Prolactin, a natriuretic hormone, interacting with the renal dopamine system

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Background. Although prolactin affects sodium and water transport across the plasma membrane and interacts with dopamine in the brain, its role in the kidney is unclear. Here we examined the effect of prolactin and its possible interaction with the intrarenal natriuretic hormone dopamine, on proximal tubular Na^+, K^+-ATPase activity in vitro and renal function in anesthetized rats.

Methods. Na^+, K^+-ATPase activity was measured as ouabain-sensitive adenosine triphosphate (ATP) hydrolysis in microdissected proximal tubular segments. Renal function was studied during euvolemic conditions by conventional clearance techniques.

Results. Prolactin induced a dose-dependent inhibition of proximal tubular Na^+, K^+-ATPase activity. A maximal inhibitory effect of 48% of control was observed at an in vitro prolactin concentration of 1 μg/mL. This effect was completely abolished by a dopamine D1 receptor antagonist. In tubules preincubated with inhibitors of aromatic amino acid decarboxylase (AADC), the rate-limiting enzyme in renal dopamine formation, prolactin had no effect on Na^+, K^+-ATPase activity. In rats, prolactin infusion resulted in an increase in urinary sodium, potassium, and water excretion. These effects were also completely abolished by the D1 receptor antagonist. Prolactin had no significant effects on glomerular filtration rate (GFR) or mean arterial blood pressure.

Conclusion. We conclude that prolactin is a natriuretic hormone which interacts with the renal dopamine system for its effects. The natriuretic response is associated with inhibition of proximal tubular Na^+, K^+-ATPase activity.

Prolactin is a polypeptide involved in various actions in the body, including lactation, luteotrophic actions, reproductive and parental behavior, immune response, an-

giogenesis, and osmoregulation [1]. It has a crucial role in regulating sodium and water balance in fish which migrate from salt to fresh water [2]. Several studies have shown that prolactin regulates ion and fluid transport across the plasma membrane. An early observation was that prolactin decreased sodium and increased potassium transport across mammary epithelial cells [3, 4]. Prolactin was also shown to regulate fluid transport across amniotic and intestinal epithelial cell membranes [5, 6]. Furthermore, other studies have shown that prolactin regulates urinary sodium excretion, albeit with conflicting results [7–12]. Prolactin receptors have been found in many different tissues, including kidneys [13, 14].

It is well known that prolactin interacts with dopamine in several tissues; lactation is inhibited by dopamine agonists [15], dopamine serves as a prolactin inhibitory factor in hypothalamus [16], and suppression of endogenous prolactin with bromocriptin prevents the onset of maternal behavior [17].

Besides its well-known effects in the brain, dopamine is an intrarenal natriuretic hormone and acts by inhibiting the activity of tubular sodium transporters, including Na^+, K^+-ATPase. By its capacity to oppose the effects of antinatriuretic substances and act permissively for other natriuretic substances, intrarenal dopamine coordinates the effect of several sodium-regulating factors [18, 19].

One of the least understood actions of prolactin is regulation of sodium and water transport. The aim of the present study was therefore to examine whether prolactin can regulate urinary sodium and water excretion during euvolemic conditions and, furthermore, to elucidate by which mechanism such an effect occurs. Three topics were studied: the renal localization of prolactin receptors (PRLR) in the adult rat; the effects of prolactin on renal function, including the effect on proximal convoluted tubule (PCT) Na^+, K^+-ATPase activity; and whether these renal effects were dependent on the renal dopamine system.

METHODS

Animals

The in vitro study was performed on male Sprague-Dawley rats (B & K Universal, Sollentuna, Sweden) aged 40 to 50 days and weighing between 200 and 250 g. The in vivo study was performed on 19 male Sprague-Dawley rats (Møllegaard Breeding Center, Copenhagen, Denmark) weighing 316 ± 9 g. All animals had free access to tap water and were fed ad libitum with standard rat chow containing 0.25% sodium. The experiments were approved by the local ethics committee for animal experimentation at Uppsala University and the Karolinska Institute.

