Effects of Prolactin on Ionic Membrane Conductances in the Human Malignant Astrocytoma Cell Line U87-MG

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Ducret, Thomas, Anne-Marie Vacher, and Pierre Vacher. Effects of prolactin on ionic membrane conductances in the human malignant astrocytoma cell line U87-MG. J Neurophysiol 91: 1203–1216, 2004. First published October 29, 2003; 10.1152/jn.00710.2003. Prolactin (PRL) is involved in numerous biological processes in peripheral tissues and the brain. Although numerous studies have been conducted to elucidate the signal transduction pathways associated with the PRL receptor, very few have examined the role of ion conductances in PRL actions. We used the patch-clamp technique in “whole cell” conﬁguration and microspectroﬂuorimetry to investigate the effects of PRL on membrane ion conductances in the U87-MG human malignant astrocytoma cell line, which naturally expresses the PRL receptor. We found that a physiological concentration (4 nM) of PRL exerted a biphasic action on membrane conductances. First, PRL activated a Ca2+-dependent K+ current that was sensitive to CTX and TEA. This current depended on PRL-induced Ca2+ mobilization, through a JAK2-dependent pathway from a thapsigargin- and 2-APB–sensitive Ca2+ pool. Second, PRL also activated an inwardly directed current, mainly due to the stimulation of calcium inﬂux via nickel- and 2-APB–sensitive calcium channels. Both phases resulted in membrane hyperpolarizations, mainly through the activation of Ca2+-dependent K+ channels. As shown by combined experiments (electrophysiology and microspectroﬂuorimetry), the PRL-induced Ca2+ influx increased with cell membrane hyperpolarization and conversely decreased with cell membrane depolarization. Thus PRL-induced membrane hyperpolarizations facilitated Ca2+ influx through voltage-independent Ca2+ channels. Finally, PRL also activated a DIDS-sensitive Cl− current, which may participate in the PRL-induced hyperpolarization. These PRL-induced conductance activations are probably related to the PRL proliferative effect we have already described in U87-MG cells.

INTRODUCTION

Initially discovered in 1928 by Stricker and Grueter (1928) for its stimulating action on rabbit mammary glands, the peptide hormone prolactin (PRL) exerts pleiotropic biological effects in a wide variety of cells and tissues (Bole-Feysson et al. 1998; Freeman et al. 2000).

In particular, PRL acts on the CNS to promote physiological and behavioral changes. Indeed, numerous studies report that the brain itself produces PRL and PRL-related peptides (Griffond et al. 1994; Paut-Pagano et al. 1993) and expresses prolactin receptors (PRL-R) (DeVito et al. 2000; Raso et al. 1999). It is now well established that several behaviors are dependent on or highly inﬂuenced by PRL: reproduction (Dudley et al. 1982), maternal behavior (Bridges et al. 1990; Grattan 2002), food intake (Gerardo-Gettens et al. 1989; Noel and Woodside 1993), etc. PRL also increases the turnover, synthesis, and release of dopamine in the median eminence (Hokfelt and Fuxe 1972). Moreover, PRL was shown to be involved in regulating cultured rat astrocyte mitogenesis (DeVito et al. 1992), cytokine expression (DeVito et al. 1995a), and the brain’s neuroimmune response to injury or trauma (DeVito et al. 1995b).

Besides these physiological effects, several studies have documented the involvement of PRL in the growth control of tumors such as breast (Manni et al. 1985), liver (Buckley et al. 1985), prostate (Johnson et al. 1985), and human meningioma cells in vitro (Boyle-Walsh et al. 1995; Jimenez-Hakim et al. 1993).

All these actions are mediated by the interaction of PRL with its speciﬁc receptor, a member of the cytokine-growth factor receptor superfamily, which includes receptors for growth hormone, erythropoietin, numerous hematopoietic interleukins, granulocyte colony stimulating factor, granulocyte macrophage colony stimulating factor, leukemia inhibitory factor, oncostatin M, thrombopoietin, leptin, etc. (Bazan 1990; Kelly et al. 1991; Taga and Kishimoto 1992). However, the molecular events between the binding of the hormone to its receptor and the expression of the biological response are not clearly understood. The PRL receptor intracellular activation pathways appear to be particularly complex, including activation of the cytoplasmic tyrosine kinase JAK2 and STAT signaling pathways (Ali 1998; Ferrag et al. 1996).

Preliminary studies of early effects of PRL on membrane ion conductances were carried out in our laboratory using a CHO cell line (CHO TSE32) stably transfected with the cDNA of the long form of rabbit mammary PRL-R (Edery et al. 1989). Briefly, we demonstrated that physiological concentrations of PRL increased the cytosolic free calcium concentration ([Ca2+]i) by Ca2+ entry and/or mobilization of Ca2+ stored in intracellular compartments (Vacher et al. 1994). At first, Ca2+ and voltage-activated potassium channels were activated by JAK2-dependent stimulation and a [Ca2+]i increase, resulting from the mobilization of Ca2+ stored in intracellular compartments (Prevarskaya et al. 1994, 1995). Then, the resulting hyperpolarization facilitated Ca2+ entry through voltage-insensitive second messenger–operated nonspeciﬁc channels (Prevarskaya et al. 1994), which required the COOH-terminal region of PRL-R (Sorin et al. 1998) and PRL-induced produc-
tion of a cytosolic messenger, suggested to be 1,3,4,5-tetakisphosphate (IP$_5$) (Ratovondraha et al. 1998a,b).

However, there are ample examples in the literature illustrating that overexpression of exogenous proteins can lead to the activation and/or up-regulation of endogenous channels, in particular in *Xenopus* oocytes and mammalian cell lines such as HEK-293, COS, or CHO (Shimbo et al. 1995; Tzounopoulos et al. 1995). For this reason, we decided to extend our previous investigation to a cellular model endowed with an endogenous PRL-R.

In this article, we study the effects of PRL on ionic membrane conductances in a cell model naturally expressing PRL-R. We used the U87-MG cultured human astrocytoma cell line, known to express PRL-R (Ducret et al. 2002), proving that U87-MG cells are fully capable of responding to PRL. PRL initially activated a Ca$_{2+}$ current via a 2-APB-sensitive voltage-dependent channel. Finally, PRL also activated a DIDS-sensitive Cl$^-$ current.

