n = 18$). Blood samples were taken again at the end of the hormone treatment.

Blood sampling and radioimmunoassay for ANF

Blood (0·8 ml) was withdrawn into a clean, dry syringe and quickly transferred to a cooled microcentrifuge tube containing EDTA (40 μl Sequester-sol) plus aprotinin (20 kallikrein inhibition units; Trasylol, Bayer AG, Leverkusen, Germany). The blood was centrifuged at 4 °C for 10 min at 14 000 *g*, and the plasma stored at −43 °C. Samples were extracted on C18 columns, and assayed in duplicate by radioimmunoassay using the materials and methods supplied by Peninsula Laboratories (Belmont, CA, USA). All samples (100 μl) were run in duplicate, care being taken to ensure that all samples for any given experiment were included within the same assay. The coefficient of interassay variation for assays done over this period was 13%. Assay sensitivity (half-maximal displacement (IC_{50})) was 4·3 pg per tube.

Statistical analysis

The significance of the changes in basal plasma ANF was estimated using one-way repeated-measures ANOVA followed by the Student–Newman–Keuls test for multiple comparisons. The significance of the increase in plasma ANF induced by atrial stretch was assessed by Student's t test for paired data. The significance of the differences in magnitude of atrial-stretch-induced increase in plasma ANF in the three groups (I, II and III) at any given time (Pre (unmated); 7 days; 14 days; 21 days; and Post (1 week postpartum)) was assessed using ANOVA followed by the Student–Newman–Keuls test for multiple comparisons. The significance of the increase in plasma ANF in response to hormonal treatment was measured by Student's t test for paired data. The significance of the differences between the changes in plasma ANF induced by the various hormonal treatments was assessed using ANOVA followed by the Student–Newman–Keuls test for multiple comparisons. All data are presented as means ± S.E.M. A probability of less than 0·05 was considered statistically significant.