Preparation of PCT segments

Kidney perfusion and tubule microdissection were performed as described [20]. The rats were anesthetized with an intraperitoneal injection of Mebumal veterinary (5 to 6 mg per 100 g body weight) (Nord Vacc, Stockholm, Sweden). Following a midline incision, the left kidney was exposed and perfused with a cold, modified Hank's solution containing 0.05% collagenase (Sigma Chemical Co., St. Louis, MO, USA) and 0.1% bovine serum albumin (BSA) (Behringwerke, Marburg, Germany). The pH was adjusted to 7.4. The kidney was removed and cut along its corticopapillary axis into small pyramids that were incubated for 20 minutes at $35°C$ in the perfusion solution containing 10^{-3} mol/L butyrate to optimize mitochondrial respiration. The solution was continuously bubbled with oxygen. After incubation, the tissue was rinsed with fresh microdissection solution, which had the same composition as the perfusion solution except that the $CaCl_2$ concentration was 0.25 mmol/L and collagenase and BSA were omitted.

The single PCT segments were manually dissected (tubular segment length 0.4 to 1.1 mm) from the outer cortex under a stereomicroscope at $4°C$. The tubule segments were individually transferred to the concavity of a bacteriologic slide in a drop of the microdissection solution and photographed for length determination using an inverted microscope at $100\times$ magnification. Tubules were stored on ice until dissection was completed, for a maximum of 30 to 60 minutes.

Preincubation with inhibitors of aromatic amino acid decarboxylase (AADC)

Renal pyramids were incubated in the microdissection solution with AADC inhibitors, carbidopa 5×10^{-4} mol/L or benserazide 10^{-5} mol/L, for 30 minutes before and during the dissection procedure. These drug doses were selected on the basis of previous studies [21, 22].

Preincubation of tubules with different drugs

Tubule segments were incubated at indicated times at room temperature, either in 1 µL of microdissection solution alone (control tubules) or in 1 µL of microdissection solution containing one or more of the agents mentioned below (experimental tubules). Prolactin was used in concentrations between 1 ng/mL and 1 µg/mL. Prolactin at these concentrations dose-dependently regulates the activity of Na^+, K^+-2 Cl^- cotransporter in epithelial cells [23]. Tubule segments were preincubated with dopamine receptor antagonists for 10 minutes before prolactin or vehicle was added. Doses of dopamine antagonists were chosen on the basis of previous studies [24, 25].

Determination of Na^+, K^+-ATPase activity

The preincubation period was stopped by cooling the tubular segments to $4°C$. The segments were made permeable by repeated freezing and thawing. This procedure allows adenosine triphosphate (ATP) and sodium free access to the interior of the cell. The tubules were incubated at $37°C$ for 15 minutes in a medium containing (in mmol/L): 70 NaCl, 5 KCl, 10 $MgCl_2$, 1 ethyleneglycol tetraacetate (EGTA), 100 Tris-HCl, 10 Tris-ATP, and γ-[^{32}P]-ATP (NEN, Boston, MA, USA), 2 to 5 Ci/mmol in trace amounts (5 nCi/mL). For determination of ouabain-insensitive ATPase activity, 2 mmol/L ouabain (USB, Cleveland, OH, USA) was added, NaCl and KCl were omitted and Tris-HCl was 150 mmol/L. The ^{32}P-phosphate liberated by hydrolysis of ATP was separated by filtration through a Millipore filter (Sartorius AG, Goettingen, Germany) after absorption of the unhydrolyzed nucleotide on activated charcoal, and radioactivity was counted in a liquid scintillation spectrometer.

In each study, total ATPase activity and ouabain-insensitive ATPase activity were measured on each of five to eight other tubule segments. Na^+, K^+-ATPase activity (pmol of ^{32}Pi hydrolyzed per mm of tubule length per hour) was calculated as the difference between the mean value for total ATPase and ouabain-insensitive ATPase activity, and expressed as an absolute value. Control and experimental tubules were always run in parallel.

Detection of prolactin receptors in renal tissue

The prolactin receptor (PRLR) was identified by a commercial available monoclonal mouse antibody, which recognizes both the long and short isoforms of PRLR, (Abcam Ltd., Cambridge Science Park, Cambridge, UK).

Briefly, renal cortex or microdissected PCT segments were homogenized in a buffer containing 65 mmol/L Tris base, 154 mmol/L NaCl, 6 mmol/L sodium deoxycholate, 1 mmol/L ethylenediamine tetraacetic acid (EDTA), adjusted to pH 7.4, followed by 10% NP-40, 1 mmol/L dithiothreitol (DTT), 5 mmol/L

phenylmethylsulfonyl fluoride (PMSF), 8 mmol/L Na$_2$SO$_4$, and complete protease inhibitor (Roche Diagnostics Scandinavia AB, Stockholm, Sweden).

