Pflugers Arch - Eur J Physiol (2003) 445:589–600
DOI 10.1007/s00424-002-0980-0

ION CHANNELS, TRANSPORTERS

C. K. Bauer · I. Wulfsen · R. Schäfer · G. Glassmeier · S. Wimmers · J. Flitsch · D. K. Lüdecke · J. R. Schwarz

HERG K^+ currents in human prolactin-secreting adenoma cells

Received: 13 December 2001 / Revised: 24 September 2002 / Accepted: 22 October 2002 / Published online: 6 December 2002

Abstract To investigate the presence and possible function of ether-à-go-go-related gene (erg) K^+ channels in human lactotroph cells (HERG channels), primary cultures were prepared from human prolactinoma tissue. In almost all primary cultures, HERG currents could be recorded in identified prolactin cells using an external high-K^+ solution. The antiarrhythmic agent E-4031, a specific blocker of erg channels, served to isolate HERG currents as the drug-sensitive currents. In cells of two tumours tested, thyrotropin-releasing hormone significantly reduced the amplitude of the HERG currents. The potential dependence of HERG current availability and the deactivation kinetics differed significantly even between prolactin cells derived from one adenoma. For comparison, corresponding values were obtained for heterologously expressed rat erg1, erg2 and erg3 channels. The expression of the three HERG channel subunits was investigated in nine human adenomas using RT-PCR. Transcripts for HERG1 were present in all adenomas and although transcripts for HERG2 and HERG3 were also detected, their expression level was more variable. The results demonstrate the functional expression of HERG channels in human prolactin-secreting tumours and are compatible with a physiological role for these channels in the control of prolactin secretion, as has been shown in normal rat lactotroph cells.

C.K. Bauer (✉) · I. Wulfsen · R. Schäfer · G. Glassmeier · S. Wimmers · J.R. Schwarz
Institut für Angewandte Physiologie,
Universitätsklinikum Hamburg-Eppendorf, Martinistrasse 52,
Universität Hamburg, 20246 Hamburg, Germany
e-mail: c.bauer@uke.uni-hamburg.de
Tel.: +49-40-428032183
Fax: +49-40-428039127

J. Flitsch · D.K. Lüdecke
Klinik für Neurochirurgie,
Universitätsklinikum Hamburg-Eppendorf, Martinistrasse 52,
Universität Hamburg, 20246 Hamburg, Germany

Keywords Pituitary adenomas · Ether-à-go-go-related gene · Thyrotropin-releasing hormone · RT-PCR · HERG1 · HERG2 · HERG3 · KCNE

Introduction

The ether-à-go-go-related gene (erg) K^+ channels belong to the EAG (ether-à-go-go) family of voltage-gated K^+ channels. During the last few years, erg K^+ currents have been found to be involved in different physiological functions (reviewed in [8]). The characteristic of erg channels is their anomalous gating behaviour, with inactivation kinetics being faster than activation kinetics and with recovery from inactivation being faster than deactivation. This results in a functional inward rectification of the erg currents [45, 50]. Accordingly, erg currents recorded in the prolactin- and growth hormone (GH)-secreting cell lines derived from a rat pituitary tumour (GH_3/B_6 [9]; GH_3 [4]) or in native rat lactotroph cells [17] were described initially as inactivating, inwardly rectifying K^+ currents. More recently, the use of specific erg channel blockers from the class of methanesulphonanilides [15] has served to isolate these erg currents as the drug-sensitive currents [5, 10, 36, 52] and has helped to demonstrate their physiological function. In native and clonal rat lactotroph cells, erg currents contribute to the maintenance of the resting membrane potential [7, 12, 52] and their inhibition by thyrotropin-releasing hormone (TRH) is thought to mediate the late phase of TRH-induced prolactin secretion (for review see [40]).

Three different erg genes have been cloned from rat [10, 41] and man (HERG1 [51]; HERG2 and HERG3 GenBank accession No. AF311913 and AF032897). In contrast to the heart, in which only erg1 is expressed [41], GH_3/B_6 cells co-express rat erg1 and erg2 [55] and native rat lactotrophs express all three erg channel subunits in different combinations [36]. This makes the presence of heteromultimeric erg channels in pituitary tissue possible,

since it has been shown recently that the three erg channel subunits are able to form heteromultimeric channels [53].

Up to now, no information has been available about the presence and function of erg currents in human prolactin-secreting cells. The present experiments performed on primary cultures derived from tissue of resected human prolactin-secreting adenomas demonstrate the functional expression of HERG channels in human prolactinoma cells. As candidate genes producing the endogenous HERG channels, HERG1 was co-expressed often with HERG2 and HERG3. Part of this work has been published in abstract form [11].

Materials and methods

Adenoma cell preparation

Experiments using tissue of human prolactin-secreting adenomas obtained by trans-sphenoidal surgery were performed with the approval of the Ethics Committee of the Hamburger Ärztekammer. Primary cultures were prepared from tissue of 13 prolactin-secreting tumours (see Table 1) obtained over a period of 4½ years. Adenoma tissues were minced into small pieces and dissociated at 37 °C for 30 min with collagenase [36]. Dispersed cells were seeded onto 35-mm plastic culture dishes previously coated with poly-D-lysine. The culture medium consisted of DMEM supplemented with 10% fetal calf serum, 1.8 mM L-glutamine and antibiotics (penicillin and streptomycin) and was changed every 3–4 days. Cells were grown in a humidified incubator (37 °C, 5% CO_2).

Primary culture cells were used for electrophysiological recordings 1–28 days after dissociation. After the experiments, the recorded cells were marked and the culture dishes processed to allow immunocytochemical identification of lactotroph cells, as previously described for rat pituitary primary cultures [17, 36]. Briefly, the cells were fixed with 5% formaldehyde in TRIS-buffered saline (TBS), which was also used to wash cells between treatments. After permeabilization with 0.1% Triton X-100, the cells were treated with 1% hydrogen peroxidase. Incubation for 1 h with normal goat serum was followed directly by 1 h incubation with a monoclonal antibody against human prolactin (1:100; clone 164.22.12; Immunotech). The cells were then incubated for 30 min with a biotinylated secondary antibody (1:500; goat anti-mouse IgG; Dianova). After 30 min incubation with an avidin biotinylated horseradish peroxidase complex (Vectastain Elite ABC Kit; Vector Laboratories) prolactin-immunopositive cells were visualized through addition of a 3,3'-diaminobenzidine (0.7 mg ml^{-1}; Sigma) and hydrogen peroxide (2 mg ml^{-1}) solution.

CHO cell culture and microinjection

CHO (Chinese hamster ovary) cells were grown in MEM supplemented with 10% fetal calf serum and 1% penicillin/streptomycin/L-glutamine in a humidified incubator (37 °C, 5% CO_2). The medium was changed every 2–3 days. For microinjection, cells were plated onto poly-D-lysine-coated grids (CELLocate, Eppendorf) in 35-mm culture dishes. For heterologous expression of rat erg K^+ channels, CHO cells were microinjected with cDNA (10–800 ng/µl) coding for rat erg1 (GenBank Acc. No. Z96106; [10]), rat erg2 (Acc. No. AF016192; [41]) or rat erg3 (Acc. No. AF016191; [41]). All cDNAs were subcloned into pcDNA3 (Invitrogen). Co-injection of EGFP-N1 in pcDNA3 encoding an enhanced green fluorescent protein (EGFP; Clontech) was used to detect successfully expressing cells.

Solutions and chemicals

The Ringer solution contained (in mM): 130 NaCl, 5 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, 5 glucose. pH was adjusted to 7.3 with NaOH. The standard external isotonic KCl solution contained (in mM): 140 KCl, 4 $MgCl_2$, 1 $CaCl_2$, 2.5 EGTA, 10 HEPES, 10 glucose (75 nM free Ca^{2+}, calculated using Eqcal; Biosoft). pH was adjusted to 7.3 with KOH. 500 nM tetrodotoxin (TTX; Calbiochem) was added to the external isotonic KCl solution. The standard pipette solution contained (in mM): 140 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 2.5 EGTA, 10 HEPES (66 nM free Ca^{2+}). pH was adjusted to 7.3 with KOH. The selective erg channel blocker E-4031 was a generous gift from Eisai (Tokyo, Japan). E-4031 was added to the bath solution to a final concentration of 10 µM. TRH was also added to the bath to a final concentration of 1 µM. If not otherwise stated, chemicals were purchased from Sigma.

