Published in final edited form as:

J Neuroendocrinol. 2011 October ; 23(10): 883–893. doi:10.1111/j.1365-2826.2011.02207.x.

Prolactin induces a hyperpolarizing current in rat paraventricular oxytocinergic neurons

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Abstract

Prolactin and oxytocin are important reproductive hormones implicated in several common adaptive functions during pregnancy, pseudopregnancy and lactation. Recently, extracellular recordings of supraoptic neurons have shown that prolactin may modulate electrical activity of oxytocinergic neurons. However, no study has been conducted to establish whether prolactin directly influences this activity in oxytocinergic paraventricular neurons. Here we addressed this question by studying the effects of prolactin on the electrical activity and voltage-current relationship of identified paraventricular neurons in rat brain slices. Whole-cell recordings were obtained and neurons were classified on the basis of their morphological and electrophysiological fingerprint (magnocellular or parvocellular) and neuropeptide phenotype (oxytocinergic or non-oxytocinergic). We report that prolactin elicited a hyperpolarizing current in 37% of the neurons in this nucleus, of which the majority (67%) were identified as putative magnocellular oxytocin neurons and the remainder (33%) were regarded as oxytocin-negative, parvocellular neuroendocrine neurons. Our results suggest that, in addition to the well-established negative feedback loop between prolactin-secreting lactotrophs and dopaminergic neurons in the arcuate nucleus, an inhibitory feedback loop also exists between lactotrophs and oxytocinergic paraventricular neurons.

Keywords

Oxytocin; prolactin; paraventricular nucleus; whole-cell recordings; immunocytochemistry, feedback

Introduction

The pituitary hormone prolactin plays an essential role in the reproductive function of female rats (1). It is important in maintaining the structural and functional integrity of the corpus luteum that, in turn, is responsible for progesterone synthesis during pregnancy (1). In the early phase of pregnancy in rats, prolactin from lactotrophs located in the anterior lobe of the pituitary gland shows a rhythmic secretion pattern characterized by nocturnal and

diurnal surges (at around 3:00 h and 17:00 h, respectively) (1). In addition, the secretion of this hormone seems to be under circadian control (2-4).

Prolactin secretion is controlled by the balanced supply of hypothalamic stimulatory and inhibitory factors delivered to the lactotrophs via long and short portal vessel systems (1). The major inhibiting factor is dopamine, which is released primarily by tuberoinfundibular neurons of the arcuate nucleus (1,5,6). Dopamine tonically inhibits the secretion of prolactin, thus the hormone can only be released when dopamine levels in the portal blood are reduced (7,8). However, the temporary reduction of dopamine does not fully account for the entire amount of prolactin released, suggesting that additional prolactin releasing factors of hypothalamic origin may be involved in the generation of the surges. Once prolactin is secreted, it stimulates dopamine synthesis and metabolism in tuberoinfundibular neurons with a time delay (9,10). This so-called short feedback loop between lactotrophs and tuberoinfundibular dopaminergic neurons (11,12) has been proposed to be largely responsible for prolactin homeostasis (5).

Recent studies from our group and others demonstrated a stimulatory effect of oxytocin in the regulation of prolactin secretion (13-16). We showed that bath application of oxytocin to cultured lactotrophs elevates intracellular Ca^{2+} levels and induces prolactin release (14). In addition to the neurons in the supraoptic nuclei (SON), those in the paraventricular nucleus (PVN) of the hypothalamus are major production sites for oxytocin (17). Importantly, oxytocinergic PVN neurons in rats display daily activity rhythms that correlate with the prolactin-releasing pattern of pregnant/pseudopregnant rats (18). PVN neurons have been classified morphologically into magnocellular and parvocellular populations, and electrophysiologically into at least two types of neurons (19). It has been proposed that magnocellular and parvocellular neurons correspond to types I and II, respectively (20,21).

Using immunocytochemical techniques, we have shown that oxytocinergic neurons in the PVN express prolactin receptors (15). This is in line with other studies that have found prolactin receptor messenger ribonucleic acid (mRNA) in neurons of the PVN (22-25). Since prolactin may gain access to the brain through a carrier-mediated transport system (26) that probably involves prolactin-binding sites in the choroid plexus (27-29), the fact that oxytocin stimulates prolactin secretion from lactotrophs and that prolactin receptors can be found on oxytocin neurons in the PVN raises the possibility that a prolactin-oxytocin feedback loop exists that is substantially involved in the regulation of prolactin secretion, in addition to the well known prolactin-dopamine feedback loop.

The goal of this work is to determine if and how prolactin affects the electrical activity of oxytocinergic neurons of the PVN. The study was carried out on acute brain slices of the hypothalamic PVN using a combination of electrophysiological (whole-cell patch clamp) and immunocytochemical techniques. Responses to prolactin were thus obtained from neurons carefully characterized by immunohistochemistry, pharmacology and electrophysiology.

Materials and Methods

Chemicals

Isoflurane used for anesthesia was purchased from Baxter (Volketswil, Switzerland). For the voltage clamp experiments, tetrodotoxin (TTX; Ascent Scientific, Bristol, UK) was diluted in artificial cerebrospinal fluid to a final concentration of $1\mu\text{M}$, to prevent spike-mediated synaptic transmission. For the perfusion protocols (see below), we used a δ opioid receptor agonist [D-Ala², D-Leu⁵]-Enkephalin (DADLE; $10\mu\text{M}$, Sigma-Aldrich, St Louis, MO, USA) and rat prolactin (100ng/ml; kindly provided by the National Institute of Diabetes and

Digestive and Kidney Diseases (NIDDK), and Dr. A.F. Parlow, Pituitary Hormones and Antisera Center, Harbor-UCLA Medical Center, Torrance, CA).

Hypothalamic brain slice preparation

PVN brain slices were obtained from juvenile female Sprague-Dawley rats (90 - 100g body weight) hosted at the animal facility (Brain Research Institute, University of Zurich). All animal procedures were approved by the local animal ethics committee and complied with the Swiss regulations for care and use of animals. The slices were cut as described previously (20). In brief, deeply anesthetized (isoflurane) animals were decapitated, their brains removed and placed in ice-cold, oxygenated artificial cerebrospinal fluid composed of (mM): NaCl 124, KCl 2.8, MgSO₄ H₂O 2, NaH₂PO₄ H₂O 1.25, NaHCO₃ 26, D-glucose (C₆H₁₂O₆) 10, ascorbic acid (C₆H₈O₆) 0.4, CaCl₂ 2 H₂O 2 (pH adjusted to 7.4 and osmolality ranging between 290 - 300mOsmol l⁻¹). Thereafter, the brains were trimmed to tissue sections of the hypothalamus (1 cm²) before cutting 300 μm thick slices of the PVN on a vibratome (Leica, VT 1200). Immediately thereafter, the brain sections were incubated in oxygenated artificial cerebrospinal fluid at 37°C for half an hour and kept for equilibration at room temperature for an additional one hour. For the experiments, slices were placed into a 1 ml recording chamber mounted on an upright microscope (Axioscope FS, Zeiss, Oberkochen, Germany). A bath perfusion system enabled continuous chamber perfusion with either artificial cerebrospinal fluid or test substances (1 - 2ml/min flow rate).

