Human atrial natriuretic peptide infusion and ovarian venous progesterone secretion in Yucatan micropigs

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Chronic indwelling catheters were implanted in the ovarian and external jugular veins of Yucatan micropigs during the luteal phase. Infusion of hypertonic saline ($0.4 \text{ mol NaCl l}^{-1}$) increased the concentration of porcine immunoreactive atrial natriuretic peptide in ovarian venous blood plasma by $0.61\text{--}1.81 \text{ fmol ml}^{-1}$ more than that of the saline control group. Conversely, endogenously released porcine immunoreactive atrial natriuretic peptide was associated with low ovarian venous progesterone concentrations. Infusion of human atrial natriuretic peptide induced a dose-dependent increase in the progesterone concentration in ovarian venous blood. The results reported here suggest a duality in the ovarian response to atrial natriuretic peptide: a decrease in progesterone secretion in response to low concentrations and stimulated progesterone secretion in response to high concentrations of this peptide.

Introduction

Atrial natriuretic peptides (ANPs) are a family of peptides that were first discovered in cardiac myocytes (de Bold *et al.*, 1981; Flynn *et al.*, 1983; de Bold, 1985). The ANP family primarily inhibits renal tubular reabsorption of Na^+ (Samson, 1992) and thus is important in fluid and electrolyte balance (Cantin and Genest, 1985), including natriuresis, kaliuresis, diuresis and vasodilation (Geller *et al.*, 1984). ANP decreases blood pressure, and vasoconstriction induced by angiotensin, adrenaline and K^+ (Samson, 1992). Extra-cardiac sources of ANP include the hypothalamus (Kawata *et al.*, 1985; Samson *et al.*, 1988, 1993), the pituitary (Kawata *et al.*, 1985) and the ovarian tissue of pigs (Kim *et al.*, 1989), cows (Vollmar *et al.*, 1988; Brunswig *et al.*, 1989), humans (Pandey *et al.*, 1987; Steegers *et al.*, 1990), rats (Zhao *et al.*, 1991; Kim *et al.*, 1992; Gutkowska *et al.*, 1993) and rabbits (Kim *et al.*, 1989). In addition to its role in regulating fluid and electrolyte balance, ANP also modulates steroidogenesis. Racz *et al.* (1985) reported that ANP inhibits the secretion of aldosterone, deoxycorticosterone and progesterone from cultured bovine zona glomerulosa and cortisol, and of corticosterone and progesterone from fasciculata cells. Human follicular fluid contains ANP (Steegers *et al.*, 1990), which could be important as an intragonadal regulator of ovarian function.

The objective of this study was to evaluate the effects of human ANP (hANP) and hypertonic saline infusion on ovarian progesterone secretion in Yucatan micropigs.

Materials and Methods

Animals and surgical procedures

Four-month-old Yucatan micropigs were purchased (Charles River Labs, Wilmington, MA) and maintained in a controlled environment. They were hand-fed 0.25 kg of a maintenance diet (Minipig Breeder No. 5082, Purina Mills, St Louis, MO) twice a day. Water was provided *ad libitum*. At 15 months of age, oestrus was synchronized by oral administration of altrenogest (0.22% oral progestin solution: 17α -allyl- 17β -hydroxyestra-4,9,11-tien-3-one; Roussel-UCLAF, Paris) at a rate of $10.10 \text{ mg day}^{-1}$ for 14 days. (The day when altrenogest treatment began was day 1.)

On days 17 and 18, after 24 h presurgical fasting, chronic indwelling catheters were implanted in the external jugular vein and in one ovarian vein using anaesthetic and laparoscopic techniques described by Musah *et al.* (1984). At the bifurcation of the utero-ovarian vein into the ovarian and uterine veins, a 15-gauge stainless steel hypodermic needle (Popper and Son Inc, New Hyde Park, NJ) was inserted into the ovarian vein about 1.0 cm from the point of the bifurcation in the direction of the ovary. Intramedic polyethylene tubing (0.575 mm i.d., 0.95 mm o.d.; Becton, Dickinson and Co, Parsippany, NJ) was threaded through the needle into the vein. Silk thread knots were used to fasten the vessel around the catheter and to attach the catheter to the broad ligaments. The catheter was exteriorized through the midventral incision and tunnelled underneath the skin of the flank region. The external portions of the catheter were enclosed in a pouch attached to the dorsolateral flank.