Electrophysiology and evaluation

The membrane potential of individual human adenoma cells was measured in the current-clamp mode of the patch-clamp technique using Ringer as the external solution. The whole-cell voltage-clamp experiments were carried out in external isotonic KCl solution. In

Table 1 Information concerning the 13 different tumours investigated. Given are sex (*f* female, *m* male) and age of the patient, the predominant hormone(s) produced by the tumour (*PRL* prolactin, *GH* growth hormone), the clinical symptoms, the period from cessation of dopamine agonist therapy to tumour resection and the material for RNA preparation. More explanations are given in the text

Tumour No.	Patient	Type	Clinical symptoms	Therapy stop	RNA prep.
#1	f; 23	PRL	Amenorrhoea, galactorrhoea	weeks	tissue
#2	f; 35	PRL	Galactorrhoea, oligomenorrhoea	weeks	tissue
#3	f; 3	GH/PRL	Giantism, galactorrhoea	1 day	tissue
#4	f; 23	PRL	Oligomenorrhoea	months	–
#5	m; 68	PRL	Chiasm compression	not stopped	12 days in culture
#6	m; 68	PRL	Recidivation	not stopped	–
#7	f; 33	PRL	Galactorrhoea, amenorrhoea	not stopped	–
#8	f; 55	PRL	Meningitis	not stopped	–
#9	f; 60	PRL	Headache, chiasm syndrome	2 days	14 days in culture
#10	f; 46	GH/PRL	Galactorrhoea	2 days*	tissue
#11	m; 30	PRL	Headache, visual disturbance	not stopped	tissue
#12	f; 22	PRL	Galactorrhoea, oligomenorrhoea	weeks	tissue
#13	f; 21	PRL	Galactorrhoea	1 year	tissue plus 12 days in culture

* Therapy also included somatostatin

human adenoma cells, the HERG-like currents were isolated from other membrane currents as difference currents before and after selective pharmacological blockade (10 μM E-4031). When filled with intracellular solution, the pipette resistance was 2–5 MΩ. Series resistance errors were compensated as far as possible (>60%). Fast and slow capacitances were compensated prior to the applied test pulse sequences. All experiments were carried out at room temperature. Stimulation, data acquisition and analysis were carried out using the Pulse/PulseFit 8.11 software in combination with an EPC-9 patch-clamp amplifier (HEKA). Further data processing was performed with Sigma Plot (SPSS, Chicago, Ill., USA). Where appropriate, data are given as means±SEM.

Reverse transcription-polymerase chain reaction (RT-PCR)

When enough tissue from a tumour was available, RT-PCR was performed from fresh tissue before dissociation (tumours #1, #2, #3, #10, #11, #12 and #13) and/or after 12–14 days from primary culture cells (tumours #5, #9 and #13). In RT-PCRs amplifying rat cDNAs, fresh pituitary tissue from a female Wistar rat prepared as described previously [55] was used. RNAs were extracted using RNAzol B (AGS, Heidelberg, Germany).

DNase digestion was performed routinely before preparing cDNA. Reverse transcription and RT-PCRs with 40 cycles of amplification were performed as described in [55]. A second amplification using nested primers was performed to get enough product for further analysis. In the PCRs with nested primers 1/50 of the amplification product of the first reaction was used as template in the second PCR. Amplified DNA fragments were analysed by agarose gel electrophoresis. All RT-PCRs were performed at least twice and all DNA fragments were verified by sequencing. Negative controls using H_2O or reverse transcription products generated without enzyme instead of cDNA as template were included. A reverse transcript from fetal human brain RNA [male, gestation 17 weeks (Stratagene)] served as template in positive controls.

Primer sequences

The following oligonucleotide primer sequences for the PCR amplifications were used (the GenBank database accession no. is given in parentheses).

Human primer sequences. HERG1: nucleotides 1398–1950 (accession no. U04270): forward 5'-CTTCAAGGCCGTGTGGGACT-3', reverse 5'-CAGGTTGTGCAGCCAGCCGA-3'. HERG2: nucleotides 2507–2993 (first amplification) (accession no. AF311913): forward 5'-GATGAACAGGCTGGAGTCCC-3', reverse 5'-GTGGCCCCAACTCCCTGCAA-3'; nucleotides 2527–2970 (second amplification): forward 5'-GCGTGTCCTCAGACCTCAGC-3', reverse 5'-CAGGATCTGAGCCATGTCTCT-3'. HERG3: nucleotides 2114–2670 (first amplification) (accession no. AF032897): forward 5'-GGAACTGCCAGGTACCACAT-3', reverse 5'-GTTAGAAAGTGATCAGAAAA-3'; nucleotides 2137–2650 (second amplification): forward 5'-GATGCTGCGAGTAAAAGAGT-3', reverse 5'-CTCAGGATACATATCCAAAA-3'. hMinK (KCNE1): nucleotides 8–402 (first amplification) (accession no. L33815): forward 5'-CAGGATGATCCTGTCTAACA-3', reverse 5'-TTCATGGGGAAGGCTTCGTC-3'; nucleotides 28–382 (second amplification): forward 5'-CCACAGCGGTGACGCCCTTT-3', reverse 5'-TCAGGAAGGTGTGTGTTGGG-3'. hMiRP1 (KCNE2): nucleotides 69–448 (first amplification) (accession no. AF071002): forward 5'-GAAGCATGTCTACTTTATCC-3', reverse 5'-TTATCAGGGGGACATTTTGA-3'; nucleotides 91–428 (second amplification): forward 5'-TTTCACACAGACGCTGGAAG-3', reverse 5'-ACCCAGCCGCACCAATGTTC-3'. hMiRP2 (KCNE3): nucleotides 70–424 (first amplification) (accession no. AF076531): forward 5'-CCCCCACCTCAATCCCTGTT-3', reverse 5'-ACCGTCCCAGCCCTCTCGTG-3'; nucleotides 90–404 (second amplification): forward 5'-GCTATGGAGACTACCAATGG-3', reverse 5'-TTAGATCATAGACACACGGT-3'. GH: nucleotides 68–426 (accession no. XM_015911): forward 5'-TGCCTTCCCAACCATTCCCT-3', reverse 5'-CCTCTAGGTCCTTCAGGTGG-3'. PRL: nucleotides 70–438 (accession no. NM_000948): forward 5'-GTGCCAGAGCGTGGCCCCCT-3', reverse 5'-ACAGCTTTGGATAGGATAGC-3'.

Rat primer sequences. rMiRP1 (KCNE2): nucleotides 15–463 (first amplification) (accession no. AF071003): forward 5'-CTCATCCTCAAGGGGGAAAC-3', reverse 5'-TGGGGCTTCAAGGCGGCA-3'; nucleotides 15–429 (second amplification): forward 5'-CTCATCCTCAAGGGGGAAAC-3', reverse 5'-GGCAGATGGACTCTCGTTCT-3'. rMiRP2 (KCNE3): nucleotides 207–583 (accession no. AJ271742): forward 5'-CTTCCCATACCTCGATTTCT-3', reverse 5'-TCTTCCATTGTCCTCAGGCA-3'.

Results

Morphology of cultured adenoma cells

Primary cultures of human pituitary adenoma cells were prepared from 13 prolactin-producing tumours from patients with various clinical backgrounds (see Table 1). Accordingly, the morphology of primary culture cells derived from different tumours was not homogeneous. In most primary cultures, the adenoma cells slightly increased in volume, extended on the bottom of the culture dish and partially reassociated to form a monolayer. These morphological changes often occurred during the first 4–5 days in culture (e.g. tumour #13 Fig. 1G, H), but this could also take more than a week (tumour #9, Fig. 1E, F) and seemed to coincide with the development of a thin layer of folliculo-stellate cells. In these cultures, almost all parenchymal cells exhibited strong immunoreactivity for prolactin except for the mixed tumours #3 (Fig. 1A) and #10, which contained a number of prolactin-negative cells.

Obviously different were the primary cultures from tumours #5 and #7. The adenoma cells derived from tumour #5 were extremely small and exhibited no immunoreactivity for prolactin on day 1 (Fig. 1C). The cell number clearly increased in culture and the resulting cell clusters started to produce prolactin as demonstrated by the positive staining for prolactin on day 7 (Fig. 1D). These findings are consistent with the clinical symptom of chiasm compression and with the absence of typical prolactin-induced symptoms in the patient concerned. The cells from tumour #7 were also very small and only a few cells stained quite well for prolactin (Fig. 1B). The cell density quickly decreased and almost no cells were left after 5 days in culture.

