Identification and characterization of Ca2+-activated K+ channels in granulosa cells of the human ovary
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Abstract
Background: Granulosa cells (GCs) represent a major endocrine compartment of the ovary producing sex steroid hormones. Recently, we identified in human GCs a Ca2+-activated K+ channel (KCa) of big conductance (BKCa), which is involved in steroidogenesis. This channel is activated by intracellular signalling molecules (e.g. acetylcholine) via raised intracellular Ca2+ levels. In this study, we aimed at characterizing 1. expression and functions of KCa channels (including BKCa beta-subunits), and 2. biophysical properties of BKCa channels.

Methods: GCs were obtained from in vitro-fertilization patients and cultured. Expression of mRNA was determined by standard RT-PCR and protein expression in human ovarian slices was detected by immunohistochemistry. Progesterone production was measured in cell culture supernatants using ELISAs. Single channels were recorded in the inside-out configuration of the patch-clamp technique.

Results: We identified two KCa types in human GCs, the intermediate- (IK) and the small-conductance KCa (SK). Their functionality was concluded from attenuation of human chorionic gonadotropin-stimulated progesterone production by KCa blockers (TRAM-34, apamin). Functional IK channels were also demonstrated by electrophysiological recording of single KCa channels with distinctive features. Both, IK and BKCa channels were found to be simultaneously active in individual GCs. In agreement with functional data, we identified mRNA encoding IK, SK1, SK2 and SK3 in human GCs and proteins of IK and SK2 in corresponding human ovarian cells. Molecular characterization of the BKCa channel revealed the presence of mRNAs encoding several BKCa beta-subunits (beta2, beta3, beta4) in human GCs. The multitude of beta-subunits detected might contribute to variations in Ca2+ dependence of individual BKCa channels which we observed in electrophysiological recordings.

Conclusion: Functional and molecular studies indicate the presence of active IK and SK channels in human GCs. Considering the already described BKCa, they express all three KCa types known. We suggest that the plurality and co-expression of different KCa channels and BKCa beta-subunits might allow differentiated responses to Ca2+ signals over a wide range caused by various intraovarian signalling molecules (e.g. acetylcholine, ATP, dopamine). The knowledge of ovarian KCa channel properties and functions should help to understand the link between endocrine and paracrine/autocrine control in the human ovary.
Background

Ion channels of ovarian granulosa cells (GCs) have been identified and functionally characterized in only a few species (human, swine, chicken) ([1-3] and references in [4]). In human and porcine GCs, several channel types are involved in the physiologically important process of progesterone production [4-7]. A $K_{Ca}$ channel of large (big) conductance ($BK_{Ca}$) in human GCs has a part in endocrine-regulated progesterone production [8]. Blocking of $BK_{Ca}$ channels results in reduction of human chorionic gonadotropin (hCG)-induced progesterone production, but does not affect basal steroidogenesis.

Moreover, $BK_{Ca}$ channels in human GCs were shown to be opened by cholinergic and oxytocinergic stimulation entailing transient membrane hyperpolarisation [8]. Acetylcholine (ACh) is produced by and also acts upon human GCs. In the human ovary, the non-neuronal cholinergic system affects several physiological functions, e.g. cell proliferation, gene transcription, and intercellular communication via gap junctions [9]. It represents just one of the local signaling systems which have been identified in recent years to complement endocrine (FSH, LH/hCG) and neuronal control of ovarian functions via autocrine/paracrine pathways. Besides ACh, other local intraovarian signaling molecules are peptide hormones (e.g. oxytocin, relaxin), catecholamines (e.g. norepinephrine, dopamine), ATP, prostaglandins, GABA and histamine. Many of these compounds (e.g. ACh, oxytocin, relaxin) exert their actions upon GCs via alteration of intracellular Ca$^{2+}$ levels [Ca$^{2+}$i] [8-12]. In human GCs, ACh and its agonist carbachol activate muscarinic receptors (e.g. M1) and increase [Ca$^{2+}$i] via Ca$^{2+}$ release from intracellular stores [9]. Activation of Ca$^{2+}$-activated ion channels such as the $BK_{Ca}$ is a well-known consequence of raised [Ca$^{2+}$i] [13-17].

