Acta Pharmacologica Sinica (2010) 31: 145–149
 1671-4083/10 $32.00
www.nature.com/aps

Original Article

Central administration of kisspeptin-10 inhibits natriuresis and diuresis induced by blood volume expansion in anesthetized male rats

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Aim: To investigate the possible role of hypothalamic kisspeptin in the regulation of body fluid metabolism and maintenance of internal homeostasis.
Methods: Natriuresis and diuresis were induced by blood volume expansion (VE) in anesthetized male rats and kisspeptin-10 was intracerebroventricularly (icv) administered. Radioimmunoassay (RIA) was used to measure the plasma arginine vasopressin (AVP) and atrial natriuretic peptide (ANP) concentrations during the VE. The mediation of the renal sympathetic nerve was also investigated in rats with bilateral renal sympathetic denervation.
Results: The increased urine flow and sodium excretion induced by VE were significantly inhibited by icv injection of 5 nmol kisspeptin-10 ($P<0.05$), which peaked 20 min after the decrease in VE. The mean arterial blood pressure and heart rate did not change during the experiment. Plasma AVP concentrations were significantly increased 20 min after icv injection of 5 nmol kisspeptin-10 during VE ($P<0.05$), while pretreatment with 5 nmol kisspeptin-10 did not significantly change plasma ANP concentrations. Furthermore, pretreatment with 5 nmol kisspeptin-10 could significantly inhibit VE-induced natriuresis and diuresis in renal sympathetic denervated rats ($P<0.05$).
Conclusion: Central administration of kisspeptin-10 inhibited VE-induced natriuresis and diuresis. This effect was likely mediated by increasing AVP release independent of plasma ANP concentration and renal sympathetic nerve activity.

Keywords: kisspeptin; natriuresis; diuresis; volume expansion; hypothalamus

Acta Pharmacologica Sinica (2010) 31: 145–149; doi: 10.1038/aps.2009.179; published online 21 December 2009

Introduction

The hypothalamo-neurohypophysial system plays a fundamental role in the maintenance of body fluid homeostasis by secreting vasopressin and oxytocin (OT) within the paraventricular nucleus (PVN) and supraoptic nucleus (SON) in response to volume expansion (VE)[1]. Blood VE increases the release of atrial natriuretic peptide (ANP) from the right atrium by stretching the atrial myocytes and activating the afferent inputs of baroreceptors to induce OT secretion in the hypothalamus. Isotonic VE also induces a decrease in renal sympathetic nerve activity and arginine vasopressin (AVP) secretion from the SON and PVN, resulting in a decrease in reabsorption of sodium and water in the kidney[2, 3]. The natriuresis and diuresis induced by VE prevents body fluid augmentations to maintain blood pressure (BP) and internal homeostasis.

Renal functions are controlled mainly by hormone factors and by the sympathetic nervous system. The renal sympathetic nerve is also involved in the volume reflex, especially during acute VE, while the detailed central nervous pathway and neurotransmitter substances underlying the reflex still remain to be elucidated[4–6]. The PVN and SON of the hypothalamus and anteroventral portion of the third ventricle (AV3V) have been shown to be important sites in the forebrain for receiving and integrating various peripheral and central signals in blood volume change[7, 8].

Kisspeptins, novel peptides encoded by Kiss-1 gene, are endogenous ligands of the G protein-coupled receptor 54 (GPR54)[9]. The distribution of kisspeptins and its receptor in many discrete hypothalamic nuclei implies that it may be related to the regulation and integration of neuroendocrine signals[10, 11]. Accumulating data suggest that kisspeptins play a major role in gonadotropin-releasing hormone (GnRH) secretion, reproductive function and puberty activation[12, 13].