A total protein of 10 μg per well was subjected to 8% sodium dodecyl sulfate-polyacryalmide gel electrophoresis (SDS-PAGE). Proteins were transferred to a polyvinylidine difluoride (PVDF) transfer membrane, Hybond-P (Amersham Biosciences, Uppsala, Sweden), and probed with the primary antibody against PRLR, followed by horseradish peroxidase (HRP)–conjugated sheep anti-mouse secondary antibody (Sigma-Aldrich, St Louis, MO, USA). Immunoreactivity was detected using enhanced chemiluminescence (ECL) plus Western blotting detection system (Amersham Biosciences). Molecular size determinations were made using Prestained SDS-PAGE standards (Bio-Rad Laboratories, Sundbyberg, Sweden).

Renal function

To study the effect of prolactin on renal function the hormone was administered intravenously into anesthetized rats. The animals were anesthetized with an intraperitoneal injection of Inactin® [5-ethyl-5-(1-methyl-propyl)-2-thio-barbiturate sodium], 120 mg/kg body weight (Sigma-Aldrich). The rats were then placed on a servo-controlled heating pad to maintain the rectal temperature at 37.5°C. Tracheostomy was performed to ensure free airways during the experiment. Both jugular veins were catheterized for infusion and the left femoral artery for continuous measurements of mean arterial blood pressure and for blood sampling. Blood pressure was continuously recorded via a transducer with a MacLab Instrument (AD Instruments, Hastings, UK) connected to a Macintosh Power PC 6100 throughout the experiment. The urinary bladder was catheterized through a suprapubic incision for urine collection.

The renal function studies were performed during euvolemic conditions. After completion of the surgical procedures, the animals were stabilized for 45 minutes and during that time given infusions of a Ringer solution (Fresenius, Kabi, Norway). To determine the glomerular filtration rate (GFR) a Ringer solution containing [^{3}H]-methoxy-inulin (4 μCi bolus followed by 2 μCi/hour/100 g body weight), was given and the total infusion rate in each animal was 5 mL/hour/kg body weight. Seventy-five microliters of arterial blood were withdrawn at the midpoint of each experimental period for estimation of the GFR and hematocrit.

Three different protocols were used. Group 1, prolactin (*N* = 7), after two consecutive 20-minute control sampling periods (C1 and C2), the animals received an intravenous bolus injection of 0.3 IU prolactin (luteotropic hormone, prolactin, from sheep pituitary glands 32 IU/mg) (Sigma-Aldrich) followed by contin-uous infusion of 3 IU/hour/kg body weight during four consecutive 20-minute experimental sampling periods (E1 to E4). Similar doses of prolactin have been reported to increase urinary sodium excretion in Long-Evans rats [9]. In group 2, control (N = 6), infusions and sampling of control rats were identical to that of prolactin-treated animals except that prolactin was omitted. In group 3, SCH 23390 (D1 receptor antagonist) (*N* = 6), was used to test the possible interaction of the effects of prolactin and the dopamine D1 receptor. This group of rats was subjected to the same prolactin treatment as group 1, but the rats were also treated intravenously with the D1-like receptor antagonist SCH23390 (Schering, Kenilworth, NJ, USA). SCH23390 was added to the infusion solution so that the animals received the antagonist throughout the experiment, including stabilization and control periods. A bolus injection 30 μg/kg body weight was given directly after surgery and then 30 μg/hour/kg body weight as a continuous infusion. This dose has previously been shown to completely inhibit the effect of dopamine on urinary sodium excretion [26, 27].

Urine analysis

The urine volumes were measured gravimetrically. The urinary sodium and potassium concentrations were determined by flame photometry (FLM3) (Radiometer, Copenhagen, Denmark). Urinary osmolality was estimated from the depression of the freezing point. The amount of [^{3}H]-methoxy-inulin in samples of plasma and urine was detected using a liquid scintillation counter (PW 4700; Philips, Amsterdam, The Netherlands). GFR was estimated from the clearance (C$_i$) of [^{3}H]-methoxy-inulin according to the equation:

$$C_i = (U_i \times V)/P_i$$

where U$_i$ and P$_i$ are the urinary and plasma concentrations of inulin, respectively, and V is the urine flow rate.

Statistical analysis

The results are presented as means ± standard errors of the mean (SEM). Comparisons within groups have been performed with Student paired *t* test and comparisons between different groups have been performed with Student unpaired *t* test. A *P* value less than 0.05 was considered statistically significant.

