Atrial natriuretic factor release during pregnancy in rats

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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

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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.

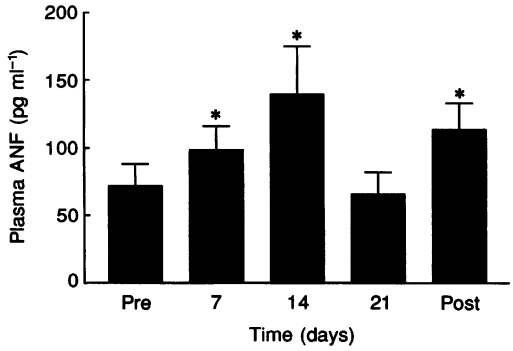

Figure 1. Basal plasma ANF levels during pregnancy and postpartum

Here and in Fig. 2, Time refers to stage of pregnancy; Pre, unmated animals; Post, 1 week postpartum. The vertical bars delineate standard error of the mean. * $P < 0{\cdot}05$.

RESULTS

Basal plasma ANF levels were significantly elevated at days 7 and 14 of pregnancy compared with the levels before mating. Although at day 21, levels had returned to the pre-pregnant values, they were again elevated at 1 week postpartum (Fig. 1). Basal plasma ANF levels in the unmated animals (time control) did not change significantly during the experimental period (repeated measures ANOVA): virgin rats, 111 ± 8 pg ml^{-1}; 7-day equivalent, 134 ± 15 pg ml^{-1}; 14-day equivalent, 142 ± 15 pg ml^{-1}; 21-day equivalent, 138 ± 18 pg ml^{-1}; postpartum equivalent, 129 ± 12 pg ml^{-1}. Although plasma levels tended to be higher in the time-control group (group II), there were no significant differences between the initial basal plasma levels in the three groups.

In response to atrial stretch, plasma ANF increased significantly in the virgin animals (Pre, Group I; Fig. 2) and in the unmated time-control rats (Pre, Group II). During pregnancy and at 1 week postpartum, this response was abolished (Group I). In contrast, there was a consistently significant increase in atrial-stretch-induced secretion in the non-pregnant animals (Group II). There

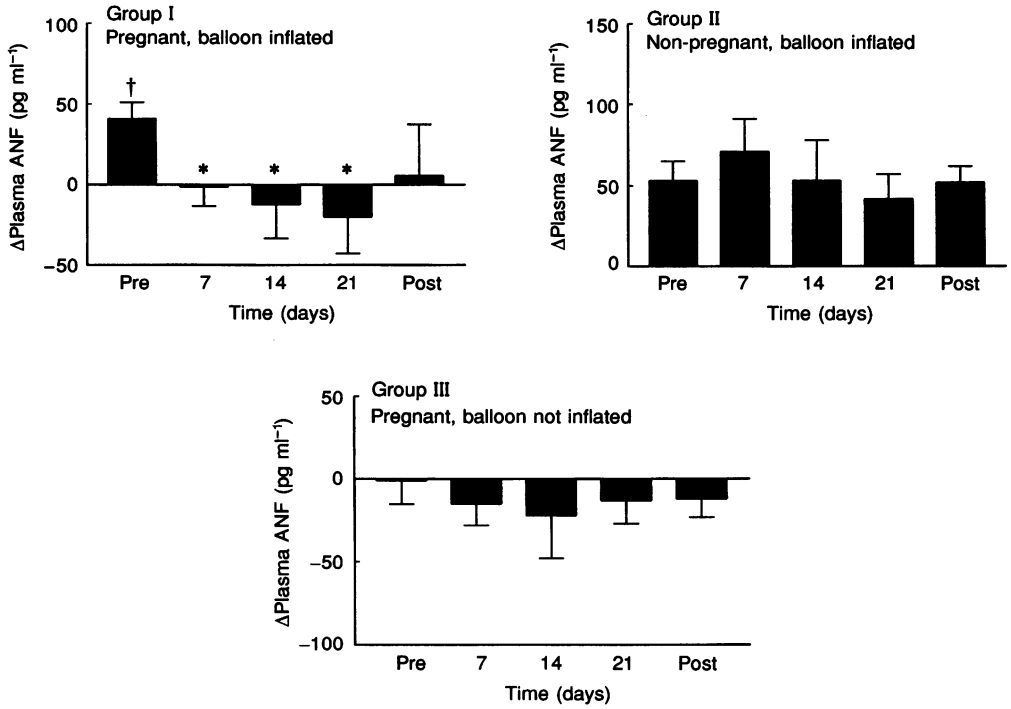

Figure 2. Change in plasma ANF in response to distension of the superior vena caval–right atrial junction with an indwelling balloon

The vertical bars delineate standard error of the mean. †Significantly different from Group III; * significantly different from Group II. There were no significant stretch-induced changes in plasma ANF in group III. Level of significance, $P < 0{\cdot}05$. See Results section for details of significance of changes.