Up to now, three $K_{Ca}$ families are known, which are classified by their single channel conductance: SK (small conductance $K_{Ca}$), IK (intermediate conductance $K_{Ca}$) and $BK_{Ca}$ [13,15-18]. They differ also regarding molecular and biophysical properties as well as their regulation by pharmacological compounds. For each class specific blockers exist which do not affect the two other classes. Three different apamin-sensitive SK channels were cloned (SK1, SK2, SK3), which exhibit single-channel conductances of $g_{sec} = 2-20$ pS [18]. The IK with $g_{sec} = 10-80$ pS is sensitive to TRAM-34 and was found in only a few non-neuronal cells, e.g. epithelial cells and erythrocytes (‘Gárdos channel’) [16,19-21]. The $BK_{Ca}$ channel has one of the highest known single channel conductances of $g_{sec} = ca. 200$ pS and is sensitive to iberiotoxin (IbTx). It is the only subtype of the $K_{Ca}$ family that exhibits a pronounced voltage-dependence in addition to its Ca$^{2+}$-sensitivity [13,14,16,22-24].

Our study aimed at the investigation of $K_{Ca}$ channels in human GCs since one member of this channel group, i.e. the $BK_{Ca}$ channel, has a part in both endocrine (i.e. role in hCG-stimulated steroidogenesis) and autocrine/paracrine pathways (i.e. cholinergic and oxytocinergic activation). The human GCs investigated originate from pre-ovulatory human follicles obtained from patients undergoing in vitro-fertilization (IVF). They represent a cell culture model of their in vivo counterparts in the antral follicle and the young active corpus luteum (CL). The study shall provide data on molecular characterization of other members of the $K_{Ca}$ channel family (SK, IK) in human GCs and their potential role in steroidogenesis. Furthermore, the Ca$^{2+}$- and voltage-dependence of the $BK_{Ca}$ channel was determined in electrophysiological single-channel recordings. As $BK_{Ca}$ channel characteristics are affected by the type(s) of accessory $\beta$ subunits present, we studied their expression in human GCs as well. There are four potential types known ($\beta$1, $\beta$2, $\beta$3, and $\beta$4), which interact with the $\alpha$ subunit and regulate $BK_{Ca}$ channel function regarding impact of Ca$^{2+}$ and voltage [25-29]. They represent also binding sites for toxins and drugs as well as phosphorylation sites [26,27,30-32].

Methods

Human GC preparation and culture

Human GCs were isolated from follicular aspirates of women undergoing IVF and cultured in DMEM/F12 (10% FCS; Sigma-Aldrich, Munich, Germany) under a humidified atmosphere at 37°C/5% CO$_2$ [33]. Use of cells was approved by the patients and the Ethics Committee of the University of Munich. All patients were treated following standard IVF protocols and negatively diagnosed for the polycystic ovary syndrome. To account for patient-to-patient variations all studies were performed on several, randomly selected cell preparations (pooled from up to 3 patients) on different days.

Human tissue samples

Human ovarian samples containing CL from consenting patients undergoing gynecological surgery (generously provided by C. Heiss, Klinik am Eichert, Göppingen, Germany) were fixed in Bouin’s fixative and embedded in paraffin. Apart from this, we used paraffin-embedded ovarian samples with follicles from the tissue archive of the Women’s Hospital in Munich, which had been taken from pre-menopausal women during autopsies [34]. The Ethics Committee of the University of Munich approved all procedures concerning use of human materials. Pathological deterioration of follicles and corpora lutea in these human ovarian samples were excluded by morphological and microscopical assessment.

Chemicals and solutions

A stock solution of apamin (Alomone Labs, Jerusalem, Israel) was prepared in distilled water. TRAM-34 (1-[[2-Chlorophenyl]diphenylmethyl]-1H-pyrazole; Sigma-Aldrich) was dissolved in DMSO (10 mM) and final
DMSO concentration in the cell culture medium did not exceed 0.01% (v/v).

**Progesterone assay**

Progesterone concentrations were measured in supernatants of human GCs cultured in 24-well plates. On day three of culture, cells were treated in triplicates with the respective compounds. After 24 h the supernatants were collected and the progesterone concentrations were measured using an ELISA (DRG Instruments, Marburg, Germany). Experiments were repeated with cells from independent cell preparations (each pooled from up to 3 patients) to account for interpatient variability. After normalization to the respective control (untreated) values, data were statistically analyzed by a repeated measures ANOVA followed by Newman-Keuls multiple comparison test.