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Received 2009-09-25 Accepted 2009-11-17

Apart from regulation of the hypothalamic-pituitary-ovary axis (HPOA), the functional role of kisspeptin in the hypothalamus remains obscure. Recent immunocytochemical staining studies have shown that kisspeptin–positive neurons and fibers are abundantly located in the anteroventral periventricular nucleus (AVPV), the PVN and the SON in the hypothalamus[10], which are related to the central regulation of volume reflex[1, 2]. Unexpectedly, we observed in our previous studies that sodium excretion and urine flow were attenuated for 20 to 60 min after intracerebroventricular (icv) administration of 0.5 or 5 nmol kisspeptin-10, an agonist of GPR54, in anesthetized male rats (unpublished data). We postulate that hypothalamic kisspeptin could be involved in the central nervous pathway for the mediation of VE-induced natriuresis and diuresis. To test the hypothesis, we investigated the effects of the central administration of kisspeptin-10 on VE-induced natriuresis and diuresis and plasma AVP and ANP concentrations in anesthetized male rats with or without renal sympathetic nerve denervation.

Materials and methods

Animals and drugs

Male Sprague-Dawley rats weighing 180−200 g were obtained from the Animal Center of Nanjing University. They were kept in an air-conditioned room with controlled lighting (light 12 h/dark 12 h) and given free access to laboratory chow and tap water. Kisspeptin-10 (the biologically active C-terminal decapeptide) was purchased from Phoenix Pharmaceutical Company (Belmont, CA) and dissolved in artificial cerebrospinal fluid (ACSF; 128 mmol/L NaCl, 2.5 mmol/L KCl, 1.4 mmol/L $CaCl_2$, 1.0 mmol/L $MgCl_2$ and 1.2 mmol/L Na_2HPO_4; pH 7.4). All other reagents and solvents were of analytical grade. All experimental protocols were approved by the local Animal Welfare and Ethics Committee.

Intracerebroventricular injection

Implantation of the cannula was performed stereotaxically under anesthesia with sodium pentobarbital (40 mg/kg, *ip*). Stereotaxic surgical procedures were used to implant one 22-gauge stainless steel guide cannula with a removable 28 gauge inner stylette to the left lateral ventricle (Bregma -1.0 mm; L: 1.5 mm; H: 3.0 mm)[14]. The device was fixed onto the skull with anchor screws. The experiments with icv injection were performed at least 7 d after surgery. The anesthetized intact or renal sympathetic nerve denervated rats were given ACSF (5 μL, as control) or kisspeptin-10 (0.05, 0.5, or 5 nmol) by icv 20 min before VE. Kisspeptin-10 dissolved in 5 μL ACSF was infused through the 28-gauge cannula using an infusion pump at a flow rate of 2 μL/min. The injection needle was kept in place for 10 min after the injection. After a 120 min observation period, all animals were injected with 4% brilliant blue by icv to verify the cannula placement (Figure 2).

Experimental procedure

All rats were water deprived for 12 h one day before the experiments. On the day of the experiment, the rats were anesthetized with inactin (100 mg/kg body wt) for renal function experiments. The trachea was intubated to facilitate ventilation. The left femoral vein was catheterized with polyethylene (PE) tubing (PE-50, filled with saline) for VE. The right femoral artery was cannulated with PE tubing (PE-50, filled with 100 U/mL heparinized saline) for monitoring arterial blood pressure and heart rate. The pressure signal was sent to a computer recording and analyzing system (PowerLab, AD Instruments) by pressure transducers. A continuous perfusion of 0.9% NaCl solution through the catheter in the right femoral vein was used for VE stimulation. The total perfusion volume was 4% of the body weight and was infused at a constant flow rate over a period of 40 min. In the test of renal sympathetic nerve denervation, bilateral renal sympathetic nerve trunks were isolated and destroyed using 95% ethanol[15].