Membrane currents recorded in external high-K^+ solution

To enable and facilitate the detection of erg currents in human prolactinoma cells, membrane currents were recorded in external isotonic KCl solution with pulse protocols similar to those previously used to describe erg currents in rat prolactin-secreting cells [9, 36]. A 2-s

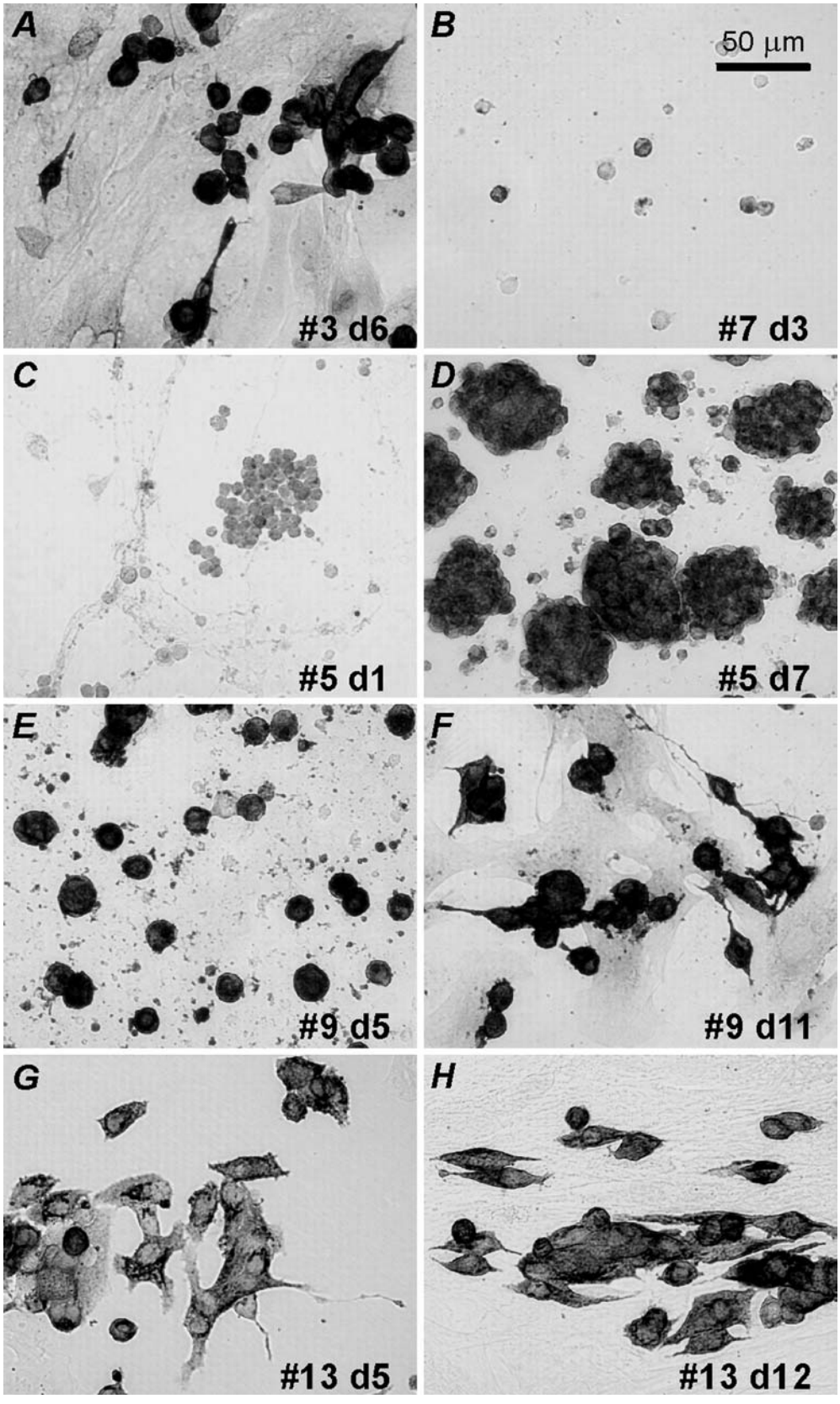


Fig. 1A–F Primary cultures of human prolactin adenoma cells. Cells were stained for prolactin. The tumour *number* (see Table 1) and the days (*d*) in culture are indicated, the *scale bar* in **B** applies to all micrographs

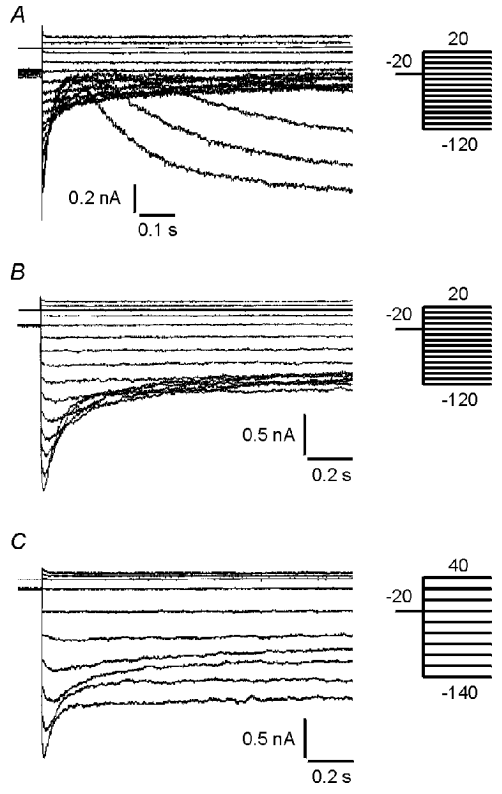


Fig. 2A–C Membrane currents recorded in external high-K^+ solution. Families of membrane currents recorded from human prolactin cells in primary cultures derived from different tumours. Currents were elicited by depolarizing and hyperpolarizing test pulses from a holding potential of –20 mV in external 150 mM K^+ solution. With a gap of 500 ms at –20 mV, a 2-s depolarizing prepulse to 20 mV preceded the test pulses to activate human ether-à-go-go-related gene (HERG) channels. Current traces were elicited with the test pulse potentials shown on the *right*

depolarizing prepulse to 20 mV preceded the test pulses to fully activate erg channels. Starting from a holding potential of –20 mV, hyperpolarizing pulses revealed different types of inwardly rectifying currents (Fig. 2) in identified prolactin cells. Hyperpolarization-activated (I_h)-like currents [14] were recorded in a number of cells from different tumours, and an example of high-amplitude I_h-like current is shown in Fig. 2A. A similar hyperpolarization-activated cation current has been reported in GH_3 cells and in a small fraction of native rat lactotrophs [44]. In many prolactinoma cells, the pulse protocol elicited membrane currents dominated by HERG currents (Fig. 2B). Characteristic for hyperpolarization-induced erg currents is an initial current hook produced by fast recovery from inactivation, followed by time- and voltage-dependent current decay [50]. Kir2.0-like non-inactivating inward rectifier currents [20] were often recorded in prolactin adenoma cells derived from tumour #13, but were only seldom observed in cells from other adenomas. Figure 2C shows an example of pronounced Kir2.0-like currents overlaid with HERG currents.

Isolation of the HERG-like current

The examples of hyperpolarization-elicited membrane currents shown in Fig. 2 demonstrate the necessity for isolating the erg currents from other recorded membrane currents. As shown in Fig. 3, the HERG currents were isolated as the E-4031-sensitive current, i.e. the difference current between control currents and those recorded in the presence of 10 μM E-4031, a substance known to specifically block erg channels [40, 41].

The current/voltage (*I*/*V*) relationship of the isolated HERG currents always exhibited clear inward rectification (Fig. 3D). The difference between peak and steady-state current amplitude mirrors the time- and voltage-dependent deactivation of HERG channels at potentials more negative than about –40 mV. Similar HERG currents could be recorded in at least some of the primary culture cells derived from all 13 tumours studied, except for tumour #7. In tumours #5 and #9, typical HERG currents were not recorded during the first days in primary culture, but after about 1 week in culture.

TRH-induced reduction of HERG currents

The effect of 1 μM TRH on HERG currents was investigated in five prolactin adenoma cells derived from two different tumours (#3 and #11). In all five experiments, TRH induced a clear and irreversible reduction of the HERG currents elicited with a short hyperpolarizing test pulse to –100 mV (Fig. 4). The TRH-induced HERG current reduction was always complete within 3 min.

For tumour #3, corresponding clinical data were available, since a TRH stimulation test had been performed in the patient from whom the tumour had been obtained: TRH elevated the prolactin concentration from 246 to 575 μg/l.