RESULTS

Several isoforms of prolactin receptors (PRLR) arise from alternative initiation sites of transcription and gene splicing. In rodents, one long and three short isoforms have been described [13, 28]. The antibody used recognized two bands at ∼70 kD and 40 kD, respectively, in renal cortex, and microdissected PCTs. The antibody
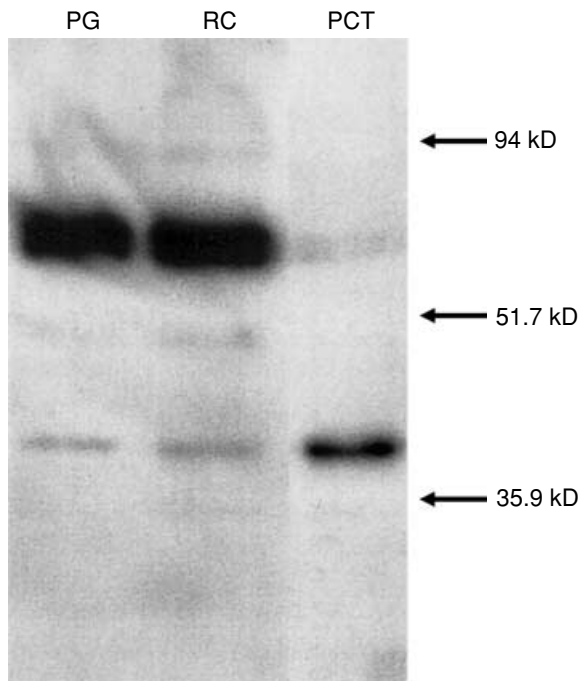
Fig. 1. Immunoreactivity of renal localization of prolactin receptors (PRLR) in renal tissue. A representative blot of PRLR in pituitary gland (PG), renal cortex (RC), and microdissected renal proximal convoluted tubular (PTC) segments.

Fig. 2. Effect of prolactin on renal proximal convoluted tubular (PCT) Na^+, K^+-ATPase activity. (*A*) Time dependence. Prolactin was used in a concentration of 1 μg/mL. Values are mean ± SEM and are expressed as pmol P_i/mm tubule/hour (N = 3 to 5 per group). *$P < 0.05$ vs. control; **$P < 0.05$ vs. control and < 0.05 vs. prolactin 15 minutes. Statistical analysis was performed with unpaired Student *t* test. (*B*) Dose dependence. Proximal tubules were incubated with prolactin for 15 minutes. Values are mean ± SEM and are expressed as pmol P_i/mm tubule/hour (N = 3 to 5 per group). *$P < 0.05$ vs. control. Statistical analysis was performed with unpaired Student *t* test. ns is not significant vs. control.

recognized bands of the same size in the positive control, the pituitary gland (Fig. 1). These bands correspond to the predicted size of the PRLRs, long and medium short isoforms. The medium short isoform was the major isoform in proximal tubular segments.