Whole-cell recordings

Patch-clamp electrodes were pulled from borosilicate glass capillaries (1.5mm, Harvard Apparatus, Edenbridge, Kent, UK) resulting in a resistance ranging from 5 - 8MΩ. Patch pipettes were filled with a solution containing (in mM): potassium D-gluconate 105, KCl 30, Hepes 10, EGTA 1.1, MgCl₂ 6H₂O 4, Na₂ATP 2, Na₂GTP 0.3. The patch-pipette solution was pH adjusted to 7.3 using KOH, and to an osmolality ranging from 300 - 310mOsmol l⁻¹. Biocytin (1%) was added to the pipette solution for subsequent immunocytochemical analysis in most of the experiments. Whole-cell patch-clamp recordings were carried out on PVN neurons in slices superfused with artificial cerebrospinal fluid at 32°C, under visual control. Recordings were performed in both current-clamp and voltage-clamp configurations using an Axopatch 200B amplifier (Molecular Devices, Palo Alto, CA, USA). All currents were low-pass filtered at 2 kHz and sampled at 5 kHz using pCLAMP9 software (Molecular Devices). Membrane voltage data were corrected for the liquid-junction potential (17mV). Series resistance ranged between 10 - 20 MΩ, and was compensated up to 70 - 80%.

Electrophysiological and pharmacological identification

According to the biocytin staining (see below), 49 neurons were identified as PVN neurons. Type I and type II PVN neurons were distinguished on the basis of the presence or absence, respectively, of a transient I_A -mediated rectification, as described by Swanson and Sawchenko (30), Tasker and Dudek (19) and Hoffman (21). To activate I_A , a current-clamp protocol was used which hyperpolarized the cell first (-400 pA, 300 ms) and then depolarized it with currents ranging from 0 pA to 100 pA in 10 pA steps (see insert in Fig. 2). In this fashion, 24 out of 49 (49%) PVN neurons analyzed were classified as type I and the remainder 25 cells (51%) as type II. Substantial evidence supports the notion that electrophysiologically defined type I and type II cells correspond to morphologically categorized magnocellular and parvocellular neurons (20,21), and will thus be henceforth termed “putative magnocellular” and “putative parvocellular”. Additional analysis of the electrophysiological recordings of putative parvocellular neurons allowed us to distinguish between neurosecretory and non-neurosecretory cells. We adopted the criteria of Luther *et al.* (31) in which non-neurosecretory parvocellular neurons generate a low-threshold spike and robust T-type Ca²⁺ current, whereas neurosecretory cells show no low-threshold spike

and only a small T-current. In our preparation we identified 80% of putative parvocellular PVN neurons as neurosecretory cells and 20% as preautonomic non-neurosecretory cells.

In addition, to classify the neurons based on the presence or absence of the I_A rectifying current, cells were initially monitored under current clamp conditions to evaluate their firing activity and membrane potential. PVN neurons that exhibited a continuous (non-phasic) firing pattern were classified as putative oxytocinergic neurons. On the other hand, those that exhibited a phasic firing pattern (characterized by a bursting pattern of action potentials with a mean intraburst firing rate range of 4 to 15 spikes per sec, a mean burst duration between 10 and 30 sec, a mean silence duration between 5 to 20 sec, minimum number of spikes per burst of 9 and minimum burst and silent duration of 3 and 2 sec, respectively) were classified as putative vasopressin neurons (32,33). In addition, PVN neurons were identified as oxytocinergic if their firing activity showed a transient inhibition in response to D-ala-D-leu-enkephalin (DADLE, see below) (32). Further identification was defined on the basis of subsequent immunocytochemical analysis.

Drug application and I-V protocol

The drug application protocol was conducted in the presence of tetrodotoxin (1 μ M) under voltage clamp conditions and included an initial control period of 3 min during which conditions prior to drug application were recorded. Thereafter, prolactin (100 ng/ml) was applied to the recording chamber for 2 min. Control artificial cerebrospinal fluid was then perfused for 10-12 min to remove prolactin (washout). Finally, the δ opioid receptor agonist DADLE was added (10 μ g/ml, 2 min) to indicate whether or not the investigated neuron was oxytocinergic. The electrical properties of the patched neurons were determined at the end of exposure to prolactin, washout or DADLE treatment period by applying the following voltage-clamp step protocol: from a holding potential of -60 mV, step to -105 mV for 350 ms, and then to a test potential in the range of -105 mV to -35 mV (in 10 mV steps) for 350 ms.

Electrophysiological parameters and curve fitting

The following electrophysiological parameters were recorded to further characterize PVN neurons and to describe prolactin-induced changes: resting membrane potential (V_m), membrane conductance ($G_m = 1/R_m$ - input resistance R_m of the cell was measured with a weak current pulse, usually -50 or -100 pA), action potential amplitude (AP) and mean firing rate (MFR). In control conditions (unstimulated, prior to TTX application), those values ($\pm$ SEM) agreed with those previously reported (20), and were: For putative magnocellular neurons, $V_m = -54 \pm 3$ mV, $G_m = 0.94 \pm 0.10$ nS, AP = 78 ± 1 mV and MFR = 8.6 ± 1.2 Hz. For putative parvocellular neurons, $V_m = -59 \pm 8$ mV, $G_m = 2.52 \pm 0.60$ nS, AP = 75 ± 20 mV and MFR = 3.8 ± 3.4 Hz.

For each type of PVN neuron studied, a number of I - V curves were selected and averaged to obtain mean I - V curves representative of the neuronal type and of the different phases of the experiment. Each of the averaged curves was then fit to an Ohmic current function with a Boltzmann conductance function:

$$I(V) = g(V)(V - V_0), \quad g(V) = \frac{A}{1 + \exp\left(-\frac{V - V_{1/2}}{s}\right)} + C$$

where V_0 is the voltage at zero current and $g(V)$ the observed membrane conductance for a voltage V , A is the amplitude parameter, $V_{1/2}$ is the half-activation voltage, s is the slope parameter, and C is the conductance offset. The fits were generally good, with R-values

close to 1. For some of the fitting parameters the variability was too large for a reliable estimation based on the 95%-confidence interval. We therefore restricted the analysis to the following parameters, which were found to vary within small intervals: V_0 , the zero-current voltage; I_{ref} , the current recorded at a reference potential of -105mV; and the average membrane conductance over the interval between -105mV and -35mV. The fitting procedures were performed in Matlab (The Mathworks, Natick, MA), and the Excel program (Microsoft) was used for part of the subsequent statistical analysis and for tabulation of the results.

