Kisspeptin Activation of Supraoptic Nucleus Neurons *in Vivo*

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Oxytocin and vasopressin are synthesized by magnocellular neurosecretory cells in the hypothalamic supraoptic and paraventricular nuclei and are released from the posterior pituitary gland into the circulation. Intravenous administration of the ligand for the G protein-coupled receptor 54 receptor, kisspeptin-10, increases plasma oxytocin levels and intracerebroventricular kisspeptin-10 increases vasopressin levels, indicating that kisspeptin might play a role in various physiological functions via stimulation of oxytocin and vasopressin secretion. Because posterior pituitary hormone secretion is dependent on action potential (spike) discharge, we used *in vivo* extracellular single unit recording to determine the effects of kisspeptin-10 on supraoptic nucleus neurons in urethane-anaesthetized female rats. Intravenous kisspeptin-10 (100 μg) increased the firing rate of oxytocin neurons from 3.7 $\pm$ 0.8 to 4.7 $\pm$ 0.8 spikes/sec ($P = 0.0004$), but only a quarter of vasopressin neurons responded to iv kisspeptin-10, showing a short (<3 sec) high-frequency (>15 spikes/sec) burst of firing. By contrast, intracerebroventricular kisspeptin-10 (2 and 40 μg) did not alter oxytocin or vasopressin neuron firing rate. To investigate the pathway involved in the peripheral action of kisspeptin-10, we used ip capsaicin to desensitize vagal afferents, which prevented the iv kisspeptin-10-induced increase of oxytocin neuron firing rate. This is the first report to show that peripheral, but not central, kisspeptin-10 increases the activity of oxytocin neurons and a proportion of vasopressin neurons and that endogenous kisspeptin regulation of supraoptic nucleus neurons is likely via vagal afferent input, with kisspeptin acting as a hormone rather than as a neuropeptide in this system. **(*Endocrinology* 152: 3862–3870, 2011)**

Magnocellular neurosecretory cells (MNC) of the hypothalamic supraoptic nucleus (SON) and paraventricular nucleus (PVN) project a single axon to the posterior pituitary gland, where they release oxytocin or vasopressin into the circulation: oxytocin is important in parturition (1) and is essential for milk ejection (2), whereas vasopressin promotes antidiuresis and vasoconstriction (3). Hormone release from the axon terminals in the posterior pituitary gland is dependent on the electrical activity of MNC (4). Therefore, any factors that influence firing rate and activity patterning of MNC will alter the release and levels of both oxytocin and vasopressin in the blood.

Kisspeptins are a recently discovered group of peptides (54-, 14-, 13-, and 10-amino acid peptides), that are ligands of the G protein-coupled receptor (GPR)54 (5). The endogenous ligand kisspeptin-54 is derived from translation of the *Kiss-1* gene, which was originally described as a novel metastasis suppressor gene (6). However, two clinical studies in 2003 showed GPR54 and the kisspeptins to be essential for puberty onset and normal human fertility (7, 8). The discovery of kisspeptin neurons in the rostral periventricular area of the third ventricle and the arcuate nucleus of the rodent brain (9–12), and the activation of GnRH neurons by kisspeptin (13, 14), firmly established the importance of neuronal kisspeptin within the GnRH neuronal network (15, 16).

Previous studies have shown that iv administration of kisspeptin-10 to female rats increases plasma oxytocin levels (5), whereas intracerebroventricular (icv) injection of

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Abbreviations: ADP, Afterdepolarisation; CCK, cholecystokinin; GPR, G protein-coupled receptor; icv, intracerebroventricular; IDFR, index of dispersion of firing rate; MFR, mean firing rate; MNC, magnocellular neurosecretory cell; NTS, nucleus tractus solitarius; PVN, paraventricular nucleus; SON, supraoptic nucleus.

kisspeptin-10 to male rats increases plasma vasopressin concentrations (17). To date, there are no reports of the effects of icv kisspeptin on oxytocin levels or of iv kisspeptin on vasopressin levels. Hence, in addition to the action of kisspeptin upon the GnRH neuron system, kisspeptin might play a role in reproductive function and body fluid regulation by acting upon MNC to stimulate oxytocin and vasopressin secretion into the blood.

Here, we investigated the effect of kisspeptin on the firing rate of oxytocin and vasopressin neurons in the SON of cycling virgin female rats. Surprisingly, we found that peripheral, but not central, kisspeptin-10 administration increased the firing rate of MNC, and that desensitization of vagal afferents blocked iv kisspeptin-10 excitation of oxytocin neurons, suggesting an indirect modulation of these neurons by circulating kisspeptin.

Materials and Methods

All animals were group housed in the University of Otago animal facility, under controlled conditions (14-h light, 10-h dark cycle; lights on at 0600 h, 22 ± 1 C) and had free access to food and water. All experimental procedures were approved by the University of Otago Animal Ethics Committee and were carried out in accordance with the recommendations of the Australian and New Zealand Council for the Care of Animals in Research and Teaching.