To examine the direct effect of prolactin on renal PCT Na^+, K^+-ATPase activity, drugs were added to microdissected tubules at specified times. Prolactin, 1 μg/mL induced a time-dependent inhibition of PCT Na^+,K^+-ATPase activity (Fig. 2), with a maximal inhibitory effect at 15 minutes' incubation (Fig. 2A). This time point was therefore used in further studies. Prolactin decreased Na^+, K^+-ATPase activity both when the enzyme was assayed with saturating Na^+ concentrations under V_{max} conditions (Na^+ 70 mmol/L) (control, 2598 ± 135; prolactin, 1 μg/mL, 1206 ± 95 pmol P_i/mm/hour) ($P < 0.05$) and when a lower nonsaturating Na^+ concentration of 20 mmol/L was used (control, 1674 ± 116; prolactin, 1 μg/mL, 1421 ± 142 pmol P_i/mm/hour) ($P < 0.05$). The inhibitory effect of prolactin during V_{max} conditions was dose-dependent with a maximal effect of 48% of control at 1 μg prolactin/mL and a threshold effect between 1 and 10 ng/mL (Fig. 2B). The basal plasma concentration of prolactin in Long-Evans rat has been estimated to be 4.95 ± 1.05 ng/mL [9]. Thus, the concentrations used in the in vitro experiments are within the physiologic range. Prolactin had no effect on ouabain insensitive ATPase
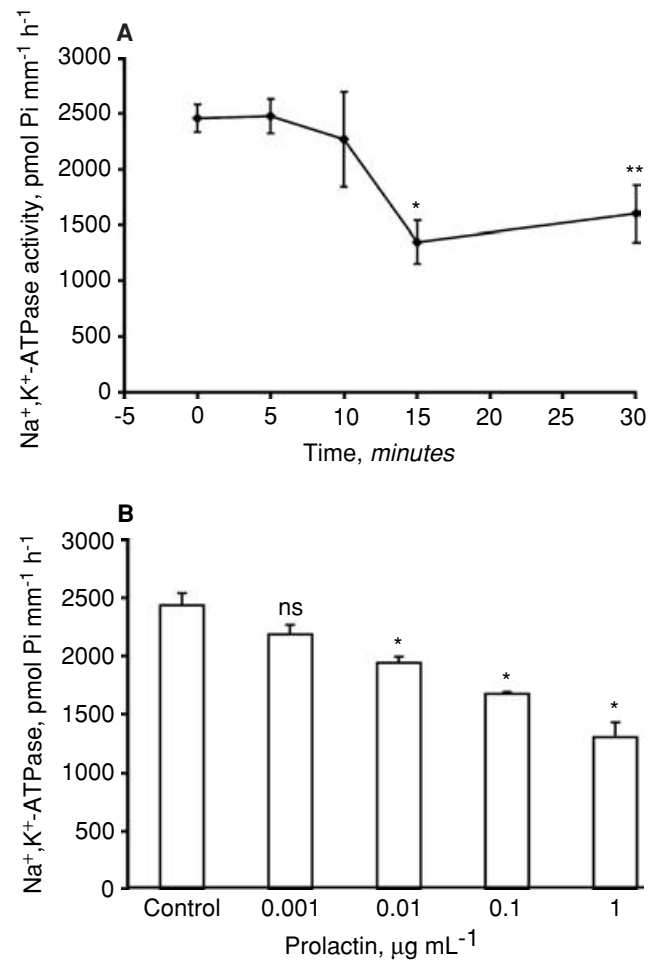
hydrolysis (control, 1397 ± 173; prolactin, 1 μg/mL, 1530 ± 178 pmol P_i/mm/hour).