**Methods**

**Cell culture**

Human malignant astrocytoma (U87-MG) cells were obtained from the European Collection of Cell Cultures. They were seeded on round glass coverslips (30 mm diam) for microspectrofluorimetry experiments and petri dishes for electrophysiological experiments. They were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco BRL) containing 10% (vol/vol) fetal calf serum (Seromed, Biochrome KG) and supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, and 1% (vol/vol) nonessential amino acids (NEAAs; Gibco BRL). They were maintained at 37°C in a humidified atmosphere gassed with 95% O$_2$ - 5% CO$_2$. Experiments were conducted 3–7 days after replating. Fifty percent of the medium was changed everyday.

**Electrophysiological recordings**

The whole cell recording mode of the patch-clamp technique was used (Hamill et al. 1981). The electrodes were pulled on a PIP5 (HEKA) puller in two stages from borosilicate glass capillaries (Harvard: 1.5 mm diam) to a tip diameter of 1.5–2.0 μm. The pipettes had a mean resistance of 2–4 MΩ when measured in standard recording conditions (i.e., using the standard extracellular solution and the K$^+$ gluconate intrapipette solution).

The cultures were viewed under phase contrast with a Nikon Diaphot inverted microscope (Nikon France). Electrodes were positioned using micromanipulators (Narishige). Grounding was achieved through a silver chloride-coated silver wire inserted into an agar bridge (4% agar in electrode solution). An RK-400 patch amplifier (Biologic) was used for whole cell recordings. Stimulus control, data acquisition, and processing were carried out on a PC computer fitted with a Labmaster TL-1 interface, using pCLAMP 5.5.1 software (Axon Instruments, interface and software). Electrode offset was balanced before forming a “giga seal.” Leakage and capacitive current subtraction protocols were composed of four or five one-fourth or one-fifth hyperpolarizing pulses, respectively, applied from a holding potential before test pulses to elicit active responses. Leak data were subtracted from the raw data during analysis. Series resistances were compensated and calculated before and after compensation. Recordings where series resistance resulted in a 5 mV or greater error in voltage commands were discarded. Currents were low-pass filtered at 2 kHz with an eight-pole Bessel filter (–3 dB) and digitized at 10 kHz for storage and analysis.

**Microspectrofluorimetric assay of cytosolic calcium**

The fluorescent probe indo-1 was used, as already described (Vacher et al. 1994). The cells plated on glass coverslips were incubated with 5 μM indo penta-acetoxymethyl ester (indo-1/AM, Sigma) and 0.02% Pluronic F127 (Molecular Probes, Eugene, OR) in Hank's balanced salt solution at 37 ± 1°C for 30 min and washed and maintained at room temperature in the same saline solution before the fluorescence measurements.

For single cell measurements, the dual emission microspectrofluorimeter was constructed from a Nikon Diaphot inverted microscope (Nikon France) fitted with epifluorescence (×100 oil immersion fluorescence objective; numerical aperture, 1.3).

For excitation of indo-1, a collimated light beam from a 100-W mercury arc lamp (Nikon) was filtered at 355 nm and reflected from a dichroic mirror (380 nm). The emitted fluorescence signal was passed through a pinhole diaphragm slightly larger than the selected cell and directed onto another dichroic mirror (455 nm). Transmitted light was filtered at 480 nm, reflected light was filtered at 405 nm, and the intensities were recorded by separate photometers (P1, Nikon). Single photon currents were converted to voltage signals, divided online by a monolithic laser-trimmed two-quadrant detector (ADS535, Analog Devices, Norwood, MA). Under these experimental conditions, the $R = F405/F480$ ratio was recorded on-line as a voltage signal and expressed as [Ca$_{2+}$], using the formula derived by Grynkiewicz et al. (1985). Ca$^{2+}$ calibrations were obtained under simultaneous whole cell clamp and microspectrofluorimetric measurements. The patch pipettes were filled with internal solution containing 10 mM EGTA (solution A), 10 mM CaCl$_2$ (solution B), or 9.2 mM EGTA, and 5.4 mM CaCl$_2$ (solution C). Solutions A and B were used to estimate minimum and maximum values, $R_{\text{min}}$ (minimal ratio) and $R_{\text{max}}$ (maximal ratio), respectively. Solution C was used to evaluate the product of the apparent dissociation constant ($K_d$) and the ratio of fluorescence of free indo-1 divided by the fluorescence of Ca$^{2+}$-bound indo-1 with 355 nm excitation and 480 nm emission ($\beta$). The latter solution had a free Ca$^{2+}$ of 300 nM, calculated using the stability constants and computer program of Fabiato and Fabiato (1979). $R_{\text{min}}$, $R_{\text{max}}$, and $K_d \beta$ averaged 0.039 ± 0.01 (n = 15), 0.65 ± 0.08 (n = 17), and 581 ± 22 nM (n = 15), respectively.

**Simultaneous electrophysiological and microspectrofluorimetric recordings**

Membrane electrophysiological properties and intracellular ionic concentration were studied as described above. These two parameters were recorded simultaneously.
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Recording solutions

For whole cell voltage-clamp studies, microspectrofluorimetry, and combined studies, the standard extracellular solution contained (in mM) 140 NaCl, 5 KCl, 2 CaCl₂, 2 MgCl₂, 0.3 Na₂HPO₄, 0.4 KH₂PO₄, 4 NaHCO₃, 5 glucose, and 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES). The osmolality of the external salt solution was adjusted to 300-2-ethanesulfonic acid (HEPES). The osmolality of the external salt solution was adjusted to 300 mOsm/kg. Solutions nominally free of Ca²⁺ were prepared by omitting CaCl₂.

For whole cell studies, the recording pipette was filled with an artificial intracellular saline containing (in mM) 150 potassium gluconate, 2 MgCl₂, 1.1 EGTA, and 5 HEPES (pH 7.3 ± 0.01 with KOH; osmolality: 290 mOsm/kg). In some experiments, the patch pipette K⁺ gluconate (KGluc) was replaced with isomolar N-methylglucamine (NMG) to eliminate K⁺ currents.

For combined patch-clamp and microspectrofluorimetry studies, free indo-1 (30 μM) was added to the artificial intracellular saline solution in the recording pipette to avoid intracellular probe dialysis by the intrapipette recording solution.

Drug application

An additional “pouring” pipette with a tip opening of 3-5 μm was used for local drug application to the investigated cell. This pipette was filled with the same extracellular saline as the bath and the drug or PRL was added in appropriate concentrations. The pipette was positioned 50-100 μm from the investigated cell. All experiments were performed at room temperature (20-22°C).

Reagents

Human PRL was kindly provided by the NIDDK (Dr. A. F. Parlow, Pituitary Hormones and Antiserum Center, Harbor-UCLA Medical Center, Torrance, CA).