In vivo electrophysiology

Female virgin Sprague Dawley rats (250–350 g) were used at various stages of the estrous cycle to preclude any possible effects of estrous cycle on the results; the phase of the estrous cycle had no effect on the firing rate (data not shown). On the day of *in vivo* electrophysiology, animals were anesthetized by an ip injection of urethane (ethyl carbamate; 1.25 g/kg), and those that were to receive an icv injection had a 28-gauge guide cannula (Plastics One, Roanoke, VA) placed in the right lateral cerebral ventricle (0.3 mm caudal and 1.3 mm lateral to bregma and 3 mm below the surface of the skull). The cannulae were secured using dental acrylic bonded to stainless steel screws (BASInc, West Lafayette, IN) inserted into the skull. At the end of the *in vivo* electrophysiology experiments, an icv injection of methyl blue dye was administered, followed by brain removal and sectioning through the area of the lateral ventricle to check cannulae placement. A catheter was inserted into the right femoral vein for iv drug injection, and the pituitary stalk and the right SON were exposed by transpharyngeal surgery. A glass recording pipette (15–40 MΩ; filled with 0.9% NaCl) was placed in the SON, and a side-by-side SNEX-200 stimulating electrode (Science Products GmbH, Hofheim, Germany) was placed on the pituitary stalk to elicit antidromic spikes in SON neurons. Kisspeptin-10 (Metastin 44-54; Calbiochem, San Diego, CA) was dissolved in 0.9% saline for iv injections and in artificial cerebrospinal fluid [in mM: 150 NaCl, 3 KCl, 1.2 $MgCl_2$, 26 $NaHCO_2$, 2.5 $CaCl_2$, and 10 glucose (Sigma, St. Louis, MO)] for icv injections. Both solutions were aliquoted and stored at −20 C until use. At the end of the experiments, the rats were killed by anesthetic overdose.

In vivo electrophysiology data analysis

Neuronal activity was recorded onto a computer and analyzed off-line using Spike2 software (Cambridge Electronic Design, Cambridge, UK). Neurons that fired less than one spontaneous spike every 10 sec were categorized as silent and were not recorded. Phasic activity was characterized using the "bursts" script in Spike2, with a burst being defined as activity lasting a minimum of 5 sec with a minimum of 20 spikes within the burst and at least a 5-sec interval between bursts, during which there was less than 1 spike every 5 sec; phasic neurons were those for which these parameters partitioned more than 95% of spikes into bursts; this activity pattern is specific to vasopressin neurons (3). In animals not pretreated with capsaicin, continuous and irregular neurons were characterized as oxytocin neurons on the basis of a transient excitation after iv cholecystokinin (CCK) injection (20 μg/kg, 0.5 ml/kg in 0.9% saline; Sigma) (18), or as vasopressin neurons either by a transient inhibition or no response after CCK injection (19). For neurons from capsaicin-treated rats, neuronal identification was further characterized by hazard function analysis as detailed below.

Neurons were confirmed as either irregular or continuously active based upon the variability of spike firing, as shown by the index of dispersion of firing rate (IDFR), which was calculated using the formula: IDFR = $SDFR^2$/mean firing rate (MFR), where SDFR is the SD of the firing rate (in 1-sec bins). All continuously active vasopressin neurons had an IDFR of less than 1.5, whereas all irregular vasopressin neurons had an IDFR more than 1.5 (20). All continuously active oxytocin neurons had an IDFR of less than 0.8, whereas all irregular neurons had an IDFR more than 0.8. The MFR of each neuron was calculated 5 min before and 5 min after iv and icv kisspeptin-10 administration.

Capsaicin treatment

To investigate whether activation of oxytocin neurons by iv kisspeptin-10 is mediated via the afferent sensory fibers in the vagus nerve, we sought to disrupt the afferent vagal pathway using ip administration of small doses of capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), which is known to desensitize the vagal afferents responsible for CCK-induced satiety without affecting the efferent fibers (21, 22). Capsaicin was dissolved in 100% ethanol and diluted to the required dose with 0.9% NaCl before use. Rats were anesthetized with isoflurane (2% in 1 liter of O_2) before each capsaicin injection and maintained under the anesthesia for 5–10 min after injection. Two different dose regimens were used (modified from Refs. 21, 23, 24). The first was an ip injection of 25 mg/kg at 0 h, followed by 50 mg/kg at 6 and 24 h (in 50% ethanol, total dose 125 mg/kg). This dose caused respiratory distress, and the rat was intubated and breathing maintained by artificial ventilation. The animals did not tolerate this dose well, and therefore, only one animal treated with this dose was used for *in vivo* electrophysiology. The second dose has been reported to cause a transient (of ~3 wk duration) vagal desensitization (21, 22): three ip injections of 10 mg/kg at 0, 6, and 24 h (in 10% ethanol, total dose 30 mg/kg). At this lower dose, no respiratory failure occurred. However, a variable change in breathing pattern was noted in the first few minutes after injection. The animals were allowed to recover for 3–6 d before *in vivo* electrophysiology.