Prolactin interacts with dopamine in the brain [1, 15, 16]. Here we examined whether prolactin also interacts with dopamine in the kidney. In tubules pretreated with an antagonist of the dopamine 2 (D2) receptor (raclopride, 10^{-6} mol/L), prolactin still inhibited Na^+, K^+-ATPase activity. In contrast, when tubules were preincubated with a dopamine D1 receptor antagonist (SCH23390, 10^{-6} mol/L) the inhibitory effect of prolactin on Na^+, K^+-ATPase activity was completely abolished, suggesting an interaction between prolactin and renal D1 receptors (Fig. 3A).
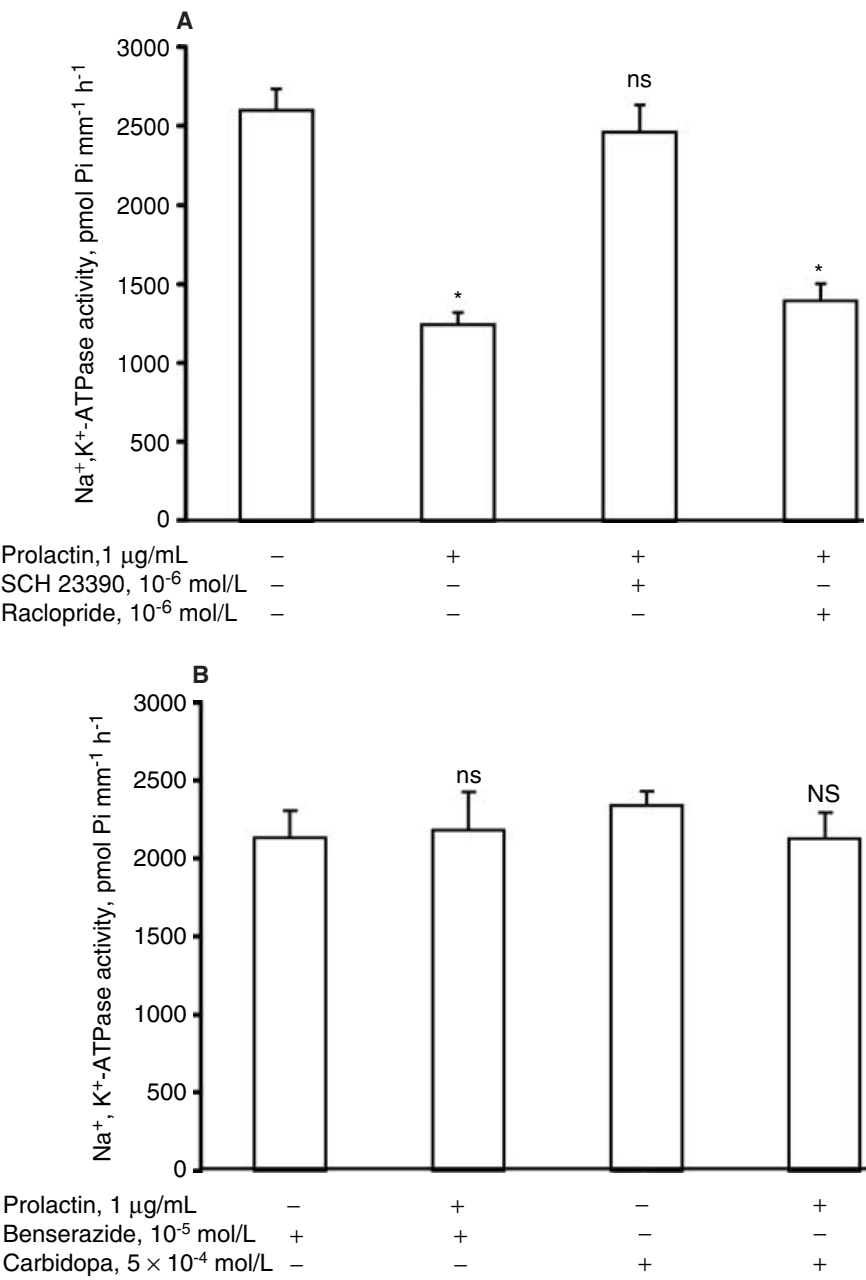
Fig. 3. Interaction between prolactin and intrarenal dopamine. Proximal tubules were incubated with prolactin, 1 μg/mL, for 15 minutes. Values are mean ± SEM and are expressed as pmol P_i/mm tubule/hour. Statistical analysis was performed with paired and unpaired Student t test. (*A*) Dopamine antagonists. Proximal tubules preincubated with SCH23390, 10^{-6} mol/L, or raclopride, 10^{-6} mol/L ($N = 4$ to 6 per group). *$P < 0.05$ vs. control. ns is not significant vs. control. (*B*) Aromatic amino acid decarboxylase (AADC) inhibitors. Proximal tubules preincubated with benserazide 10^{-5} mol/L or carbidopa 5×10^{-4} mol/L ($N = 8$ in the benserazide group and three in the carbidopa group). ns is not significant vs. benserazide alone; NS is not significant vs. carbidopa alone.

Neither SCH23390 nor raclopride alone had any effect on Na^+, K^+-ATPase activity (data not shown).

To further examine the role of intrarenal dopamine, renal slices were preincubated with two different inhibitors of AADC, the rate limiting enzyme in renal dopamine production. As shown in Figure 3B, prolactin did not decrease Na^+, K^+-ATPase activity during inhibition of intrarenal dopamine production.