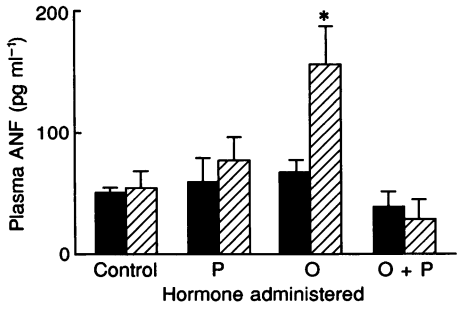

Figure 3. Effect of administration of oestradiol and/or progesterone on plasma ANF levels of ovariectomized rats

Filled bars, vehicle-injected group; hatched bars, hormone-injected groups. Control, no hormone treatment; P, progesterone-injected animals; O, oestradiol-injected animals; O + P, oestradiol plus progesterone-injected animals. The vertical bars delineate standard error of the mean. * $P < 0{\cdot}05$.

was no change in the animals comprising Group III, the pregnant animals in which the intracardiac balloons were not inflated.

In the virgin rats (Pre, Group I), the increase in plasma ANF levels after balloon inflation was significantly greater than for the balloon-not-inflated animals (Group III), but not significantly different from the virgin rats in Group II. However, throughout pregnancy and the postpartum period, the increases in plasma ANF levels in the pregnant, balloon-inflated animals (Group I) were consistently less than those from the non-pregnant, balloon-inflated animals in Group II.

The mean differences in ANF levels between pre- and postinjection blood samples for the hormone-injected and control groups are shown in Fig. 3. The only group showing a significant difference was the oestradiol-injected group, in which plasma ANF levels were significantly elevated ($P < 0{\cdot}05$). There were no significant differences between the groups with respect to pre-injection values for plasma ANF (the mean value for all groups was $70{\cdot}2 \pm 10{\cdot}9$ pg ml^{-1}, $n = 22$).

DISCUSSION

Although it seemed initially to have been established with some certainty that plasma ANF levels increase during pregnancy in humans, published data are not consistent (Hirai *et al.* 1988; Nadel *et al.* 1988; Jansakul *et al.* 1989; Castro *et al.* 1989; Hatjis *et al.* 1990; Fournier *et al.* 1991). It has been suggested that part of the difficulty in interpreting the results derives from the observation that the position of the women (sitting, supine, recumbent), has a great influence on the measured plasma ANF levels (Lowe, Macdonald & Brown, 1991).

In rats, the evidence has been somewhat confusing because of differing experimental designs employed by the various laboratories, and because there have been no studies following plasma levels sequentially throughout pregnancy. Our results indicate that ANF levels are elevated by day 7 of normal rat pregnancy; in no other studies have ANF levels been measured this early in pregnancy. Our finding that plasma ANF levels return to non-pregnant values near term is in agreement with previous data from rats (Castro *et al.* 1989) and humans (Otsuki *et al.* 1987; Steegers *et al.* 1991), but is nonetheless surprising since circulating blood volume is greatly expanded at this time (Barron, 1987) and is associated with significant atrial distension, at least in humans (Steegers *et al.* 1991). It is generally agreed that postpartum levels of ANF are significantly elevated in both the human and the rat (Nadel *et al.* 1988; Castro *et al.* 1989; Jansakul *et al.* 1989; Marlettini *et al.* 1989); our data are in agreement with these findings. It has been suggested that high circulating ANF levels contribute to the pronounced natriuresis and diuresis observed during the postpartum period (Nadel *et al.* 1988; Gregoire *et al.* 1990).

We have previously shown that the doses and duration of treatment of oestradiol and progesterone that we used yield plasma hormone levels that are characteristic of early pregnancy in the rat (Novak & Kaufman, 1991). In contrast, plasma levels of these hormones are low or undetectable in the non-treated ovariectomized animals. Plasma ANF levels were elevated in the oestradiol-treated rats, but normal in the oestradiol plus progesterone-treated rats. This is consistent with our previous finding that oestradiol increases ANF release from isolated perfused atria, and with the data from Nadel *et al.* (1988) that oestradiol increases ANF mRNA in ovariectomized rats.