Electrical properties of human prolactin-secreting adenoma cells

The electrical behaviour of the prolactinoma cells was measured in the current-clamp mode in external Ringer solution. Only a few cells were spontaneously active as shown in Fig. 5A. The majority of the identified lactotroph cells were silent (Fig. 5B), but action potentials could be elicited in these cells (Fig. 5C). The action potentials occurred upon depolarizations (on-action potentials) and were also often elicited after a hyperpolarizing current injection (off-action potentials).

The resting membrane potential was determined in identified prolactin cells derived from six different tumours. The least negative membrane potentials were observed in tumour #5 (–37.7±3.1 mV, n=5, measured after 8 days in culture), the most negative membrane potentials were recorded in cells from tumour #13 (–51.6±1.8 mV, n=10). The average of the mean values of the resting membrane potential in the six tumours was

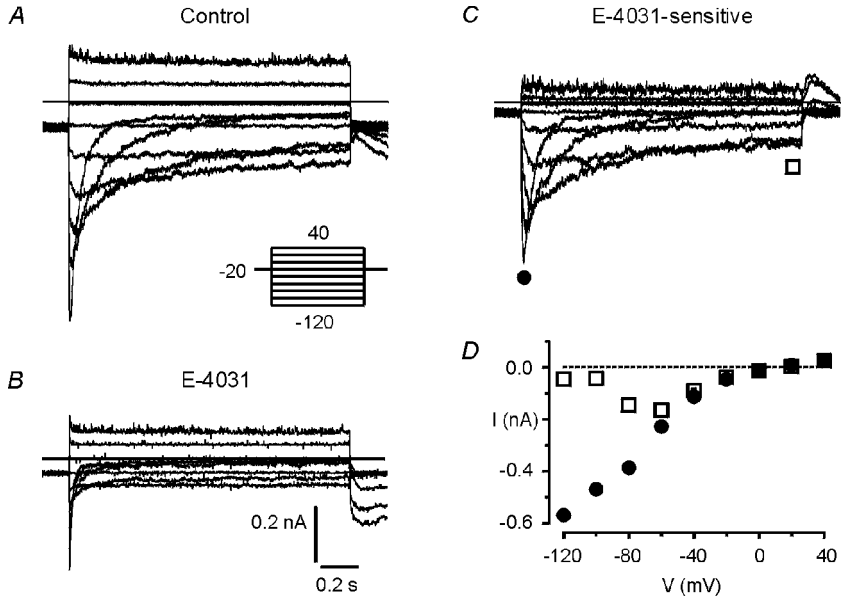


Fig. 3A–D Isolation of HERG currents as the E-4031-sensitive current. **A** Family of membrane currents recorded in external 150 mM K^+ solution. The pulse protocol consisted of variable 1.5-s test pulses between 40 mV and –120 mV in steps of 20 mV from a holding potential of –20 mV. A 2-s depolarizing prepulse to 20 mV followed by a gap of 500 ms at –20 mV preceded the test pulses to activate HERG channels. **B** Membrane currents recorded from the same cell as shown in **A** after bath application of 10 μM E-4031. **C** The E-4031-sensitive HERG currents were isolated by subtraction of the E-4031-insensitive currents (**B**) from the control currents (**A**). **D** Current/voltage (*I/V*) plot of the maximal HERG current amplitude (*filled circles*) and the current amplitude at the end of the test pulses (*open squares*)

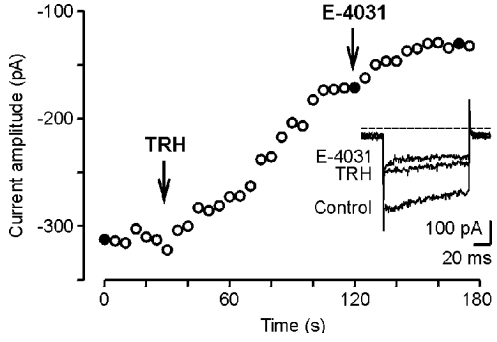


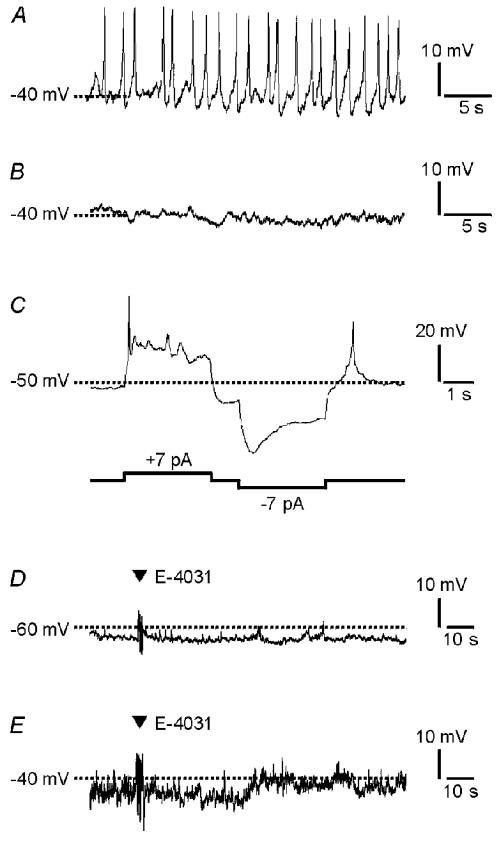
Fig. 4 Thyrotropin releasing hormone (*TRH*) reduces HERG currents. Time course of inward current reduction induced by bath application of 1 μM TRH. Currents were elicited with a 100-ms pulse to –100 mV from a holding potential of –10 mV. E-4031 (10 μM) was applied at the end of the experiment to determine the E-4031-insensitive current. *Filled circles* correspond to the current traces shown in the *inset*

–44.6±2.2 mV. In cells from tumour #13 the effect of E-4031 on the membrane potential was tested. At 70–100 s after the application of 2 μM E-4031, the mean membrane potential had depolarized slightly from –51.6 to –48.4± 3.0 mV (n=10). A depolarizing effect of E–4031 was more often visible in cells with a less negative resting membrane potential (Fig. 5D, E). The data from the ten experiments were therefore divided into two groups according to the initial value of the resting membrane potential (more negative or more positive than –50 mV). In four cells with a more negative initial membrane potential (–57.3±2.4 mV), the corresponding value 70–100 s after E-4031 application was –58.1±2.8 mV. In the other group (n=6), the value of the resting membrane potential was –47.8±0.8 mV before drug application and –41.9±1.9 mV 70–100 s after the addition of E-4031.

Expression of the different HERG channel subunits and KCNE β-subunits

Since the electrophysiological experiments demonstrated the functional expression of HERG channels in prolactin-secreting adenomas, the possible molecular correlates were investigated using RT-PCR. Altogether, material from nine of the tumours could be investigated by RT-PCR (see Table 1 in Materials and methods). The expression of the hormones prolactin and GH served as a control of the quality of the prepared cDNA. Transcripts for prolactin were expressed in all nine tumours, and the amplified growth hormone gene products were most prominent in the two mixed tumours which secreted GH in vivo (tumours #3 and #10, see Fig. 6A).

Since the sequences of HERG2 and HERG3 have been published recently in the GenBank database, it was possible to study the expression of all three human erg K^+ channel subunits. All nine tumours expressed HERG1 (Fig. 6B). Using two rounds of amplification and different primer sets, co-expression of HERG2 and HERG3 was found in almost all tumours, only HERG3 was not detected in tumour #5. After one round of amplification, transcripts for all three HERG subunits were found in four



tumours (#1, #3, #10 and #13) and, in addition to HERG1, HERG2 was detected in the tumours #5, #9 and #12 (Fig. 6B). For tumour #13, RNA was prepared from fresh tumour tissue as well as from cells after 12 days in culture. Whereas the expression of the hormones apparently did not change during the time in culture, a second round of amplification was needed to detect transcripts for HERG2 and HERG3 in the cell culture preparation, and also the signal for HERG1 transcripts became weaker (Fig. 6).