Chronic indwelling external jugular vein catheterizations were performed through a 6–8 cm ventral incision made about 4 cm lateral to the midline from the point of the mandible to the point of the shoulder. The skin and fascia were exposed and the external jugular vein located. The vein was dissected free of fascia for 5 cm along its length. Tygon microbore tubing (1.25 mm i.d., 2.25 mm o.d.; Nurton Plastic, Akron, OH) was inserted into the vein and held in place by two knots around the posterior and anterior ends of the tubing. The catheter was tunnelled subcutaneously to the dorsum of the neck and exteriorized. The external portion of the indwelling catheter was stored in a pouch on the dorsum of the neck.

Treatments

From day 10–12 after ovulation (peak luteal phase), micropigs were infused (Orion Sage Syringe Pump No. 355, Orion Research Inc, Boston, MA) at a rate of 1.0 ml min^{-1} for 10 min with saline or different doses of hANP (Sigma Chemical Co, St Louis, MO). The study consisted of seven treatment groups: control saline ($0.15 \text{ mol NaCl l}^{-1}$), hypertonic saline ($0.4 \text{ mol NaCl l}^{-1}$), 0.16, 0.32, 0.81, 1.6 and $2.4 \text{ nmol hANP l}^{-1}$. The micropigs were randomly assigned to treatments and treatment performed in rotation until all animals had received all treatments (four pigs for each treatment). At intervals of 10 min from 10 min before the start of a particular treatment to 60 min afterwards, 2–3 ml of ovarian venous blood was obtained and transferred into cryovials containing 10 U of sodium heparin. Catheters were kept patent during and after experiments by periodically flushing with heparinized saline. Plasma from blood samples was stored at -70°C .

Assay procedures

Porcine immunoreactive ANP (Ir-pANP) concentration was determined using a heterologous assay based upon a human ANP radioimmunoassay kit (Code RPA 512, Batch No. B48A, Amersham International, Amersham, Bucks). Ir-pANP was extracted from plasma using C-8 columns, and assay tubes containing an assay buffer of $0.025 \text{ mol phosphate buffer l}^{-1}$ (pH 7.2) and sodium azide (1%, w/v) were incubated with $100 \mu\text{l}$ of rabbit anti- α ANP serum at $2-8^\circ\text{C}$ for 16–24 h. After 24 h, $100 \mu\text{l}$ of ^{125}I -labelled α ANP was added to all tubes and incubated for another 24 h at $2-8^\circ\text{C}$. Antibody–ANP complex was separated from free ANP by the Amerlex-M system (Amersham International). Nonspecific binding for the heterologous assay was 4.2% and the sensitivity was 26 pg ml^{-1} .

Progesterone in ovarian venous blood was quantified using an RSL ^{125}I Progesterone Kit (ICN Biomedicals Inc, Irvine, CA). The maximum binding of the assay was 43.4% and nonspecific binding was 4.8%. The standard curve was from 0.2 to 50 ng ml^{-1} . Antiserum was generated in rabbits using 11α -hydroxyprogesterone- 11α -hemisuccinate–human serum albumin. The antibody-bound antigen complex was precipitated with a solution of goat anti-rabbit γ globulins and polyethylene glycol. The assay buffer was made of phosphate-buffered saline, pH 7.6, containing rabbit γ globulins and 0.1% sodium azide.

Fig. 1. Effects of jugular vein infusion (1 ml min^{-1} for 10 min) of control ($0.15 \text{ mol NaCl l}^{-1}$ physiological saline; thin line) and hypertonic saline ($0.4 \text{ mol NaCl l}^{-1}$; thick line) on the concentration of porcine immunoreactive atrial natriuretic peptide (ir-pANP) in ovarian venous blood plasma of Yucatan micropigs. Pooled SE is 0.19.

Statistical analysis

The experimental design was a partially balanced incomplete block design arranged in a latin square formation (Youden squares, Cochran and Cox, 1957). Yucatan micropigs ($n = 4$) represented rows (replicates) and the blocks were represented by the treatments ($n = 7$). The micropigs were randomly assigned to treatments until all animals received all treatments. The data were analysed by treatment and effects of time using, where appropriate, analysis of variance, general linear model analysis and Student's *t* test (Snedecor and Cochran, 1967) with PC-SAS (release 6.01, SAS Institute, Raleigh, NC).