**Electrophysiology**

Human GCs were grown on glass cover slips for 2–12 days and currents were recorded at room temperature by means of an EPC-9 amplifier (HEKA elektronik, Lambrecht, Germany; sample rate, 10 kHz; low pass filter, 2.5 kHz) [8]. Positive currents represent outward currents and all potentials given refer to the cytoplasmic side of the plasma membrane. Potentials were corrected for a liquid junction potential of +16 mV [35]. The extracellular solution (EC) contained (in mM) 140 NaCl, 3 KCl, 1 CaCl$_2$, 1 MgCl$_2$, 10 Hepes, and 10 glucose (pH 7.4). The intracellular solution (IC) contained (in mM) 130 K-gluconate, 5 NaCl, 2 EGTA, 1 MgCl$_2$, and 10 Hepes (pH 7.4). Single channel currents were recorded in the inside-out configuration. To assess the Ca$^{2+}$-sensitivity of the BK$_{Ca}$ channel the free Ca$^{2+}$ concentration [Ca$^{2+}$]$_i$ of the IC solutions was adjusted by using varying concentrations of CaCl$_2$, according to calculations performed by means of the software "Calcium" [36]. The following CaCl$_2$ concentrations were used: 1.000 mM ([Ca$^{2+}$]$_i$ = 100 nM), 1.870 mM ([Ca$^{2+}$]$_i$ = 1 μM), 1.977 mM ([Ca$^{2+}$]$_i$ = 5 μM), 1.996 mM ([Ca$^{2+}$]$_i$ = 10 μM), 2.013 mM ([Ca$^{2+}$]$_i$ = 20 μM), 2.100 mM ([Ca$^{2+}$]$_i$ = 100 μM), and 3.000 mM ([Ca$^{2+}$]$_i$ = 1 mM), respectively. To obtain channel current-voltage relationships a voltage protocol ranging from -80 mV to +90 mV in 10 mV steps was used with each potential applied for 200 ms. For evaluation of channel open probability $P_o$, single channel currents were recorded at +60 mV, +70 mV, +80 mV, and +90 mV for 1600 ms in each case. For analysis, frequency histograms of current traces were calculated and the two amplitude peaks corresponding to open and closed channel state were fitted using Gaussian distributions. The single channel current amplitude was measured as differences between the two peaks. Integration of the area under the curves and division of the area under the open channel peak by the area under the entire curve yielded $P_o$. In the case of two or more active individual channels in an inside-out recording, the open probability per one channel was calculated by converting the values according to the number of simultaneously active channels at each time point. [Ca$^{2+}$]$_i$ values for half-maximal activation $EC_{50}$ were determined by fitting $P_o$ as a function of [Ca$^{2+}$]$_i$ with a sigmoidal dose-response-curve with variable Hill slope and a fixed bottom value of zero. The voltage-dependence of $P_o$ was fitted using a Boltzmann sigmoidal function with the bottom value fixed to be 0 and potentials for half-maximal activation $V_{50}$ as well as slope values were obtained. The values are given with the respective 95% confidence interval (C.I.).

**RT-PCR**

Total RNA was isolated from several human GC preparations, and reverse transcribed using Superscript-RT II (Life Technologies, Karlsruhe, Germany) in combination with either a 18-mer polydeoxynthymidine primer or random hexamers of polydeoxynucleotide primers. PCR amplification was carried out with oligodeoxynucleotide primer pairs, which spanned at least one intron of the genomic sequence (except for the second BK β1 primer pair; Table 1) [37,38]. The PCR protocol consisted of 35 cycles of denaturation at 94°C (30 s), annealing at the temperatures given in Table 1 (30 s), and elongation at 72°C (45 s) using a PTC-200 Peltier Thermal Cycler (MJ Research, Bio-Rad, Munich, Germany). PCR products were separated on an agarose gel and visualized by ethidium bromide staining and ultraviolet illumination. Identity of all PCR products was verified by sequencing (Agowa, Berlin, Germany).

**cDNA array**

Total RNA of GCs cultivated for 3 days was isolated and subjected to the GEArray Q Series Human Neurosciences-1 Ion Channel and Transporter Gene Array (SuperArray Bioscience Corp., Frederick, MD). The cDNA array was analyzed by means of a chemiluminescent detection method (Roche Diagnostics, Mannheim, Germany) [39].

**Immunohistochemistry**