Hazard function

Because vagal desensitization disrupts the activation of oxytocin cells by iv CCK, we used hazard functions as an alternative method of identification. Hazard functions quantify changes in postspike excitability of neurons after spontaneous action potentials (25), indicating the probability of a cell firing a spike in a given period. Using Spike2, interspike interval histograms were constructed in 10-msec bins, for the basal firing rate period. The probability of spike firing (hazard) was calculated from the interspike interval histogram of individual cells using the formula: [hazard in bin (t, t + 10)] = [number of intervals in bin (t, t + 10)]/(number of intervals of length > t) (19). Hazard functions plot the incidence of spikes as a proportion of the size of the residual tail of the interspike interval distribution. This gives the inferred probability (as a decimal) of a cell firing a subsequent spike in any interval after a spike (at time 0) (25). Hazard functions for oxytocin cells show a constant hazard after a postspike interval of about 50 msec, and the shape of the hazard function is fitted by a single negative exponential (19). However, the hazard function for vasopressin cells does not show a constant hazard after a postspike interval of 50 msec, rather it shows an increased probability 40–200 msec after each spike, and the shape of the hazard function for a vasopressin cell is fitted by a double exponential (19). In animals pretreated with capsaicin, the oxytocin neurons were identified as those showing a hazard that was best fit by a single exponential rise to maximum ($r = 0.82–0.95$, $P < 0.0001$ for each cell).

Peak early-to-mean late hazard ratio

The peak early-to-mean late hazard ratios were calculated from these hazard plots. To compare with previous analysis, we used the peak early hazard as the maximum hazard value reached within the first 70 msec after a spike, and compared it with the mean late hazard from 200 to 300 msec, which represented steady-state hazard in each cell type. The peak early-to-mean late hazard ratios were calculated from these hazard plots, which in oxytocin neurons is less than 1.2 (19).

Statistical analyses

Statistical tests were carried out using GraphPad Prism 4.0 (GraphPad Software, Inc., San Diego, CA) or SigmaPlot (Systat Software, Inc., Germany). Between groups, the data were analyzed by Student's *t* test. For comparison between different doses, a two-way repeated measures ANOVA was used. $P \leq 0.05$ was considered statistically significant.

Results

Spontaneous activity *in vivo*

In vivo extracellular single-unit recordings were obtained from 85 identified SON neurons: 53 oxytocin neurons and 35 vasopressin neurons, from 50 rats. Vaginal smears were taken from 20 of those rats, and no difference was found in the firing rate at different stages of the estrous cycle (data not shown). The mean basal firing rate of the oxytocin neurons was 3.9 ± 0.5 spikes/sec and that of the vasopressin neurons was 5.0 ± 0.4 spikes/sec. Twenty-nine oxytocin neurons displayed continuous activity, and 24 displayed irregular activity. Of the 35 vasopressin neurons recorded, 13 were continuously active, 16 were irregular, and three showed phasic activity. The firing rate of the oxytocin neurons in the 5 min after iv CCK injection increased from 3.9 ± 0.5 to 5.1 ± 0.7 spikes/sec ($P = 0.0003$).

Intravenous administration of kisspeptin-10 excites oxytocin neurons

Intravenous administration of 25 μg of kisspeptin-10 increases plasma oxytocin levels in the blood of virgin female rats (5); therefore, we administered iv kisspeptin-10 while recording the activity of 36 oxytocin neurons (from 27 rats) to investigate whether changes in activity underpin the changes in plasma hormone concentration (5). Various doses of kisspeptin-10 were administered during the recordings: 5, 10, 25, 50, 100, and 200 μg (in 100 μl 0.9% saline), and 10 of the 36 neurons were recorded over multiple iv injections of different doses (in random order), with a minimum of 10 min separating consecutive iv kisspeptin-10 injections.

Regardless of the dose, iv kisspeptin-10 caused a short (~5 min) increase in the firing rate of all oxytocin neurons recorded (Fig. 1A). For each neuron, the firing rate in the 5 min immediately before and after each kisspeptin-10 injection was calculated. All six kisspeptin-10 doses appeared to increase the firing rate, and this was significant for four of the doses (5, 25, 100, and 200 μg) (Fig. 1B). The response to kisspeptin-10 was highly reproducible, and multiple injections during a single recording produced a similar excitation each time (Fig. 1A). The increase in firing rate began within 30 sec of iv kisspeptin-10, and the peak firing rate was typically reached by 60 sec. In almost all neurons, firing rates returned to basal levels between 5 and 10 min after the injection.

In the experiments shown here, we used kisspeptin-10, which has comparable, if not greater, biological potency to the full-length peptide (26, 27). During the recording of two oxytocin neurons, iv kisspeptin-54 (the full-length peptide) was administered (5 and 25 μg in 100 μl), and this caused an increase in firing rate similar to that seen with iv kisspeptin-10 (5 μg, 7.7–8.8 spikes/sec; 25 μg, 9.0–9.9 spikes/sec and 5.0–5.7 spikes/sec). Hence, although kisspeptin-10 may bind with greater affinity at the GPR54 (27), the activation of oxytocin neurons is similar for kisspeptin-54.