Next we examined the effect of prolactin on renal function. Rats were treated intravenously with luteotropic hormone isolated from sheep pituitary glands. The achieved plasma prolactin concentration during the present experiments can be estimated at 400 ng/mL from the data of Stier et al [12] where the rats received the same doses of prolactin and the plasma levels were measured. This approximates the elevated plasma prolactin levels found during estrus and lactation [29]. Assuming that the native hormone, rat prolactin, is as potent as ovine prolactin, the levels achieved in the current experiments may be consistent with physiologic levels under such conditions. The same levels of prolactin can also be seen during antipsychotic treatment. During chlorpromazine therapy, serum prolactin concentration increases to about 400 ng/mL [30]. These data imply that the
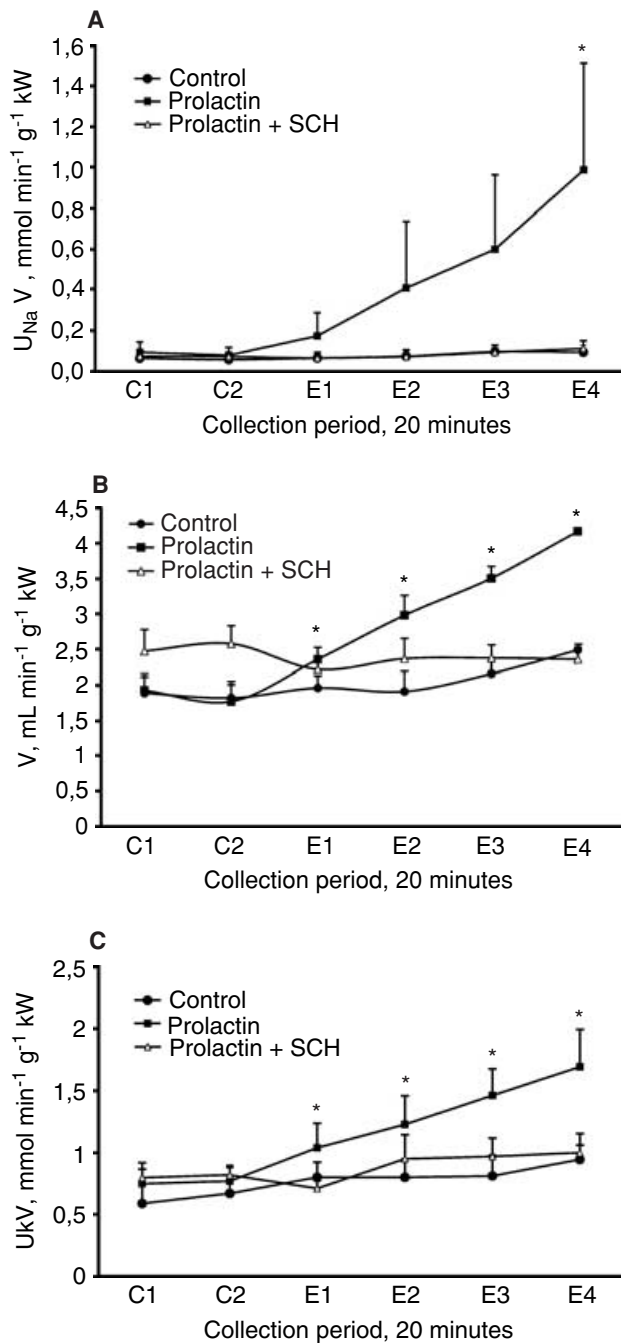
Fig. 4. Effects of prolactin on renal function. C1 and C2 correspond to collection periods 1 and 2 before and E1 to E4 correspond to collection periods 1 to 4 periods after treatment with vehicle, prolactin, and prolactin and SCH23390 ($N = 6$ to 7 per group). *$P < 0.05$ vs. control. Statistical analysis was performed with paired and unpaired Student t test. (*A*) Urinary sodium excretion. Values are mean ± SEM and are expressed as μmol/min/g kidney weight. (*B*) Urinary flow. Values are means ± SEM and are expressed as μL/min/g kidney weight. (*C*) Urinary potassium excretion. Values are mean ± SEM and are expressed as μmol/min/g kidney weight.

results in the current study may be relevant in physiologic conditions in females and during antipsychotic treatment in both men and women.

Prolactin induced an increase in urinary sodium excretion in every rat. The response was variable with a 9.1 ± 3.8-fold (E4/C1) increase in urinary sodium excretion. This effect was associated with an approximate twofold increase in urinary flow rate and urinary potassium excretion. All these effects were also abolished by the D1 receptor antagonist (Fig. 4).

Prolactin had no significant effect on urinary osmolality, GFR, mean arterial blood pressure, or hematocrit (Table 1). The constant hematocrit indicates the presence of euvolemic conditions throughout the experiment. The D1 receptor antagonist–treated rats showed a small increase in urine osmolality.

DISCUSSION

Here we present a novel function of prolactin as a potent natriuretic polypeptide, which acts by inhibiting PCT Na^+, K^+-ATPase activity. The intrarenal dopamine system acts permissively for this effect. The natriuretic potency of prolactin is in the order, or just above that for atrial natriuretic peptide [31].