Recently, the interaction of HERG1 with the β-subunits MinK (KCNE1; [32]), MiRP1 (KCNE2; [1]) and MiRP2 (KCNE3; [39]) has been reported. We therefore investigated the expression of these subunits in the human prolactin adenomas. After one round of amplification, transcripts of MiRP2 were found in all tumours, whereas the signals for MinK and MiRP1 were close to the detection threshold in most tumours. Nevertheless, a second round of amplification revealed tran-

Fig. 5A–E Membrane potential of human prolactinoma cells in primary culture recorded in the current-clamp mode in Ringer solution. **A** Example of a spontaneously active cell. **B** Example of a cell exhibiting membrane fluctuations without action potentials. **C** "On" and "off" action potentials elicited in a silent cell by a depolarizing and after a hyperpolarizing current injection, respectively. **D**, **E** Effects of the application of 2 μM E–4031 in cells with differing initial resting membrane potential

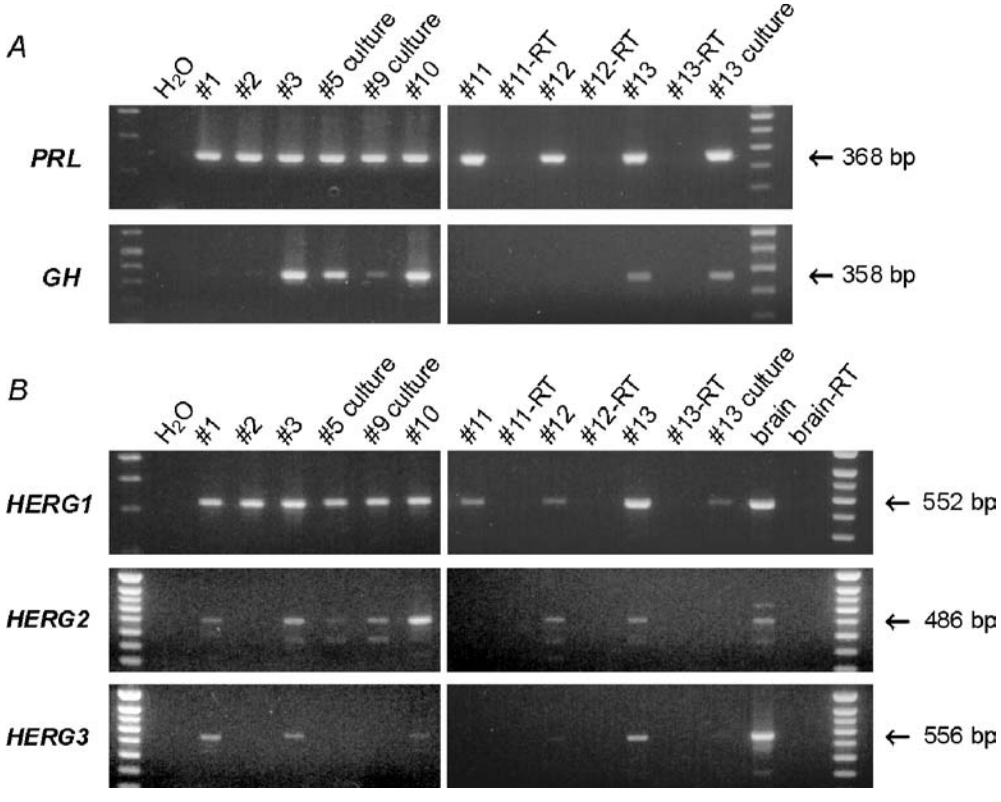


Fig. 6A, B HERG subunit expression in human prolactin adenomas. RT-PCR amplification products from the first round of amplification are shown for the pituitary hormones prolactin and growth hormone (**A**) and the three different HERG channel subunits (**B**). RT-PCR was performed as described in Materials and methods, *–RT* indicates controls performed without reverse transcriptase. The molecular weight of the amplified cDNAs is indicated on the *right*. For tumour #13, cDNA was prepared from both fresh, undissociated tissue and from primary culture cells on day 12

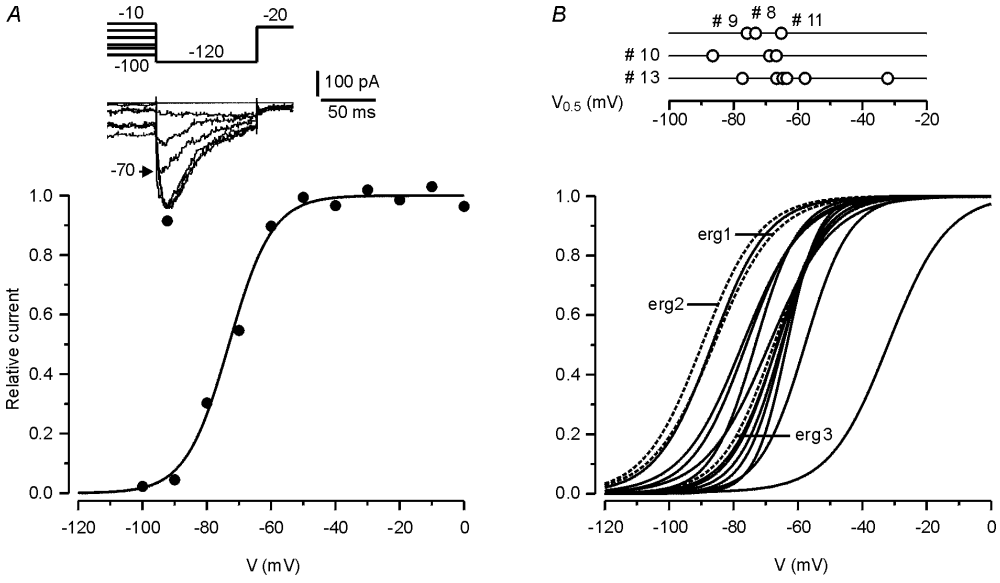


Fig. 7A, B Voltage dependence of HERG current availability. HERG current availability was investigated by 1.5-s depolarizing and hyperpolarizing pulses to potentials between 0 mV and –120 mV followed by a constant 100-ms hyperpolarizing test pulse to –120 mV. The holding potential was –20 mV and a 2-s depolarizing prepulse to 20 mV preceded the variable pulses to fully activate HERG channels. HERG currents were isolated as the E-4031-sensitive current as shown in Fig. 3. **A** Normalized maximal HERG current amplitudes elicited with the constant hyperpolarizing pulse to –120 mV as a function of the preceding test pulse potential. The *continuous line* represents a Boltzmann function fitted to the data. Values for the inflection potential and steepness are –73.2 mV and 6.8 mV, respectively. *Inset*: E-4031-sensitive current traces elicited at –120 mV following test pulses to potentials indicated in the pulse protocol *above* the traces. **B** Boltzmann functions describing the potential-dependent availability of erg K^+ currents were obtained as shown in **A**. The 12 *continuous lines* represent data from individual adenoma cells derived from five different tumours. The corresponding potentials for half-maximal activation of HERG currents are given at the *top*. For comparison, the availability curves (*broken lines*) fitted to the mean normalized erg current amplitudes are shown for heterologously expressed rat erg1, erg2 and erg3 channels

scripts of MinK and MiRP1 in all tumours (data not shown). In normal rat pituitary, the expression of MinK has been demonstrated [55], but no information is available about MiRP1 and MiRP2. Therefore, we also performed RT-PCR to detect the rat homologues rMiRP1 and rMiRP2. Both subunits were expressed in rat pituitary, although two rounds of amplification were needed to detect rMiRP1 (data not shown).

Comparative studies of HERG currents in prolactinoma cells and heterologously expressed erg channels

The potential-dependent availability of HERG currents was measured as described previously [36]. Starting from a "fully activated" state of the erg channels [42], 1.5-s test pulses to various potentials were applied and followed directly by a constant hyperpolarization to –120 mV. Due to the fast recovery from inactivation, the maximal amplitude of the erg current elicited with the constant hyperpolarizing pulses is a measure of the fraction of activated erg channels at the end of the preceding test pulse. As shown in Fig. 7A, a Boltzmann function was fitted to the data, yielding the potential at which half of the erg channels were available ($V_{0.5}$). The corresponding values obtained for twelve cells from five adenomas are given at the top of Fig. 7B.

As shown above, most human prolactinomas expressed the three erg channel subunits to varying extents. To enable a comparison, heterologously expressed erg1, erg2 and erg3 channels were studied using identical solutions and pulse protocols. Since HERG2 and HERG3 clones were not available, the three different rat erg channels were analysed following heterologous expression in CHO cells. Figure 7B shows that the potential dependence of erg current availability measured with 1.5-s test pulses is similar for erg1 ($V_{0.5}$: –84.4±1.6 mV, n=8) and erg2 ($V_{0.5}$: –89.7±1.9 mV, n=7), whereas for erg 3 the availability curve is shifted to a clearly more positive potential ($V_{0.5}$: –67.5±1.7 mV, n=6).