Effects of iv administration of kisspeptin-10 on vasopressin neurons

We recorded from 12 vasopressin neurons (from nine rats) during iv administration of kisspeptin-10 (10, 50, 100, and 200 μg in 100 μl). Comparison of the MFR 5 min before and 5 min after iv injection showed that kisspep-

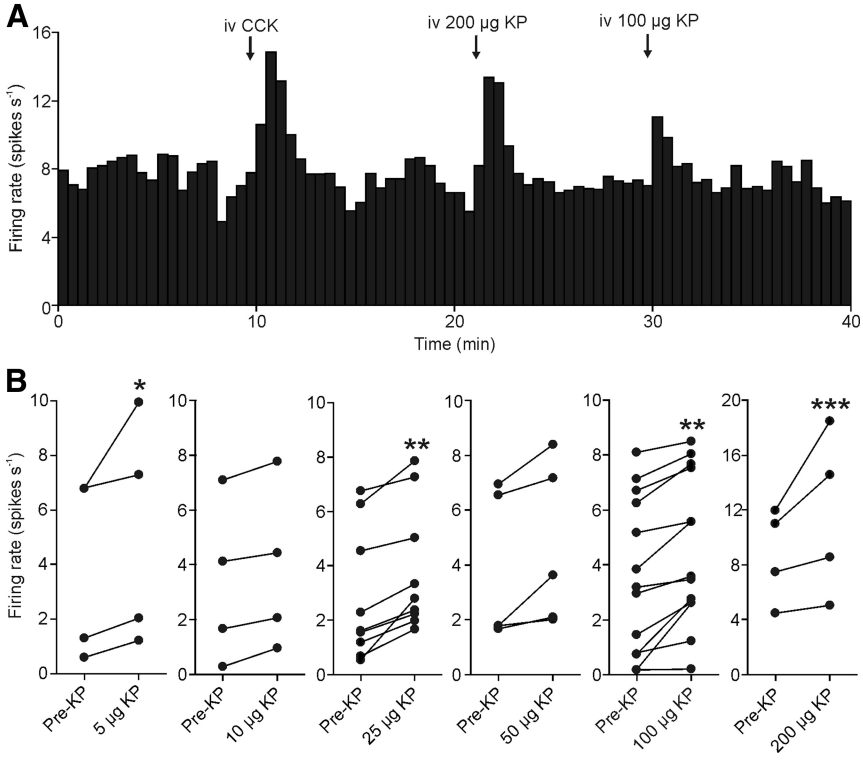

FIG. 1. Intravenous kisspeptin-10 increases the firing rate of SON oxytocin neurons. A, Representative ratemeter record (in 30-sec bins) showing a dose-dependent increase in firing rate of an oxytocin neuron (identified by a transient excitation after iv CCK) in response to iv kisspeptin-10 (KP) in a urethane-anesthetized virgin female rat. B, The MFR of identified oxytocin neurons averaged over the 5 min before and 5 min after various doses of iv kisspeptin-10, showing a consistent increase in firing rate of all cells tested. Two-way repeated measures ANOVA showed a significant effect of kisspeptin ($P < 0.001$) that was dose dependent ($P = 0.034$): 5 μg: *, $P = 0.024$; 10 μg: $P = 0.332$; 25 μg: **, $P = 0.007$; 50 μg: $P = 0.065$; 100 μg: **, $P = 0.009$; 200 μg: ***, $P < 0.001$, Student-Newman-Keuls *post hoc* tests.

tin-10 did not alter the firing rate of vasopressin neurons at any of the doses tested (Fig. 2, A and B). Nevertheless, in three of the 12 vasopressin neurons, it was apparent that a short, less than 10-sec high-frequency (>15 spikes/sec) burst occurred soon after iv kisspeptin-10 (Fig. 2C), which was obscured in the 5 min MFR analyses.

To analyze the high-frequency burst, the MFR was calculated in 2-sec bins over the 200 sec before iv kisspeptin-10 injection, and the threshold for burst recognition was defined as the MFR + 3 SD. All high-frequency bursts defined by this procedure occurred within 10 sec of kisspeptin injection in these three neurons (Fig. 2D), and no high-frequency bursts occurred at any other time in these three neurons or at any time in all other neurons recorded. In one of the kisspeptin-responsive vasopressin neurons, three iv kisspeptin injections were administered (two of 50 μg and one of 100 μg, all in 100 μl), and a high-frequency burst occurred after all three injections (Fig. 3B).

Intracerebroventricular kisspeptin-10 does not alter firing rate of oxytocin or vasopressin neurons

We recorded the firing rate of 15 oxytocin neurons and five vasopressin neurons during central (icv) administration of kisspeptin-10. Two icv doses were administered: 2 μg (in 1 or 2 μl) and 40 μg (in 2 μl). For each neuron, MFR was calculated over the 5 min before and after kisspeptin-10 icv injection. In marked contrast to the robust and repeatable excitation after iv kisspeptin, there was no change in firing rate after icv injection in any oxytocin neuron tested (Fig. 3, A and C), even in neurons excited by iv kisspeptin-10 (Fig. 3A). Similarly, no vasopressin neuron responded to icv kisspeptin-10 injection with a change in firing rate, even those neurons that responded to iv kisspeptin-10 (Fig. 3B). Finally, high-frequency burst analysis failed to show any bursts in either oxytocin or vasopressin neurons after icv kisspeptin-10. Hence, it appears likely that peripheral kisspeptin does not act within the brain to modulate the firing rate of oxytocin or vasopressin neurons.