AVP and ANP radioimmunoassay

Sequential blood samples (200 μL each) were taken through the PE tubing from the right femoral artery at 20 min intervals during the 120 min period. The same volume of physiological saline was replaced at each bleeding. Blood plasma (100 μL) was separated from each blood sample by centrifugation and stored at -20 °C until the determination of AVP. Plasma AVP and ANP concentrations were measured using Sep-Pak C18 cartridge extraction. Samples for AVP extraction were acidified with 1 mol/L HCl and extracted. Acidified plasma samples were added slowly to the columns and then washed with 0.1% trifluoroacetic acid (TFA). The absorbed AVP was eluted with 50% methanol and 0.1% TFA, and the eluates were dried in a Speed-Vac concentrator. The assay sensitivity was 1.2 pg for AVP/tube. The intra- and inter-assay coefficients of variations were 5% and 7%. The plasma ANP concentration was also determined using an RIA kit (Peninsula Laboratories, Belmont, CA, USA) after extraction of ANP from plasma with C_{18} Sep-Pak cartridges eluted with a buffer containing 60% acetonitrile in 1% TFA. Immunoreactive ANP concentration is expressed as pmol/L.

Measurement of urine and sodium excretion

The ureters were catheterized with PE-10 tubing for sequential urine collection every 20 min during the VE, and the urine volume of each period was measured. Sodium concentration and volume of each urine fraction were measured using the automatic biochemical analyzer (Beckman Coulter Corporation, Miami, USA). The data are expressed as micromoles per minute per 100-gram body weight (μmol·min^{-1} per 100 g BW) and microliters per minute per 100-gram body weight (μL·min^{-1} per 100 g BW), respectively.

Statistical analysis

All results are expressed as mean±standard deviation (SD) and were analyzed using SPSS 13.0 (SPSS, Chicago, IL). The data in Figure 1 were analyzed using a two-way repeated measures analysis of variance (ANOVA) with time as the repeated measure. The significance of difference was determined using the Newman-Keuls test. When only two treatment groups were

being compared, a Student's *t*-test was used. A probability of $P<0.05$ was considered statistically significant.

Results

Effect of kisspeptin-10 icv injection on natriuresis and diuresis induced by VE in anesthetized male rats

In the normal group, sodium excretion and urine flow began to gradually increase simultaneously with VE and reach the peak at 80 min (1.25±0.26 μmol·min^{-1} per 100 g and 22.25±3.34 μL·min^{-1} per 100 g, respectively). In animals receiving an icv injection of kisspeptin-10, however, VE-induced natriuresis and diuresis also occurred at time points from 40 to 120 min, but occurred with lower amplitudes. This increase in sodium excretion and urine flow in response to VE were attenuated by icv administration of 0.05 and 0.5 nmol kisspeptin-10 during time points from 40 to 120 min but with no statistical significance (Figure 1A and 1B). Kisspeptin-10 (5 nmol) had a significant inhibitory effect on VE-induced natriuresis and diuresis from 60 to 100 min, especially in the strongest response at 80 min (0.78±0.21 μmol·min^{-1} per 100 g and 16.94±5.53 μL·min^{-1} per 100 g) compared with controls (F=5.18 and 5.75, $P<0.05$).

Effect of kisspeptin-10 icv injection on heart rate and mean arterial pressure during VE-induced natriuresis and diuresis

Central injection of 5 nmol kisspeptin-10 was ineffective at producing any significant changes in mean arterial blood pressure or heart rate during VE. The mean arterial blood pressure of the intact rats with VE fluctuated in the range of 92±16 and 112±18 mmHg during the experiment. There was no significant change between the 5 nmol kisspeptin-10 group and the control group at all the time points. Moreover, no significant change in heart rate was observed between the two groups (range of 295±36 and 325±38, respectively).

Effect of kisspeptin-10 icv injection on plasma AVP and ANP concentrations during VE in anesthetized male rats

Central injection of 5 nmol kisspeptin-10 caused a stimulatory effect on plasma AVP concentration during VE (Figure 1C). The concentration of plasma AVP in anesthetized male rats with VE was 3.6±1.4 compared with 2.4±0.8 pg/mL in the control group during observation. There was a decreasing tendency, but this did not reach statistical significance. In comparison to the control group, plasma AVP concentrations increased significantly (from 3.2±1.2 to 9.5±2.6 pg/mL) 20 min after the icv injection of 5 nmol kisspeptin-10 (F=5.82, $P<0.05$). This effect lasted approximately 20 min. Volume expansion induced an obvious plasma ANP increase from the 40 min time point in both groups (Figure 1D). In contrast to plasma AVP, there was no statistically significant difference in ANP concentrations between the two groups throughout the experiment, showing that 5 nmol kisspeptin-10 had no significant effect on plasma ANP concentrations.