CTX, DIDS, NiCl₂, nifedipine, TEA, and TTX were purchased from Sigma (St. Louis, MO); 2-APB, AG490, and SKF-96365 were purchased from Calbiochem (Dermstadt, Germany).

Data and statistical analysis

Peak currents in whole cell recordings were measured using the automatic peak detection function in the clampam section of P-clamp software. Late currents were measured isochronally before the end of the pulse. Results are expressed as means ± SE. Each experiment was repeated several times. Student’s t-test was used for statistical comparison among means and differences, with P < 0.05 were considered significant.

RESULTS

Effects of PRL on global basal ionic conductance and membrane potential

In the standard recording medium, the membrane potential of U87-MG cells after establishment of a gigaseal and first entry into the whole cell mode was −14 ± 2 mV (n = 79). The passive membrane properties of the cells were measured from responses to −30 mV pulses applied from the holding potential (−20, −40 mV, . . .). The average cell input resistance measured with these constant current hyperpolarizing pulses was 509 ± 98 MΩ (n = 79).

Initially, whole cell recordings under standard conditions (i.e., using the standard extracellular solution and the K⁺ gluconate intrapipette solution) showed that a physiological concentration of PRL (4 nM) activated steady-state currents and increased basal ionic conductances in 26.5% of the cells investigated (61 of 230 cells). This percentage of responding cells was identical with the percentage Ca²⁺ response (24.4%) in the same cell model (Ducret et al. 2002). In fact, when the cells were clamped close to resting potential (−20 or −40 mV), PRL was able to activate two distinct steady-state currents: an outward “first phase” current and an inward “second phase” current, both associated with an increase in conductance showing that they corresponded to a current activation.

However, not all the cells responded in the same manner, and we obtained four types of PRL responses. In the majority (51%) of the responsive cells (n = 31), PRL only induced the outward current (Fig. 1Aa). In 28% of cells (n = 17), PRL only induced the inward current (Fig. 1Ba), whereas 18% of cells (n = 11) presented both (Fig. 1Ca). Surprisingly, few cells (3%) presented a merging current, i.e., a beginning of the outward current immediately followed by the inward current (Fig. 1Da). These two types of steady-state currents differed in their characteristics and kinetics. The steady-state outward current, which ranged from +15 to +750 pA with a mean of +113 ± 20 pA (n = 42), activated 51 ± 11 s following the beginning of PRL application, and lasted 113 ± 16 s. The second phase, which ranged from −15 to −540 pA with a mean of −137 ± 24 pA (n = 28), appeared more slowly, activating 249 ± 36 s after the beginning of PRL application. It was also longer in duration, returning to its basal value in 367 ± 48 s after activation. Both of these currents were associated with an increase in membrane conductance (approximately +133%), as shown by the variation in current pulses resulting from constant hyperpolarizing pulses in Fig. 1, Aa, Ba, Ca, and Da.

Taken together, these data show that PRL exerts a biphasic activation on steady-state currents. It elicited a steady-state outward current (first phase) and/or a steady-state inward current (second phase).

We then investigated the putative effects of PRL on membrane potentials. As expected, PRL induced membrane hyperpolarizations in the current-clamp configuration (Fig. 1, Ab, Bb, and Cb). Two types of PRL-induced hyperpolarization were obtained, with kinetics similar to those of the steady-state currents described above: a first type, from −4 to −25 mV, with a mean of −10 ± 2 mV (n = 14), and a second type, from −8 to −26 mV, with a mean of −13 ± 2 mV (n = 9). The first hyperpolarization (first phase) appeared within 60 s (11 ± 6 s) after the beginning of PRL application and returned rapidly to basal levels (83 ± 12 s). The second hyperpolarization (second phase) was delayed (317 ± 70 s after the beginning of PRL application). This second phase also presented longer duration (175 ± 39 s) than the first phase (83 ± 12 s). These two types of hyperpolarization were accompanied by a decrease in membrane resistance (approximately 24%), corresponding to the increase in conductance observed in voltage-clamp experiments. Thus PRL also exerted a biphasic action on membrane potential.

Three types of PRL-induced steady-state currents were obtained under current-clamp conditions. The majority of the responding cells (9 of 18 cells) presented only rapid hyperpolarization after PRL stimulation (Fig. 1Ab), whereas only a few presented delayed hyperpolarization (4 of 18 cells; Fig. 1Bb) or both (5 of 18 cells; Fig. 1Cb). The merging-type response
could not be identified in this configuration as the sum of two hyperpolarizations would be one hyperpolarization.

Characterization of the first phase

To determine the ionic origin of the first phase, we used the whole cell recording mode of the patch-clamp technique under the experimental conditions described above. The amplitude of the PRL-induced outward current response depended on the holding membrane potential. The current response was positive at holding potentials above −80 mV and became inward at more negative holding potentials. The current-voltage (I-V) plot of these data (data not shown) crosses the voltage axis at approximately −58 mV (n = 4), i.e., close to the estimated equilibrium potential for K⁺ (E_K). Furthermore, using Ohm’s law as applied to membranes (I = G(V_h − E_rev), the driving force (V_h − E_rev) underlying the long-lasting current (I) response to the peptide was estimated throughout its time course by applying brief 30-mV hyperpolarizing commands at 0.2 Hz from a V_h of −40 mV. Changes in the current responses to the latter were used to derive G, which in turn, made it possible to calculate repeated estimates of the potential reverse (E_rev). This method gave E_rev = −54 mV (n = 8), also suggesting that K⁺ were involved in this response. We therefore used pharmacological inhibitors of K⁺ channels to confirm the role of this ion and characterize the type of K⁺ channel activated by PRL.

As shown in Fig. 2A, external application of 10 mM TEACl (tetraethyl-ammonium chloride), known to inhibit voltage-dependent K⁺ channels, totally blocked the PRL-induced outward current [n = 4; a statistically significant (P < 0.05) inhibitory effect of TEA was observed]. In addition, when cells were bathed in an external medium containing 10 mM TEA, in a control (n = 38), the first phase was never recorded after PRL stimulation. In the same way, it was never observed when the patch pipette solution contained 150 mM N-methyl-D-glucamine gluconate (NMG; n = 73) instead of 150 mM K⁺ gluconate to block the K⁺ channel inner membrane by steric hindrance. However, under all these conditions, PRL still elicited delayed inward currents in 29 of the 111 cells investigated.