Capsaicin pretreatment prevents the activation of oxytocin neurons by kisspeptin-10

In vivo extracellular single-unit recordings were obtained from seven putative oxytocin neurons, from four rats with pretreated with capsaicin ip (one rat total dose, 125 mg/kg; three rats total dose, 30 mg/kg). All seven cells showed a constant hazard after 50 msec and had a shape that could be fitted by a single exponential rise (Fig. 4, C and D), indicating that they are likely to be oxytocin cells rather than vasopressin cells (19). The mean basal firing rate of the oxytocin neurons was 4.9 ± 0.6 spikes/sec, and all displayed continuous activity, similar to the spontaneous activity evident in noncapsaicin-pretreated rats. For each neuron, the firing rate in the 5 min immediately before and after CCK injection was calculated, and in all seven neurons, there was no change in firing rate after CCK injection (5.1 ± 0.6 to 5.3 ± 0.5 spikes/sec, $P = 0.165$) (Fig. 4A), indicating that the treatment with capsaicin had disrupted the vagal afferent input. Notably, unlike control animals, iv administration of kisspeptin-10 in the capsaicin-treated animals failed to increase the firing rate of oxytocin neurons (5.2 ± 0.6 to 5.3 ± 0.5 spikes/sec, $P = 0.505$) (Fig. 4, A and B). To check that capsaicin pretreatment had not altered that central responsiveness to kisspeptin-10, one animal was administered icv kisspeptin-10 (2 μg in 2 μl), and no change in firing rate was seen (5.9 ± 1.0 to 6.3 ± 0.6 spikes/sec) (Fig. 4A).

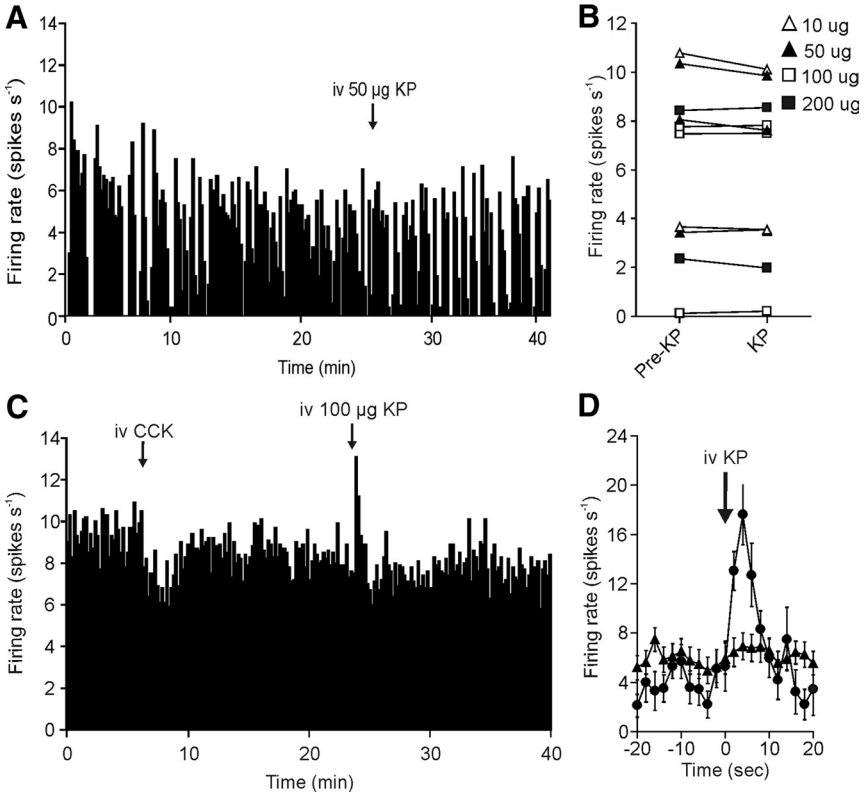

FIG. 2. Intravenous kisspeptin-10 effects on vasopressin neurons. A, Representative ratemeter record (in 10-sec bins) showing a lack of response of a phasic vasopressin neuron to iv kisspeptin-10 (KP) in a urethane-anesthetized virgin female rat. B, MFR of identified vasopressin neurons averaged over the 5 min before and 5 min after various doses of iv kisspeptin, showing a consistent lack of effect on overall firing rate in all cells tested. Two-way repeated measures ANOVA showed no significant effect of kisspeptin ($P = 0.07$). C, Representative ratemeter record (in 10-sec bins) showing a high- frequency burst in a vasopressin neuron (identified by a transient inhibition in response to iv CCK) in response to iv kisspeptin-10 in a urethane-anesthetized female virgin rat (typical of three of 12 cells tested). D, MFR (in 2-sec bins) of identified vasopressin neurons that responded to iv kisspeptin with a high-frequency burst (*circles*; five bursts recorded from three cells, each from a different rat) and those of identified vasopressin neurons that did not respond to iv kisspeptin (*triangles*; nine recordings from 12 rats). Doses of iv kisspeptin-10 administered: 10, 25, 50, and 100 μg.

Discussion

Here, we provide the first electrophysiological evidence that iv kisspeptin-10 increases MNC firing rate; all oxytocin neurons were excited (over several min), and about one quarter of vasopressin neurons showed a short high-frequency burst in response to iv kisspeptin-10.

It has been shown previously that iv kisspeptin-10 increases plasma oxytocin levels (5). Given that both oxytocin and vasopressin release from the posterior pituitary gland is dependent upon spikes initiated at the cell body (4), our novel results indicate that an increase in plasma oxytocin levels after iv kisspeptin-10 is likely due to an increase in oxytocin neuron activity. The firing rate of oxytocin neurons returned to basal levels within 5–10 min, and a second iv kisspeptin-10 injection, as little as 10 min after the first, produced a similar increase in firing rate, suggesting the short duration of excitation is not due to a long deactivation or down-regulation of GPR54. Rather, it is probable that exogenous kisspeptin-10 is rapidly broken down by matrix metalloproteinases (28), which cleave the C-terminal Gly-Leu peptide bond destroying ligand activity (29).