Results

Yucatan micropigs to which altrenogest ($10.10 \text{ mg day}^{-1}$ for 14 days) had been orally administered exhibited behavioural oestrus 5.6 ± 0.4 days after altrenogest withdrawal. Hypertonic saline ($0.4 \text{ mol NaCl l}^{-1}$) infusion increased the ir-pANP concentration from 0.49 to $0.69 \text{ fmol ml}^{-1}$ in the physiological saline control group and from 1.1 to 2.5 fmol ml^{-1} for the hypertonic saline group (Fig. 1). The mean deviation of ovarian venous blood progesterone concentrations (mean deviations from pretreatment means) were significantly depressed 10–60 min after commencement of hypertonic saline infusion (Table 1).

Human ANP administered at 0.16, 0.32, 0.81, 1.6 and $2.4 \text{ nmol ml}^{-1} \text{ min}^{-1}$ was associated with significant changes in ovarian venous progesterone concentrations. Low doses of hANP (0.16, 0.35 and $0.81 \text{ nmol ml}^{-1} \text{ min}^{-1}$) were associated with a dose-dependent increase ($P < 0.5$) in ovarian venous blood plasma progesterone concentration between 10 and 20 min after onset of infusion (Fig. 2). After an initial high secretion of progesterone, there was a subsequent decrease ($P < 0.05$) in progesterone concentration between 40 and

Table 1. Progesterone concentrations (nmol ml^{-1}) in ovarian venous blood in Yucatan micropigs after infusion of physiological and hypertonic saline

Experimental group	Time (min) after start of infusion					
	0	10	20	40	60	80
Physiological saline ($0.15 \text{ mol NaCl l}^{-1}$)	0.00	0.01	0.04	0.06	0.04	0.03
Hypertonic saline ($0.4 \text{ mol NaCl l}^{-1}$)	0.00	-0.13*	-0.24*	-0.21*	-0.39*	-0.03

Values are means from pretreatment means.

*Values between treatments are significantly different ($P < 0.05$).

Pooled SE is 0.05.

Fig. 2. Effects of jugular vein infusion (1 ml min^{-1} for 10 min) of low doses of human atrial natriuretic peptide (hANP) ($\square$, 0.16; $\blacksquare$, 0.32; and $\blacksquare$, 0.81 nmol ml^{-1}) on ovarian venous blood plasma progesterone concentrations of Yucatan micropigs. Pooled SE is 0.004.

60 min (Fig. 2). The decrease was dose dependent, reaching a maximum depression 60 min after the start of infusion.

Infusion of high doses of hANP (1.6 and 2.4 nmol ml^{-1}) induced a dose-dependent increase ($P < 0.05$) in the progesterone concentration in ovarian venous blood (Fig. 3). Peak concentration of progesterone occurred within 10 min after termination of infusion. There was no subsequent decrease in the concentration of progesterone during the blood sampling period. Progesterone concentrations were 0.08, 0.13, 0.14, 0.28 and 0.32 (pooled SE, 0.004) 10 min after treatment and -0.20 , -0.13 , -0.46 , 0.46 and 0.37 (pooled SE, 0.004) 60 min after treatment for 0.16, 0.32, 0.81, 1.6 and $2.4 \text{ nmol ml}^{-1} \text{ min}^{-1}$ hANP, respectively.

Discussion

The regulation of ovarian cyclicity and reproductive function, and the regulation of electrolyte balance (and, as a result, blood chemistry and pressure) are not normally considered to be

Fig. 3. Effects of jugular vein infusion (1 ml min^{-1} for 10 min) of high doses of human atrial natriuretic peptide (hANP) ($\square$, 1.6; and $\blacksquare$, 2.4 nmol ml^{-1}) on ovarian venous blood plasma progesterone concentrations of Yucatan micropigs. Pooled SE is 0.004.

hormonally linked. The results reported here suggest that endogenous concentrations of ANP suppress progesterone secretion, whereas exogenous values stimulate progesterone secretion, indicating a link between reproductive function and the regulation of blood plasma volume and electrolytes. Exogenously administered hANP also induced a dose-dependent increase in ovarian venous blood progesterone concentrations. However, stimulation of endogenous release of ir-pANP was associated with a decrease in progesterone concentration of ovarian veins.