Prolactin is a hormone involved in a variety of important functions in the body, including stimulation of milk production, regulation of reproductive functions, protein synthesis, osmoregulation, as well as the regulation of ion transport across the plasma membrane [1]. Prolactin has been shown to regulate fluid transport across cells in several different tissues such as mammary epithelial cells, intestinal epithelial cells, and amniotic cells, but relatively little information is available on its effects on renal ion transport and renal function. Although not examined in a systematic manner, several studies have shown that prolactin either induces an antidiuretic and antinatriuretic response [9–12] or a diuretic and natriuretic response [7–9]. These discrepancies can be explained by contamination with vasopressin in the pituitary extract, different doses, differences in rat strains, or differences in the state of hydration prior to injection. Here we studied the renal effects of prolactin during euvolemic conditions and found a pronounced natriuretic and diuretic response which was associated with a dose-dependent inhibition of proximal tubule Na^+, K^+-ATPase activity. Na^+, K^+-ATPase inhibition did not only occur during V_{max} conditions for Na^+, K^+-ATPase, but also when the enzyme was assayed with Na^+ 20 mmol/L, which is the approximate normal intracellular Na^+ concentration in renal proximal tubular cells. Prolactin treatment had no significant effects on GFR or blood pressure.

There are numerous examples of prolactin interaction with dopamine both in central nervous system and in peripheral tissues [1]. Dopamine, produced in renal

Table 1. Renal function

Collecting period	Prolactin			Prolactin and SCH23390		
	C1	E4		C1	E4	
Glomerular filtration rate *mL/min/g kidney weight*	1.42 ± 0.17	1.68 ± 0.2	ns	1.58 ± 0.11	1.46 ± 0.18	ns
Mean arterial blood pressure *mm Hg*	118 ± 3	114 ± 3	ns	116 ± 3	111 ± 3	ns
Urine osmolality *mosm/kg/H_2O*	1915 ± 65	1936 ± 69	ns	1564 ± 120	1930 ± 136	a
Hematocrit %	45 ± 0.6	45 ± 0.7	ns	45 ± 0.5	45 ± 0.6	ns

Values are mean $\pm$ SEM. ns, not significant vs. control or vs. prolactin and SCH23390.
[a] $P < 0.05$ vs. control.

proximal tubular cells, is a natriuretic hormone and acts via an inhibition of renal tubular sodium transporters [26, 27, 32, 33]. The renal effects of dopamine are mainly mediated via the D1 receptor. Intrarenal dopamine has an important role in the interactive regulation of salt balance. Many other natriuretic factors, including atrial natriuretic peptide, and nitric oxide exert their effect via the renal dopamine system [24, 34, 35]. We have shown that D1 receptor antagonists abolish the renal effects of atrial natriuretic peptide and isoproterenol and that rats pretreated with inhibitors of AADC have a blunted response to atrial natriuretic peptide [20, 24]. These effects were explained by the finding that both atrial natriuretic peptide and isoproterenol regulated the recruitment of D1 receptors from the interior of the cell to the plasma membrane where they are physiologically active [20, 24]. Herein we found that the renal effects of prolactin, including natriuresis, diuresis, kaliuresis, and Na^+, K^+-ATPase inhibition, were abolished by a D1 receptor antagonist. Furthermore, when tubules were preincubated with different inhibitors of AADC, prolactin no longer inhibited Na^+, K^+-ATPase activity.

PRLR have been identified in numerous organs, including the central nervous system, pituitary gland, heart, lung, thymus, spleen, liver, pancreas, adrenal gland, uterus, skeletal muscle, skin, and kidney [13]. Various PRLR isoforms have been described in different tissues and species. We found the long and a medium short isoform of PRLR in rat renal cortex and PCT segments. This localization is in accordance with previous studies by Mountjoy et al [14], who showed specific binding sites for prolactin primarily in the proximal tubules.

The PRLR is expressed on proximal tubular cells. Here prolactin act by inhibiting PCT Na^+, K^+-ATPase activity, the enzyme that mediates active tubular sodium transport. This effect is rapid and pronounced, and contributes to a natriuretic response. The renal dopamine system plays a permissive role in the renal effects of prolactin. During inhibition of intrarenal dopamine production as well as in the presence of a D1 receptor antagonist the renal response to prolactin is blunted. Whether prolactin and dopamine interaction involves an altered production/release of dopamine, a cross-talk among signaling pathways, heterologous recruitment of

D1 receptors and/or synergism between D1 receptors and PRLR requires further evaluation.

CONCLUSION

Prolactin is a natriuretic hormone, which interacts with the renal dopamine system for its full effect. The natriuretic response of prolactin involves inhibition of proximal tubular Na^+, K^+-ATPase activity.

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