When averaged over the 5 min before and after injection, iv kisspeptin-10 did not change the overall firing rate of vasopressin neurons. However, further analysis showed that in three of the 12 vasopressin neurons recorded, iv kisspeptin-10 caused a short high-frequency burst of spikes, reminiscent of milk-ejection bursts in oxytocin neurons during lactation (4). Such an activity pattern is highly unusual in identified vasopressin neurons, although an early report did highlight one phasic (putative vasopressin) neuron showing a burst of firing related to milk ejection (30). Administration of iv kisspeptin-10 (100 μg in 100 μl) causes a short (~5 min) increase in blood pressure (our unpublished data), which would be expected to cause a baroreceptor-induced decrease in firing rate of vasopressin neurons (31). Hence, the excitatory bursts seen in 25% of the vasopressin neurons are unlikely to be a result of changes in blood pressure.

Activity patterning in MNC is generated through interplay between intrinsic properties and synaptic inputs (3, 32). Although it is unknown why only a minority of vasopressin neurons responded to iv kisspeptin-10 with a high-frequency burst, this might result from differences in intrinsic properties and/or synaptic inputs. One possibility is that the fast afterdepolarisation (ADP) (33) is more readily activated in this subset of vasopressin neurons but fails to trigger a sustained increase in firing rate, because the slow ADP (34) is already fully activated in active neurons. Alternatively, iv kisspeptin might evoke a volley of excitatory postsynaptic potentials in these vasopressin neurons. Although the high-frequency bursts are unlike *in vivo* activity seen previously, they are (as stated above) similar to milk-ejection bursts in oxytocin neurons during lactation. In contrast to vasopressin neurons, oxytocin neurons do not show a measurable slow ADP under basal conditions (35), but over the course of pregnancy and lactation, the slow ADP becomes more prominent in oxyto-