Statistical analysis

Results are expressed as means $\pm$ SEM. For each neuronal population, statistically significant differences between the I - V relationships acquired in 3 sequential experimental conditions (control, 2 min pulse of prolactin and 10 min after hormone withdrawal) were evaluated at the level of the whole I - V relationship using the non-parametric multiple comparison test for repeated measurements (Friedman's test) as follows: Friedman Q statistics were obtained for each potential value recorded, and the results were summed. Under the null hypothesis of independent errors for differential potential values and absence of effect of the treatments, this summed Q-statistics follows an approximate Chi-squared distribution with $n \times (K-1)$ degrees of freedom, where n is the number of points in each I - V curve ($n=8$) and $K=2$ or 3 according to the number of experimental conditions being compared. Reported p values were computed according to this distribution. Equivalent parametric analysis (one-way analysis of variance followed by post-hoc Tukey's test) was also conducted with comparable results. Comparisons of the conductances measured after prolactin application and removal vs the control were analyzed using the non-parametric Wilcoxon's signed rank test for correlated samples (similar results were obtained using paired Student's t test). $P < 0.05$ was considered significant.

Immunocytochemistry (ICC)

Each of the recorded neurons was immunocytochemically investigated after the electrophysiological recordings, allowing both localization of the cell and detection of the intracellular content. To allow morphological identification of the cells, biocytin (1%) was added to the pipette solution to diffuse into the recorded neurons. After the experiments, the slices were removed from the chamber and left overnight for fixation in 4% paraformaldehyde and 0.15% picric acid diluted in phosphate-buffered saline (PBS) at 4°C. The next day, the slices were placed in cryoprotective solution (30% sucrose, 12% glycerol, 0.1 M phosphate buffer) and frozen at -20° C. For further analysis, slices were washed three times in PBS, pre-incubated overnight to block non-specific antigen binding in 0.4% Triton-X-100 containing 10% horse-serum (blocking solution), and incubated for 72 hours at 4°C in the presence of monoclonal mouse antibodies raised against oxytocin-related neurophysin in fresh blocking solution (Chemicon International, Temecula, CA, USA; dilution 1:20000) (34). Afterwards, the slices were washed three times in PBS for 1 hour and then incubated overnight at 4°C in a mixture containing Cy3-conjugated AffiniPure Goat Anti-mouse IgG (H+L) secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA USA; 1.5 mg/ml) and AlexaFluor-488-conjugated streptavidin (Molecular Probes; dilution 1:1000) for the detection and amplification of oxytocin-related neurophysin and biocytin staining, respectively. To test for the specificity of labelling, slices were incubated in buffer without primary antibody. Finally, slices were mounted on a glass slide, covered with Aqua-Poly/Mount (Poly-sciences, Warrington, PA, USA) and digitally photographed using a SPOT camera (Diagnostic Instruments, USA). The acquired images were contrast-adjusted and visualised using Adobe Photoshop (Adobe Systems, San Jose, CA, USA). Thus, double stained cells indicated oxytocin accumulation in electrophysiologically investigated neurons.

Results

Electrophysiological, morphological and pharmacological characterization of PVN neurons

Magnocellular neurons were characterized by the presence of an A-type potassium current (I_A), that is absent in putative parvicellular neurons (see Materials and Methods). The typical delayed onset of action potentials of magnocellular neurons, which is a result of the I_A current, could be demonstrated in these neurons (Fig. 1A, arrow) but not in parvicellular (Fig. 1C) neurons. In fact, the two groups of neurons could be distinguished not only by the presence/absence of I_A , but also by their activity level. Putative magnocellular neurons were typically active, with a mean overall firing rate of 8.6 ± 1.2 Hz, while only a fraction of parvicellular neurons (11 out of 25) were spontaneously active, with a mean overall firing rate of 3.8 ± 3.4 Hz.

Morphological analysis with biocytin labeling revealed further differences between putative magnocellular and parvicellular neurons, consistent with those described in the literature (35). The majority of magnocellular neurons were bipolar with long and extended somata (Fig. 1B, center panel). There were two main neuronal branches leaving the cell body on opposite sides. In contrast, the somata of parvicellular neurons were mostly compact, and there were typically three to five neuronal branches in addition to the axon (Fig. 1D, center panel).

The response of neurons to DADLE (not shown), which was used as pharmacological identification of oxytocin neurons, agreed with the immunocytochemical analysis. Oxytocin neurons were thus considered as cells that showed positive immunostaining for oxytocin-neurophysin and that hyperpolarized under DADLE. Under these criteria, 50% of the magnocellular neurons studied were regarded as oxytocin neurons. Fig. 1B shows an example of one of the magnocellular oxytocinergic cells used in the recordings. The other half of the magnocellular population was unresponsive to DADLE and showed no oxytocin-neurophysin staining and thus regarded as vasopressin-producing neurons. None of the putative parvicellular cells investigated showed positive immunostaining for oxytocin-neurophysin (Fig. 1D).

Prolactin evokes a hyperpolarizing current in responsive PVN neurons

The effects of prolactin on membrane currents of PVN neurons were determined with a voltage-clamp protocol under basal conditions and at the end of each treatment period (prolactin 100ng/ml for 2 min; a 10-min washout to remove prolactin, and DADLE 10 μ g/ml for 2 min) yielding a series of I - V curves that were analyzed by Boltzmann fits for each experimental condition (see Materials and Methods). Prolactin had an effect on 12 out of 24 (50%) of the putative magnocellular and 6 out of 25 (24%) of the putative parvicellular PVN neurons. Interestingly, all prolactin-sensitive magnocellular neurons corresponded to the entire population of oxytocin magnocellular cells studied. Results from this analysis are shown from only those neurons that exhibited a significant response to prolactin.

Figure 2 shows the corresponding I - V plots for putative oxytocinergic, magnocellular (panel A) and non-oxytocinergic, parvicellular (panel B) PVN neurons before and after a 2-min application of prolactin. In both neuronal populations, prolactin induced an outward (hyperpolarizing) current for potentials above -60mV, resulting in a left shift of the reversal potential.

In all oxytocinergic, putative magnocellular PVN neurons (Fig. 2A), the current slope conductance of the membrane (G_m) increased significantly in response to prolactin between -95mV and -75mV (Prolactin: 1.48 ± 0.2 nS vs Control: 0.9 ± 0.1 nS, $p=0.02$, paired

Wilcoxon's signed rank test) as well as in the interval -55mV to -35mV (Prolactin: 3.6 ± 0.4 nS vs Control: 3.0 ± 0.5 nS, $p=0.04$). Friedman's test followed by post-hoc analysis showed that the I - V relationship recorded at the end of the prolactin pulse was significantly different from that obtained in control conditions when comparing the whole curve ($p<10^{-4}$). The average difference current $\Delta I = I_{PRL} - I_{ctl}$ obtained by subtracting control currents from currents measured at the end of the PRL treatment (Fig 3A) reversed at -56 mV and was positive (hyperpolarizing) for larger potentials. The difference current was almost linear, suggesting that PRL activates a V -independent current. To evaluate the impact of such a current in the electrical activity of oxytocinergic magnocellular neurons, the effect of prolactin was investigated in one such cell under current clamp conditions. As shown in Fig. 4, prolactin application elicited hyperpolarization of the membrane potential with inhibition of its firing activity that was sustained for several minutes (see below).