This steady-state K⁺ outward current was further characterized by the use of charybdotoxin (CTX), a Ca²⁺-dependent K⁺ channel inhibitor in U87-MG cells (Ducret et al. 2003). Application of 50 nM CTX inhibited (P < 0.05) these currents (n = 5), identifying the steady-state outward current as a Ca²⁺-dependent K⁺ current (Fig. 2B).

To confirm the Ca²⁺ dependence of this current, we made simultaneous recordings of ionic currents and cytosolic Ca²⁺ in single U87-MG cells (n = 9) As shown in Fig. 2C, PRL-induced intracellular Ca²⁺ mobilization preceded K⁺ outward current activation, which was triggered at a given Ca²⁺ level (threshold, ~930 nM). In the same way, the current returned to its basal value when [Ca²⁺], approached the threshold (approximately 180 nM).
Taken together, all these data suggest that the first phase, but not the second one, is mainly, if not exclusively, due to a K\(^+\) efflux through the TEA and CTX-sensitive Ca\(^{2+}\)-dependent K\(^+\) channels already described in these cells (Ducret et al. 2003).

What is the origin of the Ca\(^{2+}\) involved in activating the outward current? The first phase of the PRL-induced calcium response in U87-MG cells was Ca\(^{2+}\) mobilization from intracellular compartments (Ducret et al. 2002), so we verified whether this PRL-induced Ca\(^{2+}\)-dependent K\(^+\) current activation was correlated with Ca\(^{2+}\) mobilization by bathing the cells in Ca\(^{2+}\)-free medium. Under these conditions (Fig. 2D, n = 5), combined recordings showed that PRL application still induced an outward current associated with an increase in [Ca\(^{2+}\)]. We concluded from these findings that the intracellular Ca\(^{2+}\) mobilization is responsible for the Ca\(^{2+}\)-dependent K\(^+\) current activation in the first phase (outward current and membrane hyperpolarization).

Knowing that PRL acted on a thapsigargin-sensitive Ca\(^{2+}\) pool (endoplasmic reticulum) in U87 cells (Ducret et al. 2002), we treated the cells with 2-aminoethyl diphenyl borate (2-APB; 44 \(\mu\)M, 20 min), an inositol 1,4,5-trisphosphate (IP\(_3\))-receptor inhibitor also reported to be a Ca\(^{2+}\) release-activated Ca\(^{2+}\) current inhibitor (Kukkonen et al. 2001; Prakriya and Lewis 2001), to verify the importance of this pool in the PRL electrophysiological response. Under these conditions, no response was recorded after PRL stimulation (Fig. 2E, n = 15), confirming that the PRL-induced outward current depended on an IP\(_3\)-sensitive Ca\(^{2+}\) pool mobilization.

Preliminary investigations of the CHO cell line in our laboratory demonstrated that the Ca\(^{2+}\)-dependent K\(^+\) channels implicated in the PRL-induced outward current were regulated by the JAK2 protein tyrosine kinase (Prevarskaya et al. 1994, 1995; Sorin et al. 2000). Moreover, using a cocktail of non-specific tyrosine kinase inhibitors (genistein, herbimycin A, and lavendustin A), tyrosine kinases have already been shown to participate in the PRL-induced calcium response in the U87-MG cell line (Ducret et al. 2002). AG490, a specific JAK2 inhibitor (Meydan et al. 1996), was used to investigate the implication of this tyrosine kinase in the outward current activation. After a 6-h treatment with AG490 (50 \(\mu\)M for 6 h). AG490 (50 \(\mu\)M for 6 h)}
phase activation. To verify whether JAK2 activated the K⁺/Ca²⁺-dependent channel directly via its tyrosine phosphorylation and/or indirectly by activating IP₃-receptor mediated Ca²⁺ release, we investigated the effect of PRL on [Ca²⁺]ᵢ, after a similar treatment with AG490. As shown in Fig. 2G (n = 15), under these conditions, PRL failed to induce an increase in [Ca²⁺]ᵢ, suggesting the involvement of JAK2 in Ca²⁺ mobilization and an indirect activation of the Ca²⁺-dependent K⁺ current (as debated in DISCUSSION).

All these results clearly indicate that the first event following PRL application is a Ca²⁺ mobilization from an IP₃-sensitive pool. This Ca²⁺ mobilization activates an outward steady-state current, identified as a Ca²⁺-dependent K⁺ current, which is indirectly regulated by JAK2 phosphorylation.

Characterization of the second phase

Earlier microspectrofluorometry experiments (Ducret et al. 2002) established that the second phase of the PRL Ca²⁺ response in U87-MG cells resulted from a delayed stimulation of Ca²⁺ influx. Because our first phase was associated with intracellular Ca²⁺ mobilization, we investigated whether the electrophysiological second phase was related to Ca²⁺ influx.

The reversal potential, $E_{rev}$, and the ionic basis of the PRL-induced inward current (second phase) were studied under voltage-clamp conditions, as described above for the first phase. When the patch pipette was filled with K gluconate internal solution and the cell clamped at −20 mV, $E_{rev}$ estimations of the second phase suggest that several ions were implicated (Fig. 3Ab). Indeed, $E_{rev}$ did not remain constant during the entire current response. $E_{rev}$ of the first ion involved in the PRL response was approximately +30 mV, indicative of a Ca²⁺ channel. The activation of a second channel with a more hyperpolarized $E_{rev}$ transiently resulted in a response with more negative $E_{rev}$ (approximately 0 mV). This phenomenon was amplified when the same cell was held at a more depolarized potential (+20 mV, Fig. 3Ac), with an $E_{rev}$ below −20 mV at the peak of the response. Note that, at this potential, the PRL-induced second phase became an outward current. However, when the patch pipette was filled with the NMG gluconate internal solution to block K⁺ channels (Fig. 3B), the $E_{rev}$ remained nearly constant (+30 to +20 mV) during the response (similar to the $E_{rev}$ for Ca²⁺).

The I-V relationships of the peak response (second phase) were linear over the range −80 to +20 mV and crossed the voltage axis near 0 mV (Fig. 3C). Thus an estimation of the PRL response $E_{rev}$ using this method gave a mean value of approximately 0 mV, corresponding to the activation of at least two ions.

Taken together, all these data suggest that, during the second phase, PRL activates two conductances, probably a Ca²⁺ conductance and a K⁺ conductance.

Further characterization of the inward current was carried out using simultaneous recording of ionic currents and cytosolic Ca²⁺ concentration in single U87-MG cells (n = 10).