Changes in ovarian steroid production after exogenous administration of ANP could be attributed to an alteration in blood flow and volume, since ANP is involved in the regulation of blood pressure and blood volume (Geller *et al.*, 1984; Cantin and Genest, 1986; Needleman and Greenwald, 1986). However, the anatomy and physiology of the luteal vasculature must also be considered. As a muscle relaxer, ANP acts exclusively on vascular smooth muscle and not the endothelium to cause vasodilation (Cantin and Genest, 1986). Luteal vasculature consists of sinusoidal capillaries (Dharmarajan *et al.*, 1985) that are devoid of autonomic nerves (Unsicker, 1974)

and that lack smooth muscle in rats, rabbits and monkeys (Osvaldo-Decima, 1970; Wiltbank, 1987). The corpus luteum operates on a line (maximally vasodilated) pressure curve and does not actively regulate intratissue blood flow, and is subject to acute regulation of perfusion only through changes in extraluteal vessels (Wiltbank *et al.*, 1990).

Analysis of the concentration of progesterone, luteal blood flow and mean arterial pressure indicates that although there is no correlation between serum progesterone concentrations and luteal blood flow, there is a significant correlation between luteal blood flow and mean arterial pressure (Bruce *et al.*, 1980; Bruce and Meyer, 1981; Wiltbank *et al.*, 1988). However, functional demise of the corpus luteum precedes a decline in luteal blood flow (Damber *et al.*, 1981). Acute changes in steroidogenesis and administration of exogenous hormones do not perturb luteal blood flow (Pang and Behrman, 1981; Wiltbank *et al.*, 1989), suggesting that a sustained increase in blood pressure or volume induced by ANP may not alter steroidogenesis. Any changes in luteal steroidogenesis associated with ANP may be mediated by mechanisms other than plasma volume changes.

These findings and results of previous studies suggest that factors involved in regulating fluid and electrolyte balance may have a very profound effect on ovarian steroidogenic function. The presence of ANP in ovarian (Vollmer *et al.*, 1988; Kim *et al.*, 1989; Gutkowska *et al.*, 1993) hypophyseal and hypothalamic tissues (Kawata *et al.*, 1985) indicate the possibility of separate endocrine and paracrine modes for ANP-mediated steroidogenesis in ovarian tissue. It has been suggested that an ovarian–endothelin–renin–angiotensin–ANP system is involved in a paracrine regulation of ovarian steroid production and function (Usuki *et al.*, 1992). A separate but related endocrine effect of ANP could occur at the ovarian, pituitary and hypothalamic levels.

ANP either stimulates or inhibits steroid secretion. Aldosterone, deoxycorticosterone and progesterone secretion in the zona fasciculata is inhibited (Racz *et al.*, 1985). In Leydig tumour cells, ANP inhibits gonadotrophin-stimulated progesterone synthesis in a similar way (Pandey *et al.*, 1985). These inhibitory phenomena are similar to those seen in this study in which endogenously released ir-pANP decreased progesterone secretion. However, the 3–4-fold increase in progesterone secretion by cultured human granulosa cells (Pandey *et al.*, 1987) or the dose-dependent increase in progesterone secretion by rat luteal and granulosa cells (Zhao *et al.*, 1991) exposed to ANP can be compared to the effects of exogenously administered hANP in this study.

These results imply a duality in ovarian response to ANP – a decrease in progesterone secretion in response to low concentrations of ANP and stimulated progesterone secretion in response to high concentrations of ANP. The hypothesis of this study is that hypertonic saline induces the endocrine release of ANP from cardiac sources and paracrine release of ANP from extra-cardiac sources (such as ovarian, hypophyseal and hypothalamic sites), while exogenously administered ANP may act at the endocrine level. The low paracrine concentrations of ANP would tend to suppress progesterone secretion, while endocrine release acting via other pathways stimulates progesterone secretion. ANP at the hypothalamic level typically inhibits LH release (Huang and Samson, 1991)

through C-type natriuretic peptide (CNP) (Huang *et al.*, 1992). Since progesterone and sex hormones affect the expression of mRNA encoding ANP (Hong *et al.*, 1992), there may be a feedback interaction between ovarian steroid and hypothalamic ANP. This suggests an endocrine and a paracrine mode of action of ANP involving two systems, one within the ovary and another at the hypothalamo–hypophyseal level. Further studies are needed to determine whether cardiac and ovarian ANP have different roles on ovarian steroidogenesis.

This study was presented in part at the 26th annual meeting of the Society for the Study of Reproduction (1993), Fort Collins, CO, USA. This project was funded by a St Cloud State Faculty Research Grant for 1991/92. Technical and logistics support was provided by G. Nielson (Waite Park Vet Clinic, St Cloud), E. Thrune, J. Osborn and T. Simpson (St Cloud State University), and G. Nelson (Granite City Pet Hospital, St Cloud).

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