As indicated earlier, prolactin introduced qualitatively similar changes to the electrophysiological properties of a fraction (24%) of putative parvocellular PVN neurons (Fig. 2B). The I - V relationship obtained after the 2-min prolactin application was found to be significantly different from the control when both curves were analyzed with non-parametric multiple comparison tests ($p=0.034$, Friedman's post hoc test). However, the extent of the increase of the G_m following prolactin treatment was not statistically significant, probably due to the comparatively smaller size of the sample. The reversal potential of the I - V curve was slightly left shifted following 2 min of prolactin application and the average difference current was increasing almost linearly (Fig. 3B) with a reversal potential of -57 mV.

Prolactin effects on membrane potential are long-lasting

Surprisingly, the prolactin effects persisted at least 10 min after the hormone was removed. In putative magnocellular neurons (Fig. 2A), the I - V curve obtained 10 min after prolactin washout was significantly different from that obtained under control conditions and after the 2-min prolactin application ($p<10^{-4}$ vs control, $p=2\times10^{-4}$ vs 2-min prolactin, Friedman post hoc test). This result is consistent with the sustained hyperpolarization shown earlier after prolactin application under current clamp mode (Fig. 4). It was determined that G_m measured 10 min after prolactin withdrawal in the voltage range -95mV to -75mV was significantly larger, 1.72 ± 0.3 nS, than in the control group, 0.9 ± 0.1 nS ($p<0.005$ vs control, paired Wilcoxon's signed rank test). For voltages closer to the resting potential (between -55mV and -35mV) the G_m was found to be augmented as well (Washout: 4.1 ± 0.6 nS vs Control: 3.0 ± 0.5 nS, $p=0.001$). The reversal potential for these neurons remained more negative than the one recorded previously under control conditions, but was no different from that observed at the end of the prolactin pulse (not shown). Likewise, the reversal potential of the average difference current obtained after 10 min of hormone washout (Fig 3A) was approximately -57 mV, very similar to the one previously determined at the end of the prolactin pulse.

In those putative parvocellular PVN neurons responsive to prolactin, the I - V relationship obtained after 10 min of prolactin withdrawal (Fig. 2B) was significantly different from that recorded under control conditions ($p=0.008$, Friedman's post hoc test) although it did not reach statistical significance when compared to the one obtained after 2-min of prolactin treatment ($p=0.082$). The G_m 10 min after hormone withdrawal was larger than in control conditions over the whole range of voltages tested (Fig. 2B). In fact, the increase was statistically significant (Washout: 7.5 ± 1.4 nS vs Control: 4.1 ± 1.0 nS, $p=0.03$) for voltages above -55mV. Furthermore, the reversal potential obtained after prolactin withdrawal was further shifted to the left than at the end of the hormonal pulse. In line with this, the reversal potential of the difference current (Fig 3B) was approximately -68 mV, compared to -57 mV observed at the end of the prolactin pulse (Fig 3B). In addition, the current-voltage

relationship of the difference current was no longer linear 10 min after washout, suggesting the introduction of a voltage-dependent current.

Discussion

Several lines of evidence support the idea that prolactin exerts critical modulatory actions at different hypothalamic areas involved in its regulation. First, systemic prolactin may gain access to the brain by means of a transport system at the choroid plexus (1,26,28). Second, prolactin receptor mRNA (22-25) and protein (36) have been identified in both the SON and PVN hypothalamic nuclei. In the latter nucleus, we have further demonstrated prolactin receptor immunoreactivity in oxytocinergic neurons (15). Third, both the SON and PVN undergo spectacular changes during pregnancy and lactation, and prolactin receptors are specifically upregulated in the PVN during lactation (16,36,37). Fourth, these same nuclei may produce and release prolactin locally (38,39). Fifth, in the hypothalamo-neurohypophyseal axis prolactin specifically increases oxytocin mRNA expression (40-43) and stimulates oxytocin secretion *in vitro* (44) and *in vivo* (45). Since oxytocin releases prolactin from pituitary lactotrophs and the daily activity rhythm of oxytocinergic PVN neurons correlate with the prolactin-releasing pattern of pregnant/pseudopregnant rats (18), these observations support the hypothesis that prolactin may elicit modulatory roles on the activity of oxytocinergic PVN neurons. The present work reports for the first time prolactin-induced biophysical effects on identified PVN neurons.

Forty-nine PVN neurons were electrophysiologically identified as type I, putative magnocellular neurons (n = 24, 49%) and type II, putative parvocellular neurons (n = 25, 51%), using the definitions outlined by Tasker and Dudek (19) and Hoffmann *et al.* (21). Among the magnocellular neurons investigated, 50% were classified as oxytocinergic as indicated by immunocytochemical staining and their hyperpolarizing response to DADLE application. Interestingly, all magnocellular oxytocinergic neurons were responsive to prolactin application. For membrane potentials greater than -60mV, prolactin elicited a hyperpolarizing current that persisted at least 10 min after hormone washout. This effect resulted in sustained hyperpolarization and clear inhibition of firing activity in a magnocellular oxytocinergic neuron when evaluated under current clamp mode (Fig. 4). The inhibitory effects of prolactin are in line with the *in vivo* extracellular recordings reported by Kokay et al. from magnocellular oxytocin neurons in the SON showing a decrease of neuronal activity after prolactin application (16). Of note, it has been found in another study that supraoptic oxytocin neurons of male rats show both excitatory and inhibitory responses to prolactin in horizontal hypothalamic slices (46).

Type I neurons are generally regarded as magnocellular neuroendocrine cells that supply their products to the posterior pituitary gland by means of the hypothalamo-hypophyseal tract (17). Electrophysiological studies on oxytocin neurons showed that an increase in firing activity facilitates peptide secretion (47). Because prolactin induced a hyperpolarizing current in oxytocinergic PVN neurons, we hypothesize that this decreases the neuronal activity resulting in less oxytocin release from the axon terminals of the posterior pituitary. This would result in a reduction of prolactin secretion from the anterior lobe of the pituitary gland. Such a relation between prolactin-secreting lactotrophs and oxytocinergic neurons of the PVN would thus provide a negative feedback loop where prolactin suppresses its own secretion indirectly via reduction of oxytocin release. However, as mentioned above, prolactin has been shown to stimulate oxytocin synthesis (40-43) and secretion (44,45) in lactating and steroid-primed ovariectomized female rats. This apparent discrepancy might be explained by a differential regulation of somatic vs nerve terminal release of oxytocin, as has been shown for alpha-melanocyte-stimulating hormone (48). Thus, it is possible that prolactin-induced effects in the cell bodies of oxytocin neurons are dissociated from those at

their terminals in the posterior pituitary. Alternatively, the effect of peptides may vary according to the experimental condition (*in vitro*, *in vivo*), gender, and physiological status. Indeed, central prolactin has been shown to release oxytocin in plasma in virgin female rats, but inhibit stress-induced oxytocin release (43,49).