Under control conditions, as shown in Fig. 4A, we showed that PRL application induced an increase in cytosolic Ca²⁺ (199 ± 45 nM) and a simultaneous inward current (−197 ± 69 pA) associated with the activation of one or more conductances. Note that, in this cell, inward current and Ca²⁺ influx occurred without any first phase, i.e., no intracellular Ca²⁺ mobilization. As shown in Fig. 4B, application of a Ca²⁺-free solution during the inward current abolished it and decreased the global membrane conductance (results statistically significant, $P < 0.05$), whereas the inward current reappeared immediately after stopping application, confirming that the inward current was due to Ca²⁺ entry. Moreover, when the cells were bathed in a Ca²⁺-free medium, no PRL-induced inward current was observed (n = 5).

While the correlation between the increase in [Ca²⁺]ᵢ and this inward current has been clearly established, the channels implicated in the second phase remained to be determined. We thus used various pharmacological reagents to define the type of Ca²⁺ channel responsible for Ca²⁺ entry. As shown in Fig. 4C, application of 5 mM NiCl₂, a nonselective inorganic Ca²⁺ channel blocker, totally blocked this inward current (n = 5; $P < 0.05$), confirming that it corresponded to Ca²⁺ channel activation. As U87-MG cells express L-type Ca²⁺ channels (Ducret et al. 2003), we searched for a possible involvement of these channels in the PRL-induced second phase. However, nifedipine (1–10 μM, n = 5, data not shown), a specific inhibitor of these channels, was ineffective ($P > 0.05$), thus excluding this possibility. Another hypothesis was that store-operated channels (SOCs) were responsible for the capacitative Ca²⁺ influx. Interestingly, as shown in Fig. 4D, application of 44 μM 2-APB, an IP₃-receptor antagonist also reported to be a Ca²⁺ release-activated Ca²⁺ current inhibitor (Kukkonen et al. 2001; Prakriya and Lewis 2001), totally blocked (P < 0.05) the PRL-induced delayed inward current and strongly decreased membrane conductance (n = 6), suggesting that PRL-induced Ca²⁺ entry may be via SOCs. However, the fact that the second phase may occur even in the absence of the first phase, i.e., without any intracellular Ca²⁺ mobilization (see Figs. 1Ba and 4, A–C), and vice versa (Figs. 1Aa and 2, A and C), excluded the sole involvement of store-operated calcium channels in the second phase. Moreover, 20 μM SKF-96365 (1-[β-[3-(4-methoxyphenyl)propoxy]-4-methoxyphenethyl]-1H-imidazole), also reported to be a store-operated calcium channel inhibitor (Mason et al. 1993), did not affect the second phase (n = 4, data not shown; $P > 0.05$), confirming the noninvolvement of SOCs.

The relationship between [Ca²⁺]ᵢ changes and calcium influx was studied to investigate a possible contribution of membrane hyperpolarization (resulting from K⁺ efflux, as described above) in PRL-induced Ca²⁺ entry, as already demonstrated in CHO cells (Prevarskaya et al. 1994).

As shown in Fig. 5A, using simultaneous recording of ionic currents and [Ca²⁺]ᵢ, in single U87-MG cells (n = 4), membrane potential was stepped to −40, 0, −80, and 20 mV after PRL stimulation. After a PRL-induced first phase (outward current activation and intracellular Ca²⁺ mobilization), electrical membrane hyperpolarizations led to subsequent Ca²⁺ increases, to a maximal [Ca²⁺]ᵢ value, whereas depolarization to 0 mV decreased [Ca²⁺]ᵢ, to basal level and greater depolarization (to 20 mV) led to a new, lower basal level. When the holding potential was shifted to the same values in unstimulated cells (no PRL application), no current or [Ca²⁺]ᵢ variations were recorded (Fig. 5B).

These results clearly demonstrate the importance of membrane hyperpolarization in PRL-induced Ca²⁺ entry, probably through channels with voltage-independent activation but which are voltage-sensitive due to the Nernst potential equi-
The pharmacological sensitivity of the hyperpolarization-driven Ca\(^{2+}\)/H\(^{+}\) influx was studied using 2-APB (44 \(\mu\)M, n = 4). 2-APB was ejected immediately after the first phase (to avoid inhibiting intracellular Ca\(^{2+}\) mobilization) and completely blocked Ca\(^{2+}\) entry (P < 0.05).

As in CHO cells (Prevarskaya et al. 1995; Rakovondrahona et al. 1998a), the hyperpolarization-driven Ca\(^{2+}\) influx and associated conductance responses to 4 nM PRL faded during the course of recordings, indicating that the whole cell recording technique readily depletes the soluble intracellular factors necessary to generate PRL-induced Ca\(^{2+}\) influx. However, this phenomenon was considerably slower in U87-MG than CHO cells, probably due to their larger size (>100 vs. ~10 \(\mu\)m), which makes them less sensitive to intracellular dialysis by the internal solution in the patch pipette.

**Effects of PRL on voltage-dependent currents**

Although U87-MG cells are electrically nonexcitable, preliminary studies in our laboratory have shown that they express several voltage-dependent channels (Ducret et al. 2003), particularly TEA-sensitive Ca\(^{2+}\)-dependent K\(^{+}\), 4-aminopyridine (4-AP)-sensitive transient K\(^{+}\), inwardly rectifying K\(^{+}\), 4,4'-diisothiocyanostilbene-2,2'-disulphonic acid (DIDS)-sensitive Cl\(^{-}\), TTX-sensitive Na\(^{+}\), and nifedipine-sensitive L-type Ca\(^{2+}\) currents. Moreover, as preliminary experiments in our labora-
tory had shown that PRL affected voltage-dependent channels in CHO cells (Prevarskaya et al. 1994), we verified whether similar effects occurred in U87-MG cells.

**PRL effects on voltage-dependent Ca\(^{2+}\)-sensitive K\(^+\) currents**

To investigate the physiological action of PRL on K\(^+\) conductance, U87-MG cells were voltage-clamped at \(-40\) mV in the presence of TTX (1 \(\mu\)M) and DIDS (200 \(\mu\)M) to block the Na\(^+\) and Cl\(^-\) currents, respectively, and to isolate the K\(^+\) current.

Under these conditions, sustained K\(^+\) currents, identified as Ca\(^{2+}\)-sensitive K\(^+\) currents (Ducret et al. 2003), were obtained by stepping up the membrane potential from the holding potential of \(-40\) to \(+60\) mV test potential in 10-mV increments for 400 ms (Fig. 6Aa). As shown in Fig. 6Ab, PRL application (4 nM for 1 min) induced a nearly fivefold increase in the amplitude of these currents, which were abolished by 10 mM TEA (Fig. 6Ac) or 50 nM charybdotoxin (data not shown). This effect was observed in the five cells investigated, where the mean amplitude of \(83 \pm 39\) pA for a voltage step from \(-40\) to \(+60\) mV attained \(199 \pm 46\) pA after PRL stimulation.