The deactivation kinetics of HERG and rat erg currents were determined by fitting a monoexponential function to the decay phase of the erg currents elicited with hyperpolarizing test pulses from a holding potential of –20 mV (Fig. 8). In all experiments, the time constants of deactivation were voltage dependent and decreased with more negative potentials. The deactivation kinetics were similar for erg1 and erg2, but significantly faster for erg3. The deactivation time constants determined for the isolated HERG currents are well within the range of time constants obtained for erg3 on the one hand, and erg1 and erg2 on the other hand (Fig. 8). The data obtained for

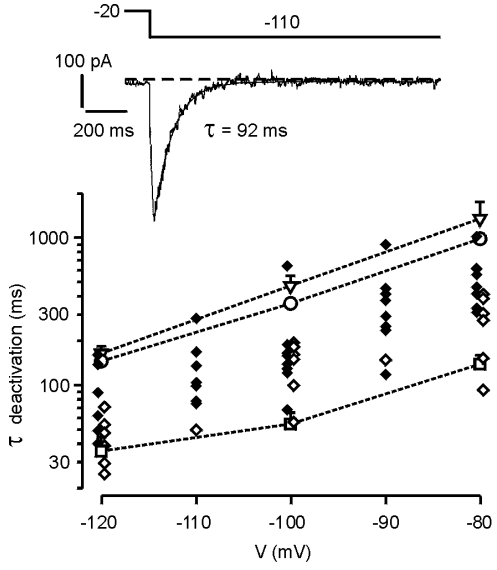


Fig. 8 Time course of HERG current deactivation. HERG currents were recorded in external isotonic KCl solution and isolated as E-4031-sensitive currents. The time course of HERG current decay upon hyperpolarizing pulses to potentials between –80 mV and –120 mV from a holding potential of –20 mV was fitted by a monoexponential function as shown in the *inset*. The deactivation time constants were determined in 15 prolactin cells derived from eight different tumours. The data obtained for tumour #13 are shown as *open diamonds*. In addition, the mean time constants (+SEM) of deactivation are given for rat erg1 (*open circles*, *n*=7), erg2 (*inverse triangles*, *n*=8) and erg3 (*open squares*, *n*=6) channels heterologously expressed in Chinese hamster ovary (CHO) cells

lactotrophs from tumour #13 are separated to demonstrate the variability in the HERG deactivation kinetics between cells derived from one tumour.

Discussion

In rat lactotroph cells, erg K^+ channels have been shown to play a role in the control of the electrical activity and, as a consequence, in the regulation of prolactin secretion (reviewed in [40]). To find out whether these results could also apply to human lactotroph cells, we investigated the expression of HERG channels in primary cultures of human prolactin-secreting adenomas.

Functional expression of HERG channels in human prolactinoma cells

Most primary cultures exhibited morphological differentiation after enzymatic dissociation during the first few days in culture, as described for primary cultures of normal human anterior pituitaries [3] and normal rat anterior pituitaries cultured under identical conditions [17]. Only the cultures derived from tumours #5 and #7 were completely different. A decrease in cell volume and even in the production of prolactin has been described to occur as a consequence of a dopamine agonist therapy in part of the treated prolactinomas (for review, see [29]). These morphological changes often reversed within about 1 week after dopamine agonist withdrawal, but permanent effects have also been described. Dopamine agonists might also account for the distinctive features of the cultures of tumours #5 and #7, since these were derived from patients in whom the dopamine agonist therapy had not been stopped before surgery (see Table 1). Interestingly, no HERG currents could be recorded in these suppressed adenoma cells. After about 1 week in culture, cells of tumour #5 produced prolactin and showed HERG currents. No information about a "delayed" functional expression of HERG channels could be obtained from tumour #7 since these cells survived only a few days in culture. HERG currents were only recorded after a few days in culture of cells from tumour #9 also. This tumour had been obtained from a patient in whom therapy had been stopped only 2 days before surgery. The primary culture appeared normal except that the onset of the morphological differentiation was delayed. Since typical HERG currents could be recorded in prolactin cells derived from all adenomas with "normal" primary cultures, it is possible that a strong suppression and dedifferentiation of prolactinoma cells by the dopamine agonist therapy coincides with a functional suppression of HERG channels.

Using RT-PCR we demonstrated the expression of HERG1 in all tested prolactinomas and co-expression of HERG2 and HERG3 in all tumours except # 5, which also exhibited the atypical morphology in primary culture. These results are compatible with data derived from the rat, where all three cloned erg channel subunits are expressed in the pituitary [55] and even in individual native rat lactotroph cells in different combinations [36]. Nevertheless, the expression level was low for HERG2 in two adenomas and for HERG3 in five adenomas. No indications were found for an enhanced HERG channel expression during cell culture, since for tumour #13, the level of HERG subunit expression was even lower after 12 days in culture than immediately after tumour resection.

The class III antiarrhythmic E-4031 was used to isolate the HERG currents as drug-sensitive difference currents because E-4031 has been shown to block all three erg channels with a similar high affinity [41]. The isolated HERG currents measured in prolactinoma cells were macroscopically similar to the erg currents of GH_3/B_6 cells [10, 52] or GH_3 cells [5]. Accordingly, the potential-dependent availability of the HERG currents was well described by a single Boltzmann function, similar to the findings in GH_3/B_6 cells [7, 36]. In contrast, availability data derived from native rat lactotrophs must be fitted with the sum of two Boltzmann functions due to the presence of an additional slowly deactivating erg current component [36].

The voltage dependence of the HERG current availability and the deactivation time course was compared with corresponding data obtained for rat erg1, erg2 and erg3 channels. At the protein level, the three rat erg

channel subunits are very similar to their human counterparts (95%, 89% and 93% amino acid identity) suggesting also similar biophysical properties. HERG1 and its rat counterpart erg1 apparently generate the same membrane currents and are comparably modulated by TRH [10, 38]. The voltage dependence of rat erg1 and erg2 channel activation differs by about 20 mV [38, 41]. Nevertheless, with respect to the parameters measured in the present study, erg1 and erg2 currents resembled each other. In contrast, erg3 exhibited a clearly faster time course of deactivation than erg1 and erg2 which is consistent with data obtained in external solution at a physiological $[K^+]$ [38, 41]. The voltage dependence of the erg3 current availability is shifted by about 20 mV to more positive potentials compared with the availability curves obtained for erg1 and erg2. This right shift results from the considerably faster deactivation of erg3 channels – despite the fact that erg3 activates at potentials about 20 mV more negative than erg1 and about 40 mV more negative than erg2 [38, 41].

The direct comparison of the HERG currents of prolactinoma cells with the corresponding data obtained for the three erg channels showed clearly that most of the values determined in individual prolactinoma cells are well within the range of values obtained for the three rat erg channels. There were no indications that the HERG currents of individual adenoma cells consisted of more than one distinct current component. This might be explained by the formation of heteromultimeric erg channels as has been demonstrated for heterologously expressed erg channel subunits [53]. For concatemers of two different erg channel subunits, the voltage dependence of activation of a heteromultimeric erg channel is intermediate compared with the corresponding homomultimeric erg channels, whereas other parameters are dominated by one subunit [54].

It is tempting to attribute the considerable cell-to-cell variability in the voltage dependence of the HERG availability curves to differing individual patterns of erg channel subunit expression. Nevertheless, other reasons might also account for the observed diversity of HERG currents in prolactinoma cells. In neuroblastoma cells, for instance, the voltage dependence of erg current activation is altered during the cell cycle [2]. The availability of erg currents might also change with different physiological states of a cell, since changes in the activity of various second messenger pathways have been described to affect the voltage dependence and/or the kinetics of erg1 channel gating [6, 18, 23, 38, 46]. The β-subunits MinK, MiRP1 and MiRP2 have also been reported to affect HERG1 channels [1, 32, 39]. Nevertheless, only small effects on the voltage dependence of erg1 channel gating are found when HERG1 is co-expressed with MinK in *Xenopus* oocytes [32] or with MiRP1 in mammalian expression systems [18, 31, 38]. We have not been able to demonstrate clear changes in erg current properties upon co-expression of MinK, MiRP1 and MiRP2 with either of the three rat erg channels in CHO cells [13]. Therefore, the physiological significance of the expression of these three KCNE subunits in human prolactinoma tissue or in normal rat pituitary tissue ([55] and present results) is still unclear with respect to the recorded HERG currents.