The electrophysiological analysis of putative parvicellular neurons showed that about a quarter of these cells responded to prolactin. Based on their lack of low-threshold spike and small T-current, these parvicellular prolactin-responsive cells are thought to be neuroendocrine cells and represent about 30% of the population of parvicellular neurons that project to the median eminence. No preautonomic (nonneurosecretory) parvicellular cells responded to prolactin. However, in contrast to what we (18) and others (50,51) have reported, we failed to observe oxytocin-positive stained cells among putative parvicellular neurons. The reason for this is not immediately clear, but we must point out here that only a minority of the oxytocinergic fibers found in the external layer of the median eminence originate in the parvicellular cell bodies in the PVN (50). Additionally, it is possible that this cell population is less abundant in the juvenile female rat used for our studies and that we would have found oxytocin-positive parvicellular neurons if our sampling size had been larger. Notwithstanding, the fact that there were nonoxytocinergic, prolactin sensitive parvicellular neurons with electrophysiological characteristics of neurosecretory cells raises the possibility that prolactin may influence the secretion of ACTH and GH through modulation of corticotrophin-releasing hormone (CRH), arginine-vasopressin, growth hormone-releasing hormone (GHRH), somatostatin and thyrotropin-releasing hormone (TRH) cell bodies (35). A well-known correlation exists between suckling-stimulated prolactin secretion and reduced hypothalamic-pituitary-adrenal axis responses in lactation, and our findings may add to the accumulating evidence suggesting a potential role of central prolactin in such diminished responses (52). Further investigations need to be carried out to elucidate details of these potential regulatory functions of prolactin.

Our data show that prolactin induces a hyperpolarizing current in putative magnocellular oxytocin neurons in the PVN that may result in inhibition of firing activity. A number of previous studies have shown that prolactin induces rapid positive (46,53) and negative (16,46) changes in the excitability of hypothalamic neurons. These diverse effects of prolactin (within and among different subsets of hypothalamic neurons) might be achieved by operating through its different prolactin receptor isoforms (1,54). In rats, short, intermediate and long forms of the receptor have been found, which differ in the length of their intracellular domains and signaling capabilities (1,54). As a result, the short form of the prolactin receptor uses mainly signaling pathways mediated through the mitogen-activated protein (MAP) kinase. However, only the long form, which in the PVN is preferentially expressed by oxytocin neurons, retains the ability to fully activate the Janus kinase/Signal transducer and activator of transcription (JAK/STAT) signaling pathway (16).

In contrast to the stimulation/inhibition of gene transcription, the activation of ion channels is one of the primary proximal (rapid) events in prolactin receptor signal transduction. For this reason, it is not likely that such rapid effects involve the JAK/STAT pathway. Nevertheless, JAK2 tyrosine kinase activity has been shown to be required for prolactin-induced phosphorylation and activation of ion channels (55,56), transporters (57,58) and kinases (59,60). Moreover, tyrosine phosphorylation is an early crucial step in prolactin receptor signaling that controls K^+ channel activation, calcium influx and/or intracellular calcium mobilization. Indeed, in Chinese hamster ovary (CHO) cells stably transfected with the long form of the prolactin receptor, it was shown that prolactin increased the activity of a 240-pS Ca^{2+} -sensitive and voltage-dependent K^+ channel (K_{Ca}) through JAK2-mediated tyrosine phosphorylation (55) and elevated the intracellular calcium concentration probably through voltage-insensitive second messenger-operated nonspecific channels (61,62). Since

PVN neurons express many types of K^+ channels, including K_v (delayed rectifier) channels, K_{ir} (inwardly rectifying) channels, KCN leak channels, two-pore (2-P) channels and both Ca^{2+} -activated large conductance (BK) and small conductance (SK) channels (63), prolactin could hyperpolarize these neurons through activation of K_{Ca} channels provided that the mechanism described in CHO cells also applies to PVN neurons. Moreover, prolactin has been shown to elicit a slow K^+ current with a time lapse to peak effect of about 10 min and an incomplete return to control levels after 10 min of washing through a mechanism that involves tyrosine phosphorylation in CHO (61), as well as in human breast MCF-7 (56) and prostate LNCaP (64) cancer cells. The persistence of the effects of prolactin reported here is comparable to these studies, lending support to the hypothesis that a predominantly K^+ conductance is responsible for mediating the prolactin-induced hyperpolarization in PVN neurons. Nonetheless, the difference current we observed in the presence of prolactin was linear and reversed at about -57mV, which is higher than the K^+ equilibrium potential, suggesting that modulation of a voltage-independent conductance with mixed ion selectivity, such as the mixed non-selective cationic current (I_{NSCC}) (65) might also play a part in the prolactin-induced hyperpolarization of PVN neurons. Participation of a chloride conductance constitutes another likely mechanism, given the observation that prolactin activates a chloride conductance thought to play a role in prolactin-induced hyperpolarization in human malignant astrocytoma cells (66). In the magnocellular system, prolactin may facilitate the sensitivity of GABA_A receptors to inhibitory inputs, since these receptors are known to be modulated by locally produced neurohormones in particular physiological stages (67). In addition, the hyperpolarization-activated current (I_h) and low-voltage-activated (LVA) Ca^{2+} current have been shown to influence neuronal excitability in the hypothalamus (65,68), and may also be modulated by prolactin in the PVN.

Recently, work of Townsend *et al.* (46) and Kokay *et al.* (16) have reported modulatory effects of prolactin on the electrical activity of oxytocinergic supraoptic neurons. Because their data were based on extracellular recordings, it is not known whether the effects of prolactin involved direct action within the supraoptic nucleus or indirect interaction with its perinuclear afferent circuitry. Here, we have provided for the first time direct evidence that prolactin elicits a rapid and long lasting hyperpolarizing current in putative magnocellular (type I) oxytocinergic neurons and putative parvocellular (type II) neurosecretory neurons of the PVN. This means that the neuronal activity of PVN neurons, particularly oxytocin-immunostained cells, is likely reduced by prolactin. Thus, besides the well known negative feedback loop between prolactin and tuberoinfundibular dopaminergic neurons (1,5), an inhibitory feedback loop exists between lactotrophs of the anterior pituitary gland and PVN neurons which supply the prolactin-releasing factor oxytocin.

Acknowledgments

This work was supported by National Institutes of Health grants DK-43200 and DA-19356. We thank Dr. Joël Tabak and Dr. Maurizio Tomaiuolo for helpful comments on the manuscript.