**FIG. 4.** Characterization of the PRL-induced inward current ("2nd phase"). Recordings obtained under voltage-clamp conditions. Cells were clamped at \(-40\) mV. As shown in A (top trace), hyperpolarizing steps were periodically injected to monitor membrane conductance. PRL (4 nM) and reagents were applied continuously during the period indicated by a continuous line. A: simultaneous recording of ionic currents (middle trace) and \([\text{Ca}^{2+}]_i\) (bottom trace). PRL-induced increase in \([\text{Ca}^{2+}]_i\) was associated with activation of an inward current and an increase in membrane conductance. B: application of Ca\(^{2+}\)-free HBSS during the PRL-induced inward current abolished it and decreased overall membrane conductance, whereas the inward current reappeared immediately after stopping application. C: application of 5 mM NiCl\(_2\), a nonselective inorganic Ca\(^{2+}\) channel blocker, totally blocked this inward current. D: application of 44 \(\mu\)M 2-APB, used as an inhibitor of capacitative Ca\(^{2+}\) influx, totally inhibited the PRL-induced "2nd phase."

**FIG. 5.** Effects of membrane hyperpolarizations on PRL-induced Ca\(^{2+}\) influx. A: combined recordings of membrane potential (top trace), ionic currents (middle trace), and \([\text{Ca}^{2+}]_i\) (bottom trace). \([\text{Ca}^{2+}]_i\) was measured in individual U87-MG cells loaded with indo-1. After PRL stimulation, electrical membrane hyperpolarizations (to \(-40\) and \(-80\) mV) led to subsequent \([\text{Ca}^{2+}]_i\) increases, whereas depolarization to 0 mV decreased \([\text{Ca}^{2+}]_i\) to basal level and greater depolarization (to 20 mV) resulted in a new, lower basal level. B: same recordings in unstimulated cells. When the holding potential was shifted to the same values in unstimulated cells (no PRL application), no current or \([\text{Ca}^{2+}]_i\) variations were recorded.
were abolished by 200 mM DIDS, which blocks the basal activity of Cl− channels, and is activated by intracellular Ca2+.

\( \text{Ca}^{2+} \text{}/\text{H} \) and TEA (10 mM, respectively). External medium contained TTX (1 \( \mu \text{M} \)) and DIDS (200 \( \mu \text{M} \)) to isolate K+ currents. b: recording of the same cell after application of 4 nM PRL. c: recording of the same cell after application of 10 nM TEA. B: current-voltage (I-V) relationships for the currents shown above (control, ▲) and after PRL (4 nM, ■) and TEA (10 mM, ◆) application. Traces are representative of 5 experiments. C: time course of voltage-dependent K+ channel activity. K+ currents were evoked by 400-ms pulses from a holding potential of −40 to +20 mV test potential, applied every 15 s.

\( P < 0.05 \), i.e., an increase of 137%. Figure 6B presents characteristic I-V relationships before and after PRL and TEA application. Figure 6C is a representative time course of the response. Note that the current amplitude decreased steadily over time (run down due to the loss of intracellular components during whole cell recording). Application of 4 nM PRL resulted in a rapid, transient activation of the K+ current followed by a slower, maintained activation, both completely blocked by TEACl. These two characteristic phases of the PRL response confirm that this TEA-sensitive channel plays a pivotal role in controlling the basal conductance in U87-MG cells and is activated by intracellular Ca2+ increases (intracellular Ca2+ mobilization and Ca2+ influx).

**PRL effects on voltage-dependent Cl− currents**

In cells held at −40 mV, it was also possible to trigger an outward current that activated slower than the K+ current (Fig. 7). To quantify the effects of PRL on this current, experimental maneuvers were used to minimize other voltage-dependent currents: Na+ and K+ channels were blocked with TTX (1 \( \mu \text{M} \)) and TEA (10 mM), respectively.

As shown in Fig. 7Ab, application of PRL (4 nM for 1 min) induced a nearly 2.5-fold increase in these currents, which were abolished by 200 \( \mu \text{M} \) DIDS (Fig. 7Ac).

This activator effect was reported in the five cells investigated, where the average amplitude of 117 ± 33 pA for a voltage step from −40 to +60 mV attained 328 ± 115 pA after PRL stimulation (\( P < 0.05 \)), i.e., an increase of 180%. Figure 7B presents representative characteristic I-V relationships before and after PRL and DIDS application. Note that the response in the presence of DIDS decreased below control levels. This can be explained by the high sensitivity of Cl− channels to DIDS, which blocks the basal activity of Cl− current completely. PRL-induced activation of the voltage-dependent Cl− channels was significant for membrane depolarization, which clamped membrane potential above 0 mV. The time course of the effect of PRL on Cl− channels (Fig. 7C) shows clearly that, as in K+ channels, PRL-induced Cl− conductance activation strictly followed the biphasic Ca2+ increase, suggesting once again the calcium dependence of the electrophysiological effects of PRL. Note that the Cl− channels seem to be less sensitive to the run down, and hence the loss of intracellular components during patch-clamp induced dialysis, than K+ channels.

**PRL effects on other voltage-dependent currents expressed in this cell line**

Although we have previously shown that U87-MG cells also possess transient outward K+, inward-rectifying K+, Na+, and L-type voltage-dependent Ca2+ currents, as well as a Na+/K+ ATPase pump (Ducret et al. 2003), we did not observe any activation of these currents or the pump by PRL application in the cells (n = 4, 5, 4, 11, and 5, respectively, data not shown).

**DISCUSSION**

Despite numerous studies of PRL-R, to our knowledge, very few laboratories have studied the cascade of ionic events induced by PRL and the nature of the ion channels involved in target cells with endogenous PRL-R. However, agreement has now been reached on several steps in the transduction pathway, including receptor dimerization (Rui et al. 1994b), JAK kinase activation (Rui et al. 1994a), and tyrosine phosphorylation of several proteins (Tourkine et al. 1995). According to the literature, a rapid increase in [Ca2+]i appeared to be the ubiquitous early step in the PRL-R transduction signal, as it had been.
observed in numerous cell models: NB2 lymphoma (Ali et al. 1991), hepatocytes of lactating rats, INS-1 insulinoma (Sekine et al. 1996), and U87-MG cells (Ducret et al. 2002). In this article, the use of “whole cell” patch-clamp configuration and microfluorimetry techniques allows us to characterize the ionic events following PRL stimulation in the U87-MG human malignant astrocytoma cell line, which naturally expresses a functional PRL-R (Ducret et al. 2002). A schematic view of the proposed pathway for PRL effects in U87-MG cells is shown in Fig. 8.