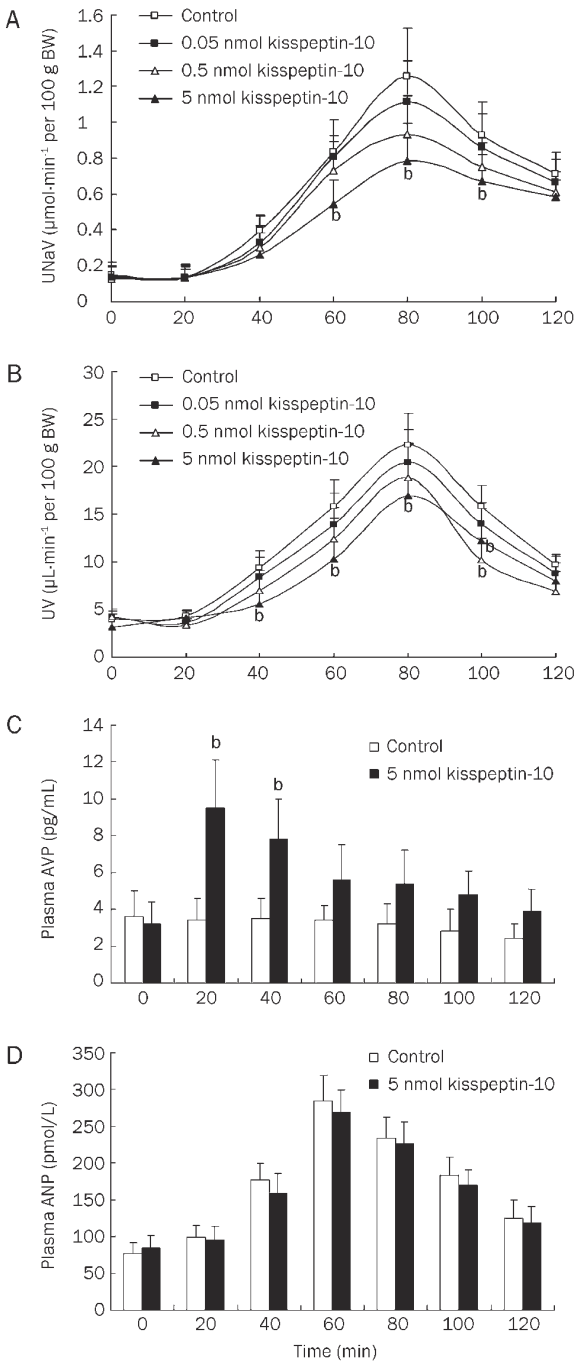

Figure 1. (A and B) Dose-dependent inhibition of kisspeptin-10 on VE-induced natriuresis and diuresis induced in anesthetized male rats. The icv administration of kisspeptin-10 or ACSF (as a control) was given at t=0. (C and D) Time-dependent increase of plasma AVP and ANP concentrations by icv injection of kisspeptin-10 during VE in anesthetized male rats. A total of 5 nmol kisspeptin-10 or ACSF (as control) was given at t=0. The sodium concentration and volume of each urine fraction were measured every 20 min for 2 h. The VE of 4% body weight lasted 40 min (from t=20 to t=60). n=10. Data are mean±SD. $^bP<0.05$ *vs* control.