References

1. Freeman ME, Kanyicska B, Lerant A, Nagy GM. Prolactin: structure, function, and regulation of secretion. *Physiol Rev.* 2000; 80:1523–1631. [PubMed: 11015620]
2. Arey BJ, Averill RL, Freeman ME. A sex-specific endogenous stimulatory rhythm regulating prolactin secretion. *Endocrinology.* 1989; 124:119–123. [PubMed: 2909362]
3. Bethea CL, Neill JD. Prolactin secretion after cervical stimulation of rats maintained in constant dark or constant light. *Endocrinology.* 1979; 104:870–876. [PubMed: 436759]
4. Kizer JS, Zivin JA, Jacobowitz DM, Kopin IJ. The nyctohemeral rhythm of plasma prolactin: effects of ganglionectomy, pinealectomy, constant light, constant darkness or 6-OH-dopamine administration. *Endocrinology.* 1975; 96:1230–1240. [PubMed: 1168130]

5. Ben-Jonathan N, Hnasko R. Dopamine as a prolactin (PRL) inhibitor. *Endocr Rev.* 2001; 22:724–763. [PubMed: 11739329]
6. Lamberts SWJ, MacLeod RM. Regulation of prolactin secretion at the level of the lactotroph. *Physiol Rev.* 1990; 70:279–318. [PubMed: 2181497]
7. De Greef WJ, Neill JD. Dopamine levels in hypophysial stalk plasma of the rat during surges of prolactin secretion induced by cervical stimulation. *Endocrinology.* 1979; 105:1093–1099. [PubMed: 487998]
8. McKay DW, Pasieka CA, Moore KE, Riegle GD, Demarest KT. Semicircadian rhythm of tuberoinfundibular dopamine neuronal activity during early pregnancy and pseudopregnancy in the rat. *Neuroendocrinology.* 1982; 34:229–235. [PubMed: 6803182]
9. Hentschel K, Moore KE, Lookingland KJ. Effects of prolactin on expression of Fos-related antigens in tyrosine hydroxylase-immunoreactive neurons in subdivisions of the arcuate nucleus. *Brain Res.* 2000; 857:110–118. [PubMed: 10700558]
10. Moore KE, Demarest KT, Johnston CA. Influence of prolactin on dopaminergic neuronal systems in the hypothalamus. *Fed Proc.* 1980; 39:2912–2916. [PubMed: 7409209]
11. Grattan DR, Xu J, McLachlan MJ, Kokay IC, Bunn SJ, Hovey RC, Davey HW. Feedback regulation of PRL secretion is mediated by the transcription factor, signal transducer, and activator of transcription 5b. *Endocrinology.* 2001; 142:3935–3940. [PubMed: 11517172]
12. Milenkovic L, Parlow AF, McCann SM. Physiological significance of the negative short-loop feedback of prolactin. *Neuroendocrinology.* 1990; 52:389–392. [PubMed: 2124664]
13. Bertram R, Egli M, Toporikova N, Freeman ME. A mathematical model for the mating-induced prolactin rhythm of female rats. *Am J Physiol - Endocrinol Metab.* 2006; 290:E573–E582. [PubMed: 16467488]
14. Egli M, Bertram R, Sellix MT, Freeman ME. Rhythmic secretion of prolactin in rats: action of oxytocin coordinated by vasoactive intestinal polypeptide of suprachiasmatic nucleus origin. *Endocrinology.* 2004; 145:3386–3394. [PubMed: 15033917]
15. Egli M, Bertram R, Toporikova N, Sellix MT, Blanco W, Freeman ME. Prolactin secretory rhythm of mated rats induced by a single injection of oxytocin. *Am J Physiol - Endocrinol Metab.* 2006; 290:E566–E572. [PubMed: 16467487]
16. Kokay IC, Bull PM, Davis RL, Ludwig M, Grattan DR. Expression of the long form of the prolactin receptor in magnocellular oxytocin neurons is associated with specific prolactin regulation of oxytocin neurons. *Am J Physiol- Regul Integr Comp Physiol.* 2006; 290:R1216–R1225. [PubMed: 16410399]
17. Gimpl G, Fahrenholz F. The Oxytocin receptor system: structure, function, and regulation. *Physiol Rev.* 2001; 81:629–683. [PubMed: 11274341]
18. Arey BJ, Freeman ME. Activity of oxytocinergic neurons in the paraventricular nucleus mirrors the periodicity of the endogenous stimulatory rhythm regulating prolactin secretion. *Endocrinology.* 1992; 130:126–132. [PubMed: 1727695]
19. Tasker JG, Dudek FE. Electrophysiological properties of neurones in the region of the paraventricular nucleus in slices of rat hypothalamus. *J Physiol.* 1991; 434:271–293. [PubMed: 2023120]
20. Luther JA, Tasker JG. Voltage-gated currents distinguish parvocellular from magnocellular neurones in the rat hypothalamic paraventricular nucleus. *J Physiol.* 2000; 523:193–209. [PubMed: 10673555]
21. Hoffman NW, Tasker JG, Dudek FE. Immunohistochemical differentiation of electrophysiologically defined neuronal populations in the region of the rat hypothalamic paraventricular nucleus. *J Comp Neurol.* 1991; 307:405–416. [PubMed: 1856330]
22. Bakowska JC, Morrell JI. Atlas of the neurons that express mRNA for the long form of the prolactin receptor in the forebrain of the female rat. *J Comp Neurol.* 1997; 386:161–177. [PubMed: 9295145]
23. Bakowska JC, Morrell JI. The distribution of mRNA for the short form of the prolactin receptor in the forebrain of the female rat. *Brain Res -Mol Brain Res.* 2003; 116:50–58. [PubMed: 12941460]
24. Chiu S, Wise PM. Prolactin receptor mRNA localization in the hypothalamus by in situ hybridization. *J Neuroendocrinol.* 1994; 6:191–199. [PubMed: 8049718]