We showed that the first event following PRL application was the development of an outward steady-state current. Its sensitivity to TEA (10 mM) and NMG identified this outward current as a K+ efflux. Moreover, coupling electrophysiology with microfluorimetry, we characterized this current as a CTX-sensitive Ca2+-dependent K+ current, with activation dependent on Ca2+ mobilization from IP3-sensitive intracellular stores. These results agree with those of previous studies in our laboratory using PRL-R transfected CHO cells. Briefly, we demonstrated that physiological concentrations of PRL increased [Ca2+]i, by Ca2+ entry and/or mobilization of Ca2+ stored in intracellular compartments (Vacher et al. 1994). Then, Ca2+-dependent K+ channels were activated by an increase in [Ca2+]i, resulting from this mobilization and JAK2-dependent stimulation (Prevarskaya et al. 1994, 1995).

Using AG490 (a JAK2 inhibitor), we found that the channels implicated in this first phase in U87-MG cells were also regulated by JAK2 tyrosine phosphorylation, but only in an indirect way via an increase in [Ca2+]i. Indeed, the basal activity (without PRL stimulation) of the Ca2+-dependent K+ current was not sensitive to AG490 treatment (data not shown). While the implication of JAK2 in PRL response had already been described in CHO cells transfected with PRL-R (Prevarskaya et al. 1995), this result also confirmed previous findings on the implication of tyrosine kinases in the PRL-induced calcium response demonstrated in U87-MG cells using a cocktail of nonspecific tyrosine kinase inhibitors (Ducret et al. 2002). Moreover, a recent study has shown that JAK2 regulates PRL-mediated Cl− transport in HC11 cells through tyrosine phosphorylation of Na+−K+−2Cl− cotransporters (Selvaraj et al. 2000), confirming the importance of this tyrosine kinase in PRL-induced ion transport.

It is important to note that this K+ efflux was expressed as a hyperpolarization of the plasma membrane, as discussed below.

In the second stage, PRL also activated an inward steady-state current corresponding mainly to Ca2+ entry via as-yet unidentified channels. Indeed, this current did not operate via L-type Ca2+ channels, because it was insensitive to nifedipine. This was in agreement with our preliminary results in CHO transfected cells where the increase in [Ca2+]i, was unrelated to these voltage-dependent Ca2+ currents, which were, on the contrary, reduced by PRL (Prevarskaya et al. 1995).

If the increase in [Ca2+]i did not result from the activation of Ca2+ entry via a voltage-dependent Ca2+ current, another possibility was the implication of SOC, which allow Ca2+ influx after the depletion of intracellular Ca2+ stores. This Ca2+ current, called “capacitative influx” by Putney (1986, 1990), refills intracellular stores and also extends the calcium signal with a long-lasting [Ca2+]i plateau. The electrophysiological properties of this current, known as I_{CRAC} or I_{SOC} (for calcium release activated current or store operated current), were described by Hoth and Penner (1992). However, to date, the channels responsible for I_{CRAC} have not been identified, even if trp channels appear to be the most likely candidates (Gailly and Colson-Van Schoor 2001). Their activation mech-
anisms have also been much debated. There are two main theories (for review see Elliott 2001). The first suggests a conformational coupling between endoplasmic reticulum IP3 receptors and SOC (Berridge 1995b; Irvine 1990). The second involves the intervention of a diffusible second messenger between the endoplasmic reticulum and the plasma membrane (Randriamampita and Tsien 1993). However, the nature of this diffusible messenger, or calcium influx factor (CIF), has yet to be determined.

In an attempt to determine the implication of SOC in the second PRL-induced electrophysiological phase in U87-MG cells, we used 2-APB, an IP3-receptor inhibitor also reported to be a Ca2+ release-activated Ca2+ current inhibitor (Kukkonen et al. 2001; Prakriya and Lewis 2001), and SKF-96365. 2-APB totally inhibited this delayed inward current, suggesting that PRL-induced Ca2+ entry could be via SOCs. However, the fact that this second phase occurred even when the first phase (associated with Ca2+ mobilization) was absent, and vice versa, indicated that store-operated Ca2+ channels could not be solely responsible for the second phase. A similar observation, i.e., the implication of trp channels in calcium influx without depletion of calcium stores, was recently reported. Indeed, Chu et al. (2002) found that, in CHO cells transfected with an erythropoietin receptor, which belongs to the same superfamily...
as PRL-R, erythropoietin-modulated Ca\(^{2+}\) influx through trp channels, probably by a direct receptor-operated activation mechanism. The implication of trp channels in the second PRL-induced phase in U87-MG is currently under investigation in our laboratory using an anti-sense strategy, following the identification of trp channel expression in U87-MG cells (as used by Gailly and Colson-Van Schoor 2001). The fact that SKF-96365, also reported to be a store-operated Ca\(^{2+}\) channel inhibitor (Mason et al. 1993), failed to block the PRL-induced inward current also confirmed the nonexclusive intervention of store-operated calcium channels in the PRL-induced inward current.

Another hypothesis was the intervention of hyperpolarization-driven second messenger-operated channels. The intervention of this type of channel has been clearly demonstrated in CHO cells stably expressing PRL receptors. Indeed, Rato-vondrakona et al. (1998a), used the patch-clamp technique in the cell-attached configuration to record single Ca\(^{2+}\) currents following PRL application. These currents were voltage-independent and required the activation of a soluble cytoplasmic component, possibly Ins(1,3,4,5)P\(_4\) and/or InsP\(_6\). Another study based on mutated PRL receptors (Sorin et al. 1998) showed that the last 141 amino acids of the COOH-terminal PRL receptor are involved in the production of the intracellular messenger that opens these voltage-independent Ca\(^{2+}\) channels. In addition, these second messenger-operated Ca\(^{2+}\) channels, identified in many cell types, including mast cells (Penner et al. 1988), B lymphocytes (MacDougall et al. 1988), neutrophils (von Tscharner et al. 1986), platelets (Hallam and Rink 1985), and lacrimal gland cells (Llano et al. 1987), were probably involved in the PRL-induced second phase in U87-MG cells.