Effect of kisspeptin-10 icv injection on VE-induced natriuresis and diuresis in anesthetized male rats with bilateral renal sympathetic denervation

In renal sympathetic nerve denervated rats, similar increases in VE-induced urine flow and sodium excretion were observed, with a slight increase that reached a peak at 80 min (1.41±0.31 μmol·min^{-1} per 100 g and 24.28±3.85 μL·min^{-1} per 100 g BW, respectively). A significant inhibitory effect on VE-induced natriuresis and diuresis also occurred in the 5 nmol kisspeptin-10 group (Figure 2C and 2D). The sodium excretion and urine flow were decreased significantly by pretreatment with icv administration of 5 nmol kisspeptin-10 at 60 to 80 min compared with the control group. The highest response level also occurred at 80 min (0.95±0.23 μmol·min^{-1} per 100 g and 18.84±3.13 μL·min^{-1} per 100 g BW, respectively) (F=4.65 and 4.92, P<0.05).

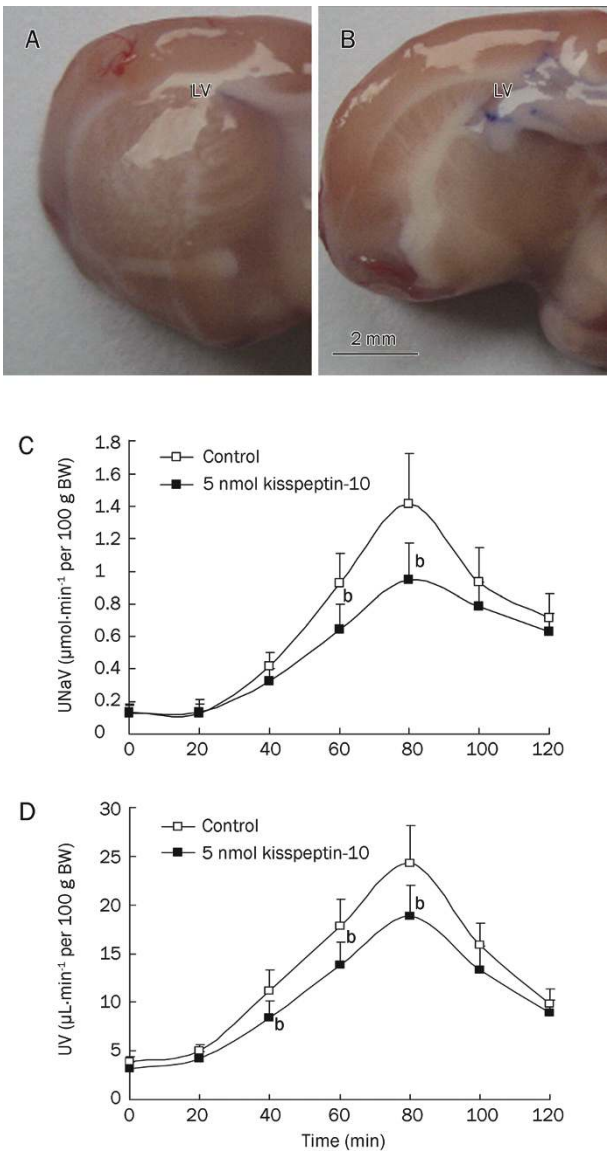

Figure 2. (A and B) The lateral ventricles were stained by 4% brilliant blue injected through the internal cannula after 120 min of observation to verify the proper cannula placement. (C and D) Time-dependent effect of icv injection of kisspeptin-10 on VE-induced natriuresis and diuresis in anesthetized male rats with bilateral renal sympathetic denervation. A total of 5 nmol kisspeptin-10 or ACSF (as a control) was given at t=0. The sodium concentration and volume of each urine fraction were measured every 20 min for 2 h. The VE of 4% body weight lasted 40 min (from t=20 to t=60). n=10. Data are mean±SD. ^bP<0.05 *vs* control.

Discussion

Kisspeptin and its receptor are densely distributed in several nuclei of the hypothalamus, suggesting that kisspeptin might mediate and modulate hypothalamic functions[11]. Previous studies have demonstrated that kisspeptin and GPR54 signaling in the brain serve as an important conduit for controlling GnRH secretion in developing and adult animals[12, 16]. In the present study, we observed that central administration of kisspeptin-10 could inhibit VE-induced natriuresis and diuresis in anesthetized male rats. To our knowledge, this is the first study suggesting that hypothalamic kisspeptin might participate in the central regulation of body fluid homeostasis.