25. Pi XJ, Grattan DR. Differential expression of the two forms of prolactin receptor mRNA within microdissected hypothalamic nuclei of the rat. *Brain Res -Mol Brain Res*. 1998; 59:1–12. [PubMed: 9729233]
26. Walsh RJ, Slaby FJ, Posner BI. A receptor-mediated mechanism for the transport of prolactin from blood to cerebrospinal fluid. *Endocrinology*. 1987; 120:1846–1850. [PubMed: 3569115]
27. Posner BI, van Houten M, Patel B, Walsh RJ. Characterization of lactogen binding sites in choroid plexus. *Exp Brain Res*. 1983; 49:300–306. [PubMed: 6832262]
28. Silverman WF, Walsh RJ, Posner BI. The ontogeny of specific prolactin binding sites in the rat choroid plexus. *Brain Res*. 1986; 389:11–19. [PubMed: 3004665]
29. Di Carlo R, Muccioli G, Papotti M, Bussolati G. Characterization of prolactin receptor in human brain and choroid plexus. *Brain Res*. 1992; 570:341–346. [PubMed: 1617425]
30. Swanson LW, Sawchenko PE. Paraventricular nucleus: a site for the integration of neuroendocrine and autonomic mechanisms. *Neuroendocrinology*. 1980; 31:410–417. [PubMed: 6109264]
31. Luther JA, Daftary SS, Boudaba C, Gould GC, Halmos KC, Tasker JG. Neurosecretory and non-neurosecretory parvocellular neurones of the hypothalamic paraventricular nucleus express distinct electrophysiological properties. *J Neuroendocrinol*. 2002; 14:929–932. [PubMed: 12472873]
32. Richardson CM, Wakerley JB. Supraoptic oxytocin and vasopressin neurones show differential sensitivity to the neurosteroid pregnenolone sulphate. *J Neuroendocrinol*. 1998; 10:829–837. [PubMed: 9831259]
33. Poulain, DA.; Brown, D.; Wakerley, JB. Statistical analysis of patterns of electrical activity in vasopressin and oxytocin-secreting neurones.. In: Leng, G., editor. *Pulsatility in neuroendocrine systems*. CRC; Boca Raton, FL: 1988. p. 119-154.
34. Shahrokh DK, Zhang TY, Diorio J, Gratton A, Meaney MJ. Oxytocin-dopamine interactions mediate variations in maternal behavior in the rat. *Endocrinology*. 2010; 151:2276–2286. [PubMed: 20228171]
35. Liposits Z. Ultrastructure of hypothalamic paraventricular neurons. *Crit Rev Neurobiol*. 1993; 7:89–162. [PubMed: 8102325]
36. Pi XJ, Grattan DR. Increased prolactin receptor immunoreactivity in the hypothalamus of lactating rats. *J Neuroendocrinol*. 1999; 11:693–705. [PubMed: 10447808]
37. Crumeyrolle-Arias M, Latouche J, Jammes H, Djiane J, Kelly PA, Reymond MJ, Haour F. Prolactin receptors in the rat hypothalamus: autoradiographic localization and characterization. *Neuroendocrinology*. 1993; 57:457–466. [PubMed: 8321418]
38. Clapp C, Torner L, Gutierrez Ospina G, Alcantara E, Lopez Gomez FJ, Nagano M, Kelly PA, Mejia S, Morales MA, Martinez de la Escalera G. The prolactin gene is expressed in the hypothalamic-neurohypophyseal system and the protein is processed into a 14-kDa fragment with activity like 16-kDa prolactin. *Proc Natl Acad Sci USA*. 1994; 91:10384–10388. [PubMed: 7937959]
39. Mejia S, Morales MA, Zetina ME, Martinez de la Escalera G, Clapp C. Immunoreactive prolactin forms colocalize with vasopressin in neurons of the hypothalamic paraventricular and supraoptic nuclei. *Neuroendocrinology*. 1997; 66:151–159. [PubMed: 9380272]
40. Ghosh R, Sladek CD. Prolactin modulates oxytocin mRNA during lactation by its action on the hypothalamo-neurohypophyseal axis. *Brain Res*. 1995; 672:24–28. [PubMed: 7749745]
41. Ghosh R, Sladek CD. Role of prolactin and gonadal steroids in regulation of oxytocin mRNA during lactation. *Am J Physiol - Endocrinol Metab*. 2005; 269:E76–E84.
42. Popeski N, Amir S, Diorio J, Woodside B. Prolactin and oxytocin in the paraventricular and supraoptic nuclei: effects on oxytocin mRNA and nitric oxide synthase. *J Neuroendocrinol*. 2003; 15:687–696. [PubMed: 12787053]
43. Torner L, Toschi N, Nava G, Clapp C, Neumann ID. Increased hypothalamic prolactin expression in lactation: involvement in behavioral and neuroendocrine stress responses. *Eur J Neurosci*. 2002; 15:1381–1389. [PubMed: 11994132]
44. Parker SL, Armstrong WE, Sladek CD, Grosvenor CE, Crowley WR. Prolactin stimulates the release of oxytocin in lactating rats: evidence for a physiological role via an action at the neural lobe. *Neuroendocrinology*. 1991; 53:503–510. [PubMed: 1883415]

45. Sarkar DK. Evidence for prolactin feedback actions on hypothalamic oxytocin, vasoactive intestinal peptide and dopamine secretion. *Neuroendocrinology*. 1989; 49:520–524. [PubMed: 2498758]
46. Townsend J, Cave BJ, Norman MR, Flynn A, Uney JB, Tortones DJ, Wakerley JB. Effects of prolactin on hypothalamic supraoptic neurones: evidence for modulation of STAT5 expression and electrical activity. *Neuroendocrinol Lett*. 2005; 26:125–130. [PubMed: 15855883]
47. Cazalis M, Dayanithi G, Nordmann JJ. The role of patterned burst and interburst interval on the excitation-coupling mechanism in the isolated rat neural lobe. *J Physiol*. 1985; 369:45–60. [PubMed: 4093889]
48. Sabatier N, Caquineau C, Dayanithi G, Bull PM, Douglas AJ, Guan XM, Jiang M, van der Ploeg I, Leng G. α -Melanocyte-stimulating hormone stimulates oxytocin release from the dendrites of hypothalamic neurons while inhibiting oxytocin release from their terminals in the neurohypophysis. *J Neurosci*. 2003; 23:10351–10358. [PubMed: 14614094]
49. Carter DA, Lightman SL. Oxytocin responses to stress in lactating and hyperprolactinaemic rats. *Neuroendocrinology*. 1987; 46:532–537. [PubMed: 3696384]
50. Ajika K, Arai K, Okinaga S. Electronmicroscopic localization of vasopressin and oxytocin in the median eminence of normal and hypophysectomized rats. *Neuroscience*. 1987; 22:S138.
51. Sarkar DK, Frautschy SA, Mitsugi N. Pituitary portal plasma levels of oxytocin during the estrous cycle, lactation, and hyperprolactinemia. *Ann NY Acad Sci*. 1992; 652:397–410. [PubMed: 1626839]
52. Brunton PJ, Russell JA. Attenuated hypothalamo-pituitary-adrenal axis responses to immune challenge during pregnancy: the neurosteroid opioid connection. *Journal of Physiology*. 2008; 586:369–375. [PubMed: 17991694]
53. Moss RL, Chan A, Dudley CA. Hyperprolactinemia: its electrophysiologic and pharmacologic effect on neurons of the ventromedial nucleus of the hypothalamus. *Brain Res*. 1985; 346:301–309. [PubMed: 3902150]
54. Bole-Feysot C, Goffin V, Edery M, Binart N, Kelly PA. Prolactin (PRL) and its receptor: Actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. *Endocr Rev*. 1998; 19:225–268. [PubMed: 9626554]
55. Prevarskaya NB, Skryma RN, Vacher P, Daniel N, Djiane J, Dufy B. Role of tyrosine phosphorylation in potassium channel activation. Functional association with prolactin receptor and JAK2 tyrosine kinase. *J Biol Chem*. 1995; 270:24292–24299. [PubMed: 7592639]
56. Faouzi M, Chopin V, Ahidouch A, Ouadid-Ahidouch H. Intermediate Ca^{2+} -sensitive K^{+} channels are necessary for prolactin-induced proliferation in breast cancer cells. *J Membr Biol*. 2010; 234:47–56. [PubMed: 20177667]
57. Selvaraj NG, Omi E, Gibori G, Rao MC. Janus kinase 2 (JAK2) regulates prolactin-mediated chloride transport in mouse mammary epithelial cells through tyrosine phosphorylation of Na^{+} - K^{+} - 2Cl^{-} cotransporter. *Mol Endocrinol*. 2000; 14:2054–2065. [PubMed: 11117534]
58. Deachapunya C, Poonyachoti S, Krishnamra N. Regulation of electrolyte transport across cultured endometrial epithelial cells by prolactin. *J Endocrinol*. 2008; 197:575–582. [PubMed: 18492821]
59. Ma FY, Grattan DR, Goffin V, Bunn SJ. Prolactin-regulated tyrosine hydroxylase activity and messenger ribonucleic acid expression in mediobasal hypothalamic cultures: The differential role of specific protein kinases. *Endocrinology*. 2005; 146:93–102. [PubMed: 15388649]
60. Rider L, Shatrova A, Feener EP, Webb L, Diakonova M. JAK2 tyrosine kinase phosphorylates PAK1 and regulates PAK1 activity and functions. *J Biol Chem*. 2007; 282:30985–30996. [PubMed: 17726028]
61. Prevarskaya N, Skryma R, Vacher P, Daniel N, Bignon C, Djiane J, Dufy B. Early effects of PRL on ion conductances in CHO cells expressing PRL receptor. *Am J Physiol - Cell Physiol*. 1994; 267:C554–C562.
62. Sorin B, Vacher AM, Djiane J, Vacher P. Role of protein kinases in the prolactin-induced intracellular calcium rise in Chinese hamster ovary cells expressing the prolactin receptor. *J Neuroendocrinol*. 2000; 12:910–918. [PubMed: 10971816]