Physiological function of HERG currents in prolactin-secreting pituitary cells

The value of the resting membrane potential is important for the physiological function of erg channels. Only in cells that lack pronounced classical inwardly rectifying K^+ currents may erg currents be involved in the setting of the resting membrane potential (reviewed in [40]). Kir2.0-like inward rectifier currents were observed only rarely in human prolactinoma cells. Only the primary culture of tumour #13 contained a considerable number of cells with non-inactivating inward rectifier currents similar to those described in rat corticotrophs [30]. In normal rat lactotroph cells [12, 17, 36] and in GH cell lines [4, 9], Kir2.0-like inward rectifier currents have not been reported, although the rat Kir2.3 channel has been cloned from GH_3/B_6 cells [21].

The resting membrane potentials of most human prolactinoma cells were in the range –50 to –40 mV, i.e. a potential range in which erg1 and erg3 channels exhibit steady-state outward currents [38, 41]. The resting membrane potentials of human prolactinoma cells determined in the present study correlate well with those reported for native rat lactotrophs [12] and prolactin-secreting clonal tumour cells [43, 47]. Only slightly more negative values were obtained in early publications on human prolactin adenoma cells using intracellular microelectrodes [26]. In accordance with a previous report [26], the majority of the human prolactin adenoma cells investigated in the present study were silent, but electrically excitable. Off-potentials elicited on the cessation of hyperpolarizing current pulses have often been described in various prolactin-secreting pituitary cells (e.g. [26, 35]). In these cells, erg currents are present and the hyperpolarization-induced deactivation of erg currents combined with the slow activation kinetics of the erg currents should support the generation of off-action potentials. The electrical activity is important for the control of prolactin secretion since it determines the intracellular free $[Ca^{2+}]$. Ca^{2+} influx occurs via Ca^{2+} action potentials [26, 28] and as small steady-state Ca^{2+} currents through voltage-dependent Ca^{2+} channels that are already activated at the resting membrane potential [33, 37].

Direct evidence for a contribution of erg currents to the maintenance of the resting membrane potential has been obtained by a selective pharmacological block of erg channels, which results in moderate membrane depolarization in native rat lactotrophs [12], GH_3/B_6 cells [7, 52] and GH_3 cells [5]. The present data obtained from tumour #13 regarding the effect of E-4031 on the resting membrane potential of human prolactinoma cells are absolutely compatible with the results obtained in the rat. The mean depolarization produced by E–4031 in cells

with a resting membrane potential positive to –50 mV was about 5 mV and similar in magnitude to that described for native and clonal rat lactotroph cells. Probably due to the presence of Kir2.0-like inward rectifier currents, tumour #13 exhibited the most negative resting potential. The observed lack of an effect of HERG current block in cells with a more negative membrane potential is readily explained by the potential dependence of HERG channel activation, suggesting that most HERG channels are deactivated at potentials close to –60 mV.

Supporting the concept of the stimulus-secretion coupling [19], the electrical activity is also important for the action of hypothalamic inhibiting and releasing hormones. TRH enhances prolactin secretion by releasing intracellularly stored Ca^{2+} and by increasing the electrical activity of the lactotroph cells (for reviews, see [16, 24, 34]). The latter is thought to be due to TRH-induced reduction of erg currents, as described in clonal prolactin-secreting cells [4, 7, 9] and in native rat lactotrophs [17, 36]. The present results show that TRH application resulted in an effective HERG current reduction also in human prolactin-secreting adenoma cells. The changes in the biophysical properties of erg channels underlying the TRH-induced current reduction have been studied in detail for erg1, erg2 and erg3 [38]. The most prominent and consistent TRH effects were a shift in the voltage dependence of erg channel activation to more positive potentials and a reduction of the maximal available erg current amplitude.

The finding that TRH can modulate HERG currents in human prolactinoma cells was not a matter of course, because the plasma prolactin response to TRH has been reported to be absent in most patients with prolactinoma [48]. Nevertheless, TRH increased prolactin levels in the patient with tumour 3 [22], consistent with the electrophysiological data obtained from the adenoma cells in the corresponding primary culture. The reduction of erg currents by TRH is part of the physiological stimulation of prolactin secretion. In the last few years, a number of different drugs have been described that inhibit HERG1 currents [8] and investigations of the potency of these drugs in blocking the recently cloned HERG3 channel have just begun [27]. Since a pharmacological block of the erg currents of native rat lactotrophs increases prolactin secretion [12], the corresponding side-effects of drugs with erg channel blocking properties (e.g. class III antiarrhythmics, antipsychotics or antihistaminics) should be considered in patients with prolactinomas.

The present experiments yield information concerning HERG currents in human prolactinoma cells. This is the first tissue in which co-expression of all three HERG channel subunits has been described together with the demonstration of functional HERG currents. Prolactinomas are generally believed to be well-differentiated adenomas and adenomatous and non-adenomatous human lactotrophs behave very similarly in culture [25]. The observed functional diversity among prolactinomas corresponds to the heterogeneity of normal lactotroph cells in the intact pituitary [16, 49]. Therefore, the detection of functional HERG channels in prolactinoma cells combined with the knowledge about erg currents in normal rat lactotrophs points strongly to a physiological role of HERG currents in clonal as well as in normal human prolactin-secreting cells.

Acknowledgements We thank Dr. J. Dixon (Stony Brook, N.Y.) for the rat erg2 and erg3 cDNA, Waltraud Krüger and Dorrit Schiemann for technical help and the Deutsche Forschungsgemeinschaft for financial support.

References

1. Abbott GW, Sesti F, Splawski I, Buck ME, Lehmann MH, Timothy KW, Keating MT, Goldstein SAN (1999) MiRP1 Forms I_{Kr} potassium channels with HERG and is associated with cardiac arrhythmia. Cell 97:175–187
2. Arcangeli A, Bianchi L, Becchetti A, Faravelli L, Coronnello M, Mini E, Olivotto M, Wanke E (1995) A novel inward-rectifying K^+ current with a cell-cycle dependence governs the resting potential of mammalian neuroblastoma cells. J Physiol (Lond) 489:455–471
3. Bacsy E, Gyevai A, Szabo M, Nagy I, Sotonyi P (1992) Morphological and functional characterization of cell cultures from adult human adenohypophyses. Acta Histochem 86:231–237
4. Barros F, Delgado LM, del Camino D, de la Pena P (1992) Characteristics and modulation by thyrotropin-releasing hormone of an inwardly rectifying K^+ current in patch-perforated GH3 anterior pituitary cells. Pflugers Arch 422:31–39
5. Barros F, del Camino D, Pardo LA, Palomero T, Giraldez T, de la Pena P (1997) Demonstration of an inwardly rectifying K^+ current component modulated by thyrotropin-releasing hormone and caffeine in GH3 rat anterior pituitary cells. Pflugers Arch 435:119–129
6. Barros F, Gomezvarela D, Viloria CG, Palomero T, Giraldez T, de la Pena P (1998) Modulation of human erg K^+ channel gating by activation of a G protein-coupled receptor and protein kinase C. J Physiol (Lond) 511:333–346
7. Bauer CK (1998) The erg inwardly rectifying K^+ current and its modulation by thyrotrophin-releasing hormone in giant clonal rat anterior pituitary cells. J Physiol (Lond) 510:63–70
8. Bauer CK, Schwarz JR (2001) Physiology of EAG K^+ channels. J Membr Biol 182:1–15
9. Bauer CK, Meyerhof W, Schwarz JR (1990) An inward-rectifying K^+ current in clonal rat pituitary cells and its modulation by thyrotrophin-releasing hormone. J Physiol (Lond) 429:169–189
10. Bauer CK, Engeland B, Wulfsen I, Ludwig J, Pongs O, Schwarz JR (1998) RERG is a molecular correlate of the inward-rectifying K^+ current in clonal rat pituitary cells. Receptors Channels 6:19–29
11. Bauer CK, Flitsch J, Wulfsen I, Glassmeier G, Lüdecke D, Schwarz JR (2000) HERG currents in human prolactinoma cells (abstract). Pflugers Arch 439:R294
12. Bauer CK, Schäfer R, Schiemann D, Reid G, Hanganu I, Schwarz JR (1999) A functional role of the erg-like inward-rectifying K^+ current in prolactin secretion from rat lactotrophs. Mol Cell Endocrinol 148:37–45
13. Buhk JH, Burgarth S, Wulfsen I, Schwarz JR, Bauer CK (2002) Coexpression studies of erg K^+ channel subunits with KCNE β subunits in CHO cells (abstract). Pflugers Arch 443:S278
14. Chen S, Wang J, Siegelbaum SA (2001) Properties of hyperpolarization-activated pacemaker current defined by coassembly of HCN1 and HCN2 subunits and basal modulation by cyclic nucleotide. J Gen Physiol 117:491–504