We observed that icv injection of 5 nmol kisspeptin-10 significantly inhibited VE-induced natriuresis and diuresis and that this inhibitory effect lasted at least 80 min. Due to the limitation of our observation intervals, we cannot exclude the possibility that kisspeptin-10 may only postpone the sodium and water excretion surge or prolong the duration after VE. Expansion of the blood volume induces natriuresis and diuresis, and this volume reflex plays an important role in the regulation of water and electrolytes balance[1]. The release of ANP from the atrial myocytes[17], changes in renal sympathetic nerve activity[4, 5] and hypothalamic AVP release from the PVN[1] are regarded as the chief efferent regulators in the volume reflex, including the experimentally induced VE performed in our experiments. Sympathetic innervation in the kidney elicits important effects on the regulation of renal blood flow, rennin release and reabsorption of sodium and water[1, 5]. Inconsistent with our anticipation, the inhibitory effect of kisspeptin is not likely to be mediated by the renal sympathetic nerves because bilateral renal denervation could not abolish the attenuation caused by central injection of 5 nmol kisspeptin-10. The lack of significant change in mean blood pressure and heart rate suggests that the effect of central administration of kisspeptin did not lead to the activation of the efferent visceral sympathetic nervous system.

The current study concentrated on the possible role of the renal sympathetic nerves in the mediation of the effect of kisspeptin-10. We observed differences in values between intact and renal sympathetic nerve denervated rats during VE, especially peak differences. In denervated rats, VE-induced increases in urine flow and sodium excretion at the peak were slightly elevated compared with the intact control group; however, the difference did not reach statistical significance. We performed bilateral renal sympathetic denervation according to the method described by Patel *et al*[15]. This method has been shown to decrease the norepinephrine level in kidneys to an undetectable range. It should be noted that we did not confirm the effectiveness of this method by measuring the NE

content in the surgically denervated kidneys, as done in Patel *et al*[15]. Moreover, due to the complexity of our surgical procedures, we did not simultaneously record the activity from the renal sympathetic nerve. We therefore cannot determine if the function of the renal sympathetic nerves were blocked completely.

The neuroendocrine system plays a vital role in the maintenance of body fluid homeostasis by secreting AVP and OT in response to a variety of signals, including osmotic stimulus or volume expansion[1]. The PVN, SON, and AVPV in hypothalamus have been deemed important integrating sites governing AVP and ANP release[1, 2, 17]. Using immunocytochemistry, the recent detailed account of kisspeptin neuroanatomy has shown that kisspeptin neurons are more abundantly distributed in the AVPV than elsewhere in hypothalamus. Meanwhile, kisspeptin fibers are densely located in the PVN and SON, running along the wall of the third ventricle[10]. The negative effect of kisspeptin on volume reflex is probably related to processes of the neuroendocrine system, such as increasing AVP release or decreasing ANP into circulation. As shown in our studies, the inhibitory effect of kisspeptin-10 on VE-induced natriuresis and diuresis is not likely to be mediated by altering plasma ANP release because the 5 nmol kisspeptin-10 icv injection had no significant effect on plasma ANP concentrations. Our studies demonstrated that plasma AVP concentration increased 20 min after central injection of 5 nmol kisspeptin-10 during VE, suggesting that the negative effect of kisspeptin-10 on VE-induced natriuresis and diuresis is partly mediated by its stimulatory influence on AVP release. We postulate that the swift effect of the icv injection of kisspeptin-10 on AVP release are probably mediated by nuclei around the third ventricle, where it could diffuse and reach in a short period of time. The administered kisspeptin is likely to diffuse along with ventricular cerebrospinal fluid and modulate neuronal activity in nuclei located in the periventricular area of the third ventricle, such as neurons within PVN and SON. Certainly, the mechanism underlying hypothalamic kisspeptin-10 in neuroendocrine control of AVP release remains to be elucidated.