63. Hindmarch C, Yao S, Beighton G, Paton J, Murphy D. A comprehensive description of the transcriptome of the hypothalamoneurohypophyseal system in euhydrated and dehydrated rats. *Proc Natl Acad Sci USA*. 2006; 103:1609–1614. [PubMed: 16432224]
64. Van Coppenolle F, Skryma R, Ouadid-Ahidouch H, Slomianny C, Roudbaraki M, Delcourt P, Dewailly E, Humez S, Crepin A, Gourdou I, Djiane J, Bonnal J-L, Mauroy B, Prevarskaya N. Prolactin stimulates cell proliferation through a long form of prolactin receptor and K⁺ channel activation. *Biochem J*. 2004; 377:569–578. [PubMed: 14565846]
65. Lyons DJ, Horjales-Araujo E, Broberger C. Synchronized network oscillations in rat tuberoinfundibular dopamine neurons: Switch to tonic discharge by Thyrotropin-releasing hormone. *Neuron*. 2010; 65:217–229. [PubMed: 20152128]
66. Ducret T, Vacher AM, Vacher P. Effects of prolactin on ionic membrane conductances in the human malignant astrocytoma cell line U87-MG. *Journal of Neurophysiology*. 2004; 91:1203–1216. [PubMed: 14586031]
67. Koksma J-J, Van Kesteren RE, Rosahl TW, Zwart R, Smit AB, Lüddens H, Brussaard AB. Oxytocin regulates neurosteroid modulation of GABAA receptors in supraoptic nucleus around parturition. *Journal of Neuroscience*. 2003; 23:788–797. [PubMed: 12574407]
68. Israel J-M, Poulain DA, Oliet SHR. Oxytocin-induced postinhibitory rebound firing facilitates bursting activity in oxytocin neurons. *J Neurosci*. 2008; 28:385–394. [PubMed: 18184781]

Figure 1.

Electrophysiological, morphological and immunohistochemical characterization of PVN neurons. A) Magnocellular (type I) neurons. Typical traces recorded from a type I neuron showing prominent action potential onset delay (I_A current, arrow). Inset displays the current protocol used. B) *Left panel:* Immunocytochemical staining for oxytocin-neurophysin (OT; arrow pointing to the location of the recorded cell). *Center panel:* Biocytin labeling of the recorded neuron (dye applied through the patch-pipette). *Right panel:* Merged images. Measuring bar = 20 μ m. C) Parvicellular (type II) neurons. Typical traces recorded from a type II neuron with no onset delay. D) OT and biocytin staining as shown in B.

Figure 2.

Representative I - V curves of prolactin-sensitive magnocellular and parvicellular PVN neurons before (circles), at the end of a 2 min pulse of prolactin 100 ng/ml (squares) and after 10 min of hormone withdrawal (triangles). Neurons responded to prolactin with a hyperpolarizing current that reversed at a potential more negative than the control current and persisted after 10 min of removal of the agonist. (A) 50% of putative magnocellular neurons were immunoreactive to oxytocin-neurophysin and were prolactin-responsive ($n=12$). *Inset*, voltage step protocol used for all I - V curves. (B) 24% of putative parvicellular neurons were prolactin-responsive ($n=6$). None of these neurons were immunoreactive to oxytocin-neurophysin. Non-parametric multiple comparison test for repeated measures

(Friedman's test) followed by Dunn's post-hoc analysis showed that the I - V relationship was significantly different after prolactin treatment in both neuronal cell types (see text for further details). Errors bars indicate SE. Note the different current amplitudes in magnocellular and parvicellular neurons.

Figure 3.

Average current difference. Difference currents were obtained by subtracting control current from currents measured at the end of a 2 min prolactin application (squares) and after 10 min of hormone removal (triangles). (A) Average difference current obtained from prolactin-responsive, oxytocinergic magnocellular cells. Data were calculated from I - V plots shown in Fig. 2A. The reversal potential of this difference current did not significantly change between these two conditions and was about -56 mV to -57 mV. (B) Average difference current obtained in the prolactin-responsive parvocellular cells shown in Fig. 2B. The reversal potential of this difference current after the 2 min prolactin treatment was -57 mV but shifted to -68 mV after 10 min of removal of the agonist. Error bars indicate standard error. Note the different current amplitudes in magnocellular and parvocellular neurons.

Figure 4. Response of a magnocellular oxytocinergic PVN neuron to prolactin (PRL). The neuronal activity decreases as soon as prolactin-containing artificial cerebrospinal fluid (100 ng/ml prolactin) reaches the cell. Horizontal bar indicates time of PRL application.