Plasma ANF levels reflect both basal release of the peptide, and stimulated release resulting from transient changes in atrial pressure due to changes in respiration, posture, etc. Although we found basal plasma ANF levels to be elevated in early and mid-pregnancy (Fig. 1), stretch-induced

stimulation of ANF release was markedly attenuated (Fig. 2). Presumably therefore, only basal secretion was increased at this time. This is in agreement with our *in vitro* results showing that oestradiol increased only basal, not stretch-induced, ANF release from isolated atria (Deng & Kaufman, 1993).

By the third week of pregnancy in the rat, basal plasma ANF levels were no longer elevated, and the lack of response to atrial distension was maintained. Several possibilities have been put forward to explain the failure of plasma ANF to rise in late pregnancy despite the increase in circulating blood volume (Barron, 1987) and the echocardiographic evidence for atrial distension (Steegers *et al.* 1991). It has been suggested that elevated ANF secretion in late pregnancy might be balanced by an increased rate of clearance, so that circulating levels are unchanged (Nadel *et al.* 1988). However, recent evidence shows that there are no pregnancy-induced changes in ANF clearance (Castro *et al.* 1994). We suggest another possibility: that the hormonal profile of late pregnancy, which differs markedly from that of early pregnancy, alters the atrial tissue itself so it becomes less sensitive to stimuli for ANF release. In support of this proposition, we have evidence that isolated perfused atria derived from late-pregnant rats do not increase ANF secretion in response to distension, unlike those from unmated or early-pregnant animals (Kaufman & Deng, 1993). Moreover, ultra-structural examination of atrial tissue from pregnant rats reveals reduced evidence of synthetic activity and atrial-specific granule formation, and absence of granule exocytosis (Gall, Alcorn, Fernley, Coghlan & Ryan, 1990).

Nonetheless, it is difficult to correlate the plasma ANF levels found during pregnancy with changes in steroid hormones, since both progesterone and oestradiol increase during pregnancy. The results are more readily explained by examining the levels of 20α-OH progesterone (Taya & Greenwald, 1981). Plasma levels of this metabolite of progesterone are depressed from days 7 to 20, at which time we find ANF levels to be elevated. On day 21, the levels rise sharply. It is tempting to speculate that it is this metabolite which prevents oestrogen from stimulating ANF release. This concept is especially attractive in the light of recent evidence that another progesterone metabolite, 3α-OH dihydroprogesterone, mimics the effects of pregnancy on baroreflex control of sympathetic outflow (Heesch & Rogers, 1995). Although there have been no other studies on the effects of steroid hormones on blood volume or blood pressure regulation, Barron, Schreiber & Lindheimer (1986) did find that hormone treatment enhanced osmotic stimulation of vasopressin.

It might be suggested that structural remodelling of the atrium of pregnant animals, as has been reported for the ventricle (Robson, Hunter, Moore & Dunlop, 1987), could result in the atrial balloon no longer providing effective distension of the vein–atrial junction. We have measured unstressed atrial volume in isolated atria derived from virgin and pregnant rats, and we have found no significant changes associated with pregnancy (Kaufman *et al.* 1994). In addition, we have always examined the atrial balloons in animals that had to be withdrawn from our studies because cannulae became blocked. In all cases when the balloon was correctly positioned, regardless of the stage of pregnancy, 50 μl of saline would stretch the vein–atrial junction. There was thus no possibility that the *complete lack of response* observed in the pregnant animals could be explained as being due to ineffectual distension of the vein–atrial junction by the balloon. The stimulus–response curve for stretch-induced release of ANF clearly undergoes a marked rightward shift during pregnancy.

In summary, we have determined that basal ANF levels are significantly elevated by day 7 of normal rat pregnancy, but that they return to non-pregnant levels by the third week of gestation. ANF levels are again significantly elevated postpartum. Although atrial distension elicits an increase in ANF secretion in virgin rats, this response is markedly attenuated throughout pregnancy and postpartum. Oestradiol causes a significant increase in plasma ANF levels whereas treatment with exogenous progesterone, or a combination of oestradiol plus progesterone, has no effect. These results are consistent with our data obtained from isolated perfused atria, and support our proposition that reproductive steroid hormones may influence secretion of ANF from the atrial myocytes. However, the precise mechanisms underlying suppression of ANF release in late pregnancy are still not elucidated. Oestradiol levels continue to increase during pregnancy, and would therefore be expected to stimulate, rather than reduce, ANF secretion (this study; Hong *et al.* 1992; Deng & Kaufman, 1993). Although plasma progesterone levels also increase, the progesterone : oestrogen ratio is, if anything, reduced at term (Taya & Greenwald, 1981). We suggest that the inhibitory principle may not be progesterone itself, but rather an active metabolite such as 20α-OH-progesterone or 3α-OH-dihydroprogesterone.

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