To our knowledge, this is the first study to show that central administration of kisspeptin-10 inhibits natriuresis and diuresis induced by blood volume expansion in anesthetized male rats. This effect is probably mediated by increasing AVP secretion and is independent of plasma ANP concentration and renal sympathetic nerve activity. The role of kisspeptin in the regulation of body fluid homeostasis remains to be elucidated.

Acknowledgements

Project was supported by Nature Scientific Foundation of Jiangsu Province (BK2008490).

Author contribution

Xiao-fei AN designed the research; Xu HAN, Xiao-fei AN, and Ming YAN performed the research; Ming HE and Jiang-yi YU contributed new analytical tools and reagents; Ming YAN and Xiao-fei AN analyzed the data; and Xiao-fei AN wrote the paper.

References

1 Antunes-Rodrigues J, Castro MD, Klias LK, Valenc MM, McCann SM. Neuroendocrine control of body fluid metabolism. Physiol Rev 2004; 84: 169–208.
2 Coote JH. A role for the paraventricular nucleus of the hypothalamus in the autonomic control of heart and kidney. Exp Physiol 2005; 90: 169–73.
3 Leng G, Brown CH, Russell JA. Physiological pathways regulating the activity of magnocellular neurosecretory cells. Prog Neurobiol 1999; 57: 625–55.
4 Wongmekiat O, Johns E. Role of nitric oxide and renal nerves in the renal responses to acute volume expansion in anaesthetized rats. Exp Physiol 2001; 86: 47–54.
5 Young PJ, Miller JH. Renal nerve and alpha 2-adrenergic action during acute volume expansion in the anaesthetized rat. Clin Exp Pharmacol Physiol 1999; 26: 608–13.
6 Li YF, Mayhan WG, Patel KP. Role of the paraventricular nucleus in renal excretory responses to acute volume expansion: role of nitric oxide. Am J Physiol Heart Circ Physiol 2003; 285: H1738–46.
7 Bealer SL. Anteroventral third ventricle periventricular tissue contributes to cardiac baroreflex responses. Clin Exp Pharmacol Physiol 2000; 27: 460–4.
8 Rinaman L. Visceral sensory inputs to the endocrine hypothalamus. Front Neuroendocrinol 2007; 28: 50–60.
9 Messager S, Chatzidaki E, Ma D, Hendrick AG, Zahn D, Dixon J, *et al*. Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. Proc Natl Acad Sci USA 2005; 102: 1761–6.
10 Clarkson J, Tassigny X, Colledge WH, Caraty A, Herbison AE. Distribution of kisspeptin neurons in the adult female mouse brain. J Neuroendocrinol 2009; 21: 673–82.
11 Mikkelsen JD, Simonneaux V. The neuroanatomy of the kisspeptin system in the mammalian brain. Peptides 2009; 30: 26–33.
12 Gottsch ML, Clifton DK, Steiner RA. Kisspepeptin-GPR54 signaling in the neuroendocrine reproductive axis. Mol Cell Endocrinol 2006; 25: 91–6.
13 Smith JT, Clifton DK, Steiner RA. Regulation of the neuroendocrine reproductive axis by kisspeptin-GPR54 signaling. Reproduction 2006; 131: 623–30.
14 Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 5th ed. San Diego: Academic Press; 2005.
15 Patel KP, Kline RL. Influence of renal nerves on noradrenergic responses to changes in arterial pressure. Am J Physiol Regul Integr Comp Physiol 1984; 247: R615–R620.
16 Dungan HM, Clifton DK, Steiner RA. Minireview: kisspeptin neurons as central processors in the regulation of gonadotropin-releasing hormone secretion. Endocrinology 2006; 147: 1154–8.
17 Antunes-Rodrigues J, Favaretto AL, Ballejo G, Gutkowska J, McCann SM. ANP as a neuroendocrine modulator of body fluid homeostasis. Rev Bras Biol 1996; 56: 221–31.