We also found that, as is the case in CHO cells, membrane hyperpolarizations in the presence of PRL in U87-MG cells induced an increase in Ca\(^{2+}\)\(_i\), suggesting the importance of the K\(^+\) efflux and the resulting membrane hyperpolarizations in the PRL response. We conclude that membrane hyperpolarization increases the driving force for Ca\(^{2+}\)\(_i\), thus resulting in Ca\(^{2+}\)\(_i\) entry through 2-APB-sensitive Ca\(^{2+}\) channels, which have not yet been characterized. A similar observation has already been made concerning epidermal growth factor receptor activation in A431 human carcinoma cells (Peppelenbosch et al. 1991). Indeed the authors reported that stimulation of the receptor was followed by an increase in [Ca\(^{2+}\)]\(_i\), and Ca\(^{2+}\)-dependent K\(^+\) channels, causing hyperpolarization of the cell membrane. This in turn activated a class of Ca\(^{2+}\) channels sensitive to hyperpolarization. Note that, as in the case of PRL, EGF signal transduction implied the activation of tyrosine kinases.

In addition, we showed that hyperpolarizing the plasma membrane in nonstimulated cells did not result in an increase in [Ca\(^{2+}\)]\(_i\). This indicates that the production of a second messenger is necessary for Ca\(^{2+}\)\(_i\) influx through 2-APB-sensitive Ca\(^{2+}\) channels. Furthermore, this messenger is diffusible, as the PRL effects disappeared over time during cell dialysis by the patch pipette. As activation was not direct but via a second messenger, the channels responsible for Ca\(^{2+}\)\(_i\) influx should be classified as second messenger-operated channels.

We also demonstrated that, during the second phase, in addition to a Ca\(^{2+}\) conductance, PRL activated a K\(^+\) conductance by Ca\(^{2+}\)-indirect activation (Ca\(^{2+}\) influx). As membrane resting potential has already been shown to be at least partly controlled by K\(^+\) fluxes in U87-MG cells (Ducret et al. 2003), this K\(^+\) conductance activation may explain the delayed PRL-induced hyperpolarization observed in some responding cells.

In addition to steady-state current activation, PRL was also shown to act on voltage-dependent currents. Indeed, in addition to the voltage-dependent conductances already described in U87-MG cells, PRL activated a TEA-sensitive Ca\(^{2+}\)-dependent K\(^+\) current and a DIDS-sensitive Cl\(^-\) current (Ducret et al. 2003).

However, not all the cells responded in the same manner, i.e., PRL can induce the first or second phase only, or both of them. Although we do not have an immediate explanation for this variability between cells, it is possible that, the expression of the PRL-related intracellular transduction components (PRL-R expression, production of messenger responsible for calcium entry and/or release, as well as the sensitivity of intracellular Ca\(^{2+}\) pools) varies according to the cell cycle.

In view of the variety of PRL-activated ionic conductances in U87-MG cells and the importance of ionic species in many physiological events, it is reasonable to suppose that PRL plays an important role in our cell line.

In particular, we reported in a previous publication (Ducret et al. 2002) that PRL exerted a dose-dependent effect on U87-MG cell proliferation. The next step will be to elucidate the contribution of each membrane ion channel to this phenomenon.

A number of studies carried out on several cell types (GH3 pituitary cells, Czarnecki et al. 2000; Vaur et al. 1998; LNCaP human cancer prostate cells, Skryma et al. 1997; primary rat spinal cord cultures, Pappas et al. 1994; and U87-MG cells, Chin et al. 1997) have shown that cell proliferation depends on changes in intracellular K\(^+\) concentrations. Indeed, proliferation of these cells is modulated by potassium channel blockers (TEA, 4-AP, etc.) (Chin et al. 1997; Lee et al. 1986).

Numerous authors (Berridge 1995a; Durham and Walton 1982; Lu and Means 1993) have also emphasized the important role played by Ca\(^{2+}\) in cell proliferation. For example, increases in [Ca\(^{2+}\)]\(_i\) have been associated with the induction of proliferation-associated immediate early genes, including c-fos and c-jun (Pribnow et al. 1992). Moreover, calcium influx through store-operated Ca\(^{2+}\) channels also seems to play a role in the activation of MAPK pathways (Rodland et al. 1997). In addition, studies using Ca\(^{2+}\) channel inhibitors (diltiazem, nifedipine, and verapamil), as well as the ryanodine receptor antagonist, dantrolene, have demonstrated the involvement of calcium in controlling the proliferation of CNS-derived cells (Lee et al. 1994). In most cases, cell proliferation requires millimolar external Ca\(^{2+}\) concentrations (Durham and Walton 1982). In general, cell proliferation is inhibited when cells are cultured in a Ca\(^{2+}\)-deprived medium or one containing Ca\(^{2+}\)-channel blockers. At least two regulated stages in the cell cycle, the G1/S transition and M, are highly Ca\(^{2+}\)-dependent, resulting in the activation of transcription factors (e.g., c-Fos, c-Jun, CRE, and SRE) and key mitotic kinases (Berridge 1995a; Lu and Means 1993).

The role of ion channels in PRL-induced cell proliferation is currently under investigation in our laboratory. Moreover, as ion channels play a key role in proliferation and astrocytoma have a high proliferative capacity, the link between PRL-induced proliferation and ion channel activation may represent a molecular target for new antineoplastic treatments.
We can also speculate that PRL intervenes in other physiological events, as astrocytes play an important role in the CNS: regulation in neuronal external medium ion homeostasis (Barres et al. 1990; Newman 1986) and extracellular pH during neuronal activity (Jendelova and Sykova 1991; Rose and Deitmer 1994), as well as neuron-glial communication (see Charles 1998 for review).

In conclusion, this study extended our previous investigation into the early effects of PRL on ion channels and intracellular ion concentrations in CHO cells transfected with PRL-R cDNA to a cell model that naturally expresses the long form of PRL-R. This work demonstrates that, in addition to the increase in [Ca2+]i already described in U87-MG cells (Ducret et al. 2002), PRL also activates ionic conductances quite similar to those previously obtained in transfected CHO cells (Prevarskaya et al. 1994, 1995; Vacher et al. 1994). Taken together, these data confirm the usefulness of CHO cells for studying the signal transductions associated with PRL-R. Moreover, as already investigated (Sorin et al. 1998), mutated PRL-R expression in CHO may help us to understand the functions of distinct cytoplasmic regions of the PRL-R in activating ion conductances.

However, cell lines naturally expressing PRL-R remain necessary for characterizing the physiological and pathological effects of PRL. In particular, further work is needed to investigate the probable link between the PRL-induced current activations reported in this work and the PRL proliferative effects already described (Ducret et al. 2002).

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