15. Colatsky TJ, Argentieri TM (1994) Potassium channel blockers as antiarrhythmic drugs. Drug Dev Res 33:235–249
16. Corrette BJ, Bauer CK, Schwarz JR (1995) Electrophysiology of anterior pituitary cells. In: Scherübl H, Hescheler J (eds) The electrophysiology of neuroendocrine cells. CRC Press, Boca Raton, pp 101–143
17. Corrette BJ, Bauer CK, Schwarz JR (1996) An inactivating inward-rectifying K current present in prolactin cells from the pituitary of lactating rats. J Membr Biol 150:185–195
18. Cui J, Kagan A, Qin D, Mathew J, Melman YF, McDonald TV (2001) Analysis of the cyclic nucleotide binding domain of the HERG potassium channel and interactions with KCNE2. J Biol Chem 276:17244–17251
19. Douglas WW (1968) Stimulus-secretion coupling: the concept and clues from chromaffin and other cells. Br J Pharmacol 34:451–474
20. Doupnik CA, Davidson N, Lester HA (1995) The inward rectifier potassium channel family. Curr Opin Neurobiol 5:268–277
21. Falk T, Meyerhof W, Corrette BJ, Schäfer J, Bauer CK, Schwarz JR, Richter D (1995) Cloning, functional expression and mRNA distribution of an inwardly rectifying potassium channel protein. FEBS Lett 367:127–131
22. Flitsch J, Lüdecke DK, Stahnke N, Wiebel J, Saeger W (2000) Transsphenoidal surgery for pituitary gigantism and galactorrhea in a 3.5 year old child. Pituitary 2:261–267
23. Heath BM, Terrar DA (2000) Protein kinase C enhances the rapidly activating delayed rectifier potassium current, I_{Kr}, through a reduction in C-type inactivation in guinea-pig ventricular myocytes. J Physiol (Lond) 522:391–402
24. Hinkle PM, Nelson EJ, Ashworth R (1996) Characterization of the calcium response to thyrotropin-releasing hormone in lactotrophs and GH cells. Trends Endocrinol Metabolism 7:370–374
25. Ishibashi M, Yamaji T (1985) Mechanism of the inhibitory action of dopamine and somatostatin on prolactin secretion from human lactotrophs in culture. J Clin Endocrinol Metab 60:599–606
26. Israel J-M, Jaquet P, Vincent J-D (1985) The electrical properties of isolated human prolactin-secreting adenoma cells and their modification by dopamine. Endocrinology 117:1448–1455
27. Kang J, Chen XL, Wang L, Rampe D (2001) Interactions of the antimalarial drug mefloquine with the human cardiac potassium channels KvLQT1/mink and HERG. J Pharmacol Exp Ther 299:290–296
28. Kidokoro Y (1975) Spontaneous calcium action potentials in a clonal pituitary cell line and their relationship to prolactin secretion. Nature 258:741–742
29. Kobras G (1992) The clinical, morphological and biological characteristics of prolactinomas following dopamine agonist treatment – review of the literature. Pathologe 13:183–189
30. Kuryshev YA, Haak L, Childs GV, Ritchie AK (1997) Corticotropin releasing hormone inhibits an inwardly rectifying potassium current in rat corticotropes. J Physiol (Lond) 502:265–279
31. Mazhari R, Greenstein JL, Winslow RL, Marban E, Nuss HB (2001) Molecular interactions between two long-QT syndrome gene products, HERG and KCNE2, rationalized by in vitro and in silico analysis. Circ Res 89:33–38
32. McDonald TV, Yu ZH, Ming Z, Palma E, Meyers MB, Wang KW, Goldstein SAN, Fishman GI (1997) A minK-HERG complex regulates the cardiac potassium current I_{Kr}. Nature 388:289–292
33. Mollard P, Theler JM, Guerineau N, Vacher P, Chiavaroli C, Schlegel W (1994) Cytosolic Ca^{2+} of excitable pituitary cells at resting potentials is controlled by steady state Ca^{2+} currents sensitive to dihydropyridines. J Biol Chem 269:25158–25164
34. Ozawa S, Sand O (1986) Electrophysiology of excitable endocrine cells. Physiol Rev 66:887–951
35. Sand O, Chen B, Li Q, Karlsen HE, Bjoro T, Haug E (1989) Vasoactive intestinal peptide (VIP) may reduce the removal rate of cytosolic Ca^{2+} after transient elevations in clonal rat lactotrophs. Acta Physiol Scand 137:113–123
36. Schäfer R, Wulfsen I, Behrens S, Weinsberg F, Bauer CK, Schwarz JR (1999) The erg-like potassium current in rat lactotrophs. J Physiol (Lond) 518:401–416
37. Scherübl H, Hescheler J (1991) Steady-state currents through voltage-dependent, dihydropyridine-sensitive Ca^{2+} channels in GH3 pituitary cells. Proc R Soc Lond B Biol Sci 245:127–131
38. Schledermann W, Wulfsen I, Schwarz JR, Bauer CK (2001) Modulation of rat erg1, erg2, erg3 and HERG K^+ currents by thyrotropin-releasing hormone in anterior pituitary cells via the native signal cascade. J Physiol (Lond) 532:143–163
39. Schroeder BC, Waldegger S, Fehr S, Bleich M, Warth R, Greger R, Jentsch TJ (2000) A constitutively open potassium channel formed by KCNQ1 and KCNE3. Nature 403:196–199
40. Schwarz JR, Bauer CK (1999) The ether-à-go-go-related gene K^+ current: functions of a strange inward rectifier. News Physiol Sci 14:135–142
41. Shi WM, Wymore RS, Wang HS, Pan ZM, Cohen IS, McKinnon D, Dixon JE (1997) Identification of two nervous system-specific members of the erg potassium channel gene family. J Neurosci 17:9423–9432
42. Shibasaki T (1987) Conductance and kinetics of delayed rectifier potassium channels in nodal cells of the rabbit heart. J Physiol (Lond) 387:227–250
43. Simasko SM (1991) Evidence for a delayed rectifier-like potassium current in the clonal rat pituitary cell line GH3. Am J Physiol 261:E66–E75
44. Simasko SM, Sankaranarayanan S (1997) Characterization of a hyperpolarization-activated cation current in rat pituitary cells. Am J Physiol 35:E405–E414
45. Spector PS, Curran ME, Zou AR, Sanguinetti MC (1996) Fast inactivation causes rectification of the I_{Kr} channel. J Gen Physiol 107:611–619
46. Taglialatela M, Castaldo P, Pannaccione A, Giorgio G, Genovese A, Marone G, Annunziato L (1999) Cardiac ion channels and antihistamines: possible mechanisms of cardiotoxicity. Clin Exp Allergy 29:182–189
47. Vacher P, McKenzie J, Dufy B (1989) Arachidonic acid affects membrane ionic conductances of GH3 pituitary cells. Am J Physiol 257:E203–E211
48. Vance ML, Thorner MO (1995) Prolactin-hyperprolactinemic syndromes and management. In: de Groot LJ (ed) Endocrinology. Saunders, Philadelphia, pp 394–405
49. Velkeniers B (2001) From prolactin cell to prolactinoma. Verh K Acad Geneeskd Belg 63:561–573
50. Wang SM, Liu SG, Morales MJ, Strauss HC, Rasmusson RL (1997) A quantitative analysis of the activation and inactivation kinetics of HERG expressed in *Xenopus* oocytes. J Physiol (Lond) 502:45–60
51. Warmke W, Ganetzky B (1994) A family of potassium channel genes related to eag in *Drosophila* and mammals. Proc Natl Acad Sci USA 91:3438–3442
52. Weinsberg F, Bauer CK, Schwarz JR (1997) The class III antiarrhythmic agent E-4031 selectively blocks the inactivating inward-rectifying potassium current in rat anterior pituitary tumor cells (GH_3/B_6 cells). Pflugers Arch 434:1–10
53. Wimmers S, Wulfsen I, Bauer CK, Schwarz JR (2001) Erg1, erg2 and erg3 K^+ channel subunits are able to form heteromultimers. Pflugers Arch 441:450–455
54. Wimmers S, Bauer CK, Schwarz JR (2002) Biophysical properties of heteromultimeric erg K^+ channels. Pflugers Arch DOI 10.1007/s00424-002-0936-4
55. Wulfsen I, Hauber HP, Schiemann D, Bauer CK, Schwarz JR (2000) Expression of mRNA for voltage-dependent and inward-rectifying K channels in GH_3/B_6 cells and rat pituitary. J Neuroendocrinol 12:263–272