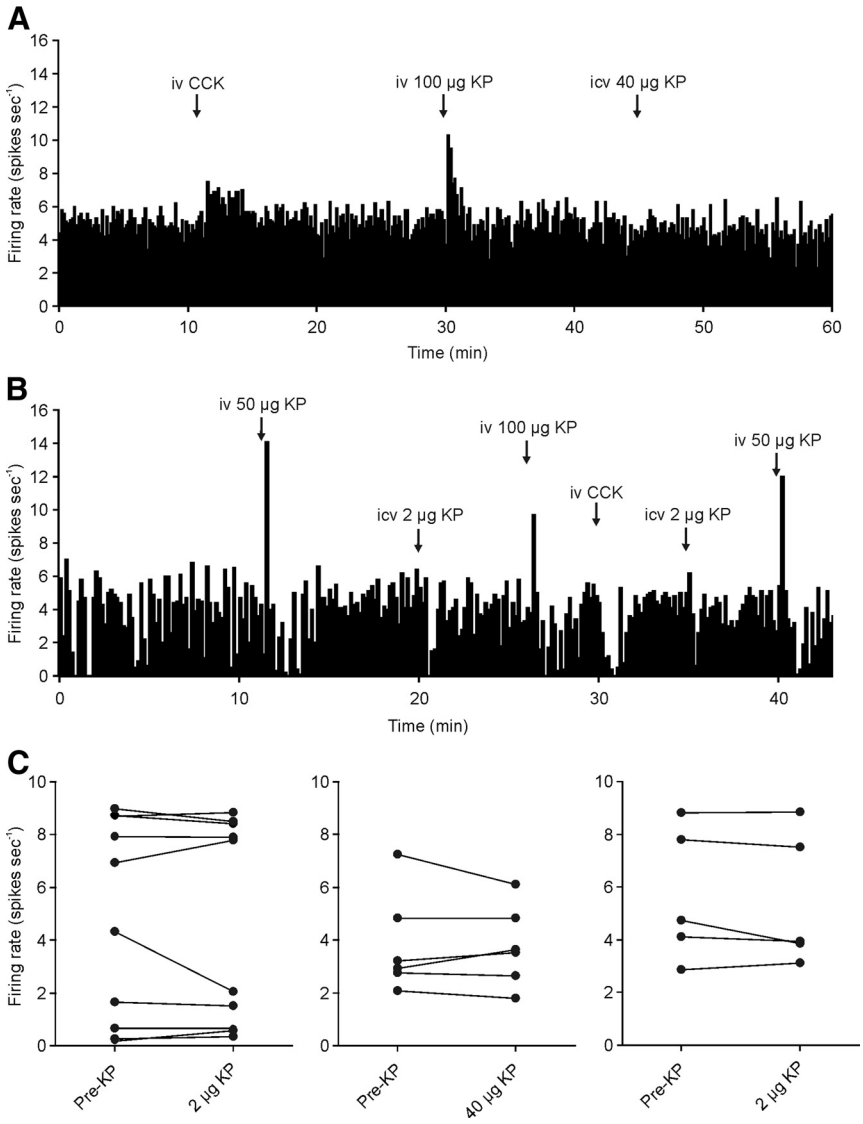

FIG. 3. Intracerebroventricular kisspeptin-10 does not affect the firing rate of oxytocin or vasopressin neurons. A, Representative ratemeter record (in 10-sec bins) of an oxytocin neuron (identified by transient excitation after iv CCK), showing an increase in firing rate after iv kisspeptin-10 (KP) but no response to icv kisspeptin-10, in a urethane-anesthetized virgin female rat. B, Representative ratemeter record (in 10-sec bins) of a phasic vasopressin neuron, showing high-frequency bursts after iv kisspeptin-10 but no response to icv kisspeptin-10, recorded from a urethane-anesthetized female virgin rat. C, MFR of identified oxytocin neurons (*left and middle panels*) and vasopressin neurons (*right panel*) showing no change in firing rate of oxytocin neurons (two-way repeated measures ANOVA; $P = 0.52$) in response to icv kisspeptin at 2 μg (*left panel*) or 40 μg (*middle panel*), or of vasopressin neurons [at 2 μg (*right panel*); paired *t* test, $P = 0.899$].

cin neurons (36). If the presence of ADP underpins the ability of kisspeptin-10 to trigger high-frequency bursts in vasopressin neurons evident here, circulating kisspeptin might be capable of triggering bursts in oxytocin neurons during pregnancy or lactation, when the ADP are expressed more strongly. Clearly, it will be important to characterize the effect of iv kisspeptin on oxytocin neurons during pregnancy and lactation.

Electrophysiological studies have shown that kisspeptin acts directly on GnRH neurons, arcuate nucleus, proopiomelanocortin neurons, and hippocampal neurons in brain slices (13, 37, 38). By contrast, we saw no effect of icv kisspeptin-10 on the activity of any oxytocin or vasopressin neuron recorded. GPR54-expressing GnRH neurons can be activated by icv administration of 1 nmol kisspeptin-10 (39). Even administering a 30-fold higher dose of kisspeptin-10, we saw no effect of central kisspeptin on the firing rate of oxytocin or vasopressin neurons, suggesting that the lack of excitation is unlikely to reflect a failure to deliver sufficient kisspeptin-10 to the SON.

The lack of response of MNC to icv kisspeptin-10, combined with a robust response to iv kisspeptin, suggests that iv kisspeptin-10 does not cross the blood brain barrier to act directly on MNC, or on GPR54 receptors on central inputs to the SON. GPR54 is expressed throughout the hypothalamus (40). However, to date, there has been no evidence showing GPR54 expression (or lack of GPR54 expression) in the rat SON, but transgenic GPR54/lacZ mice do not express GPR54 in the SON (41), consistent with our lack of effect of central kisspeptin on MNC.

A recent study demonstrated that icv kisspeptin-10 increases plasma vasopressin after 30 min, with a concurrent decrease in sodium excretion and urine production (17). These observations are most easily be explained by increased vasopressin secretion, which would be expected to result from an increased firing rate of vasopressin neurons. However, contrary to these observations, we found that no vasopressin (or oxytocin) neurons changed firing rate after icv kisspeptin-10 administration. Hence, it appears that, if icv kisspeptin increases plasma vasopressin levels, it does not do so by increasing the firing rate of vasopressin neurons in the SON. It is unclear what underlies these different observations. However, although the vasopressin neurons of the SON and PVN are generally considered to behave identically (42), SON and PVN vasopressin neurons could respond differently to icv kisspeptin, perhaps via activation of different inputs; although many central projections to the SON and PVN are the same, there are known differences,

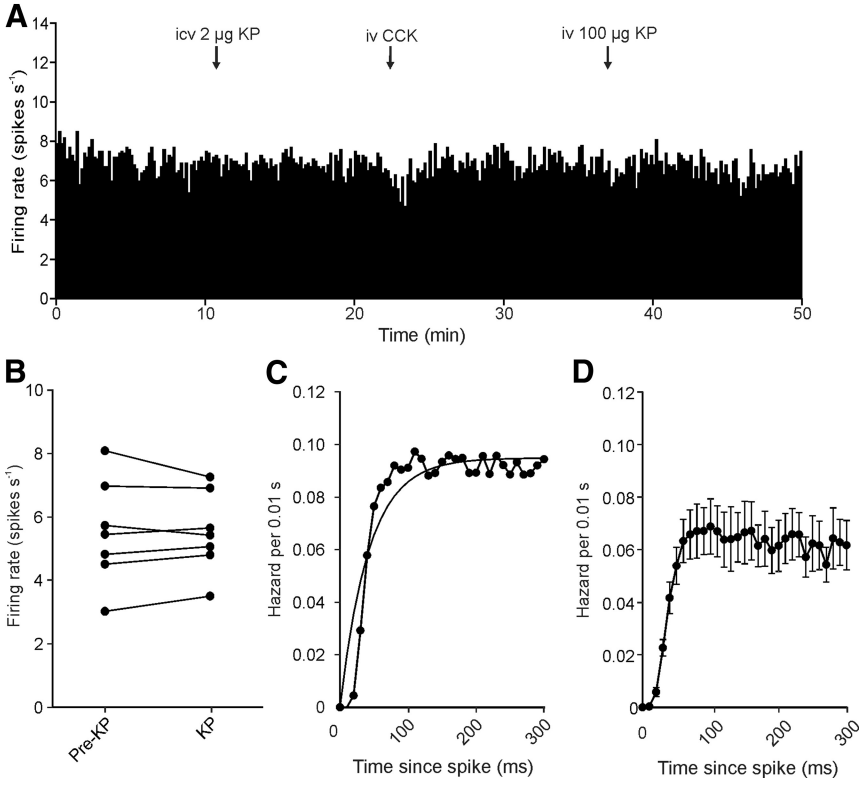

FIG. 4. Capsaicin treatment prevents the activation of oxytocin neurons by kisspeptin-10. A, Representative ratemeter record (in 10-sec bins) of an oxytocin neuron (identified by hazard function), showing a lack of response to icv kisspeptin-10, iv CCK, and iv kisspeptin-10 in a urethane-anesthetized virgin female rat. B, MFR of identified oxytocin neurons averaged over the 5 min before and 5 min after various doses of iv kisspeptin (KP), showing a lack of effect of kisspeptin on overall firing rate of oxytocin neurons in capsaicin-treated rats. C, The hazard function of the oxytocin neuron shown in A. The curve fitted over the hazard function is a single exponential rise to maximum, indicative of an oxytocin neuron. D, A consensus hazard function for the seven oxytocin neurons recorded from the four capsaicin-treated rats. The hazard functions for all seven cells could be fitted by single exponential rise to maximum ($r = 0.82–0.95$, all $P < 0.0001$).

and the two nuclei have some functional independence based upon their afferent inputs (42).

The lack of effect of kisspeptin-10 icv on MNC indicates that, in contrast to the well-characterized direct central effects of kisspeptin on GnRH neurons, kisspeptin does not influence MNC directly but acts as a hormone on peripheral targets with projections to the SON. Both secretin (43) and CCK (44, 45) excite oxytocin neurons indirectly, via vagal afferents to the nucleus tractus solitarius (NTS) in the brain stem, with subsequent excitatory noradrenergic input to the SON-activating adrenergic receptors, which increase the firing rate of oxytocin neurons (4). We hypothesized that circulating kisspeptin-10 might also converge on this pathway to act on the SON, because GPR54 receptors are expressed in peripheral tissues, including the stomach and small intestine (5), and might be present on the vagus nerve. The results we present here indicate that this is indeed the case, because pretreatment with ip capsaicin to desensitize the vagus nerve prevented the increase in firing rate of oxytocin neurons induced by iv kisspeptin-10, as well as that induced by iv CCK. Capsaicin is an agonist of the transient receptor potential channel-vanilloid receptor subtype 1 and desensitizes unmyelinated C and thin myelinated Aδ nerve fibers within the abdominal vagus nerve after ip injection (46, 47). It is likely that NTS relays the vagal input to the SON through adrenergic input. However, further work is required to fully establish the pathway from the vagus to the SON.

This hormonal action of kisspeptin, mediated in part via vagal afferents, might become important during pregnancy, when there is a 10,000-fold increase of kisspeptin in human plasma (during the third trimester) (48). Circulating kisspeptin is secreted from syncytiotrophoblasts (48) (placental endocrine cells), which produces a number of other hormones important in pregnancy (49). Currently, the physiological role of placental kisspeptin is not known (50), and an increase in plasma kisspeptin during human pregnancy has yet to be confirmed in rodent models. Nevertheless, given the importance of oxytocin in pregnancy, parturition, and lactation (51, 52), and our observations that kisspeptin-10 excites oxytocin neurons in virgin rats, it appears that oxytocin neurons are likely to be an important target of circulating kisspeptin during pregnancy.

The increase in oxytocin neuron activity that we observe after iv kisspeptin-10 would presumably increase oxytocin release (5). A rise in plasma kisspeptin over pregnancy (48) would therefore be expected to increase plasma oxytocin concentrations, which is seen in human pregnancy (53, 54). Logically, this would be expected to stimulate premature uterine contractions, but during pregnancy, the uterus is quiescent due in part to low oxytocin receptor expression and low oxytocin sensitivity until the time of parturition (55, 56). Furthermore, the pattern of oxytocin secretion is important in parturition; pulsatile oxytocin secretion is required to elicit the rhythmic contractions of the uterus that expel the fetus, rather than a constant high level of oxytocin (57, 58). Alternatively, chronically elevated levels of endogenous kisspeptin during pregnancy might desensitize GPR54, because chronic administration of kisspeptin-10 desensitizes GPR54 on

GnRH neurons, with an associated loss of LH responses (8). Nevertheless, it will require further work to reconcile the high levels of the excitatory hormone, kisspeptin, in late pregnancy with the need to prevent premature activation of the oxytocin system.

In summary, we provide the first report of electrical excitation of oxytocin neurons and vasopressin neurons by iv kisspeptin-10. A chemical vagotomy using ip capsaicin administration prevented the activation of oxytocin neurons by kisspeptin-10, indicating that the peripheral pathway is mediated through an afferent vagal pathway, although presently, the precise location of the GPR54 is unknown. The role that kisspeptin may be having on MNC via the vagus, and presumably NTS, might be of critical physiological importance during pregnancy and lactation, when circulating kisspeptin levels are increased (48), and is the focus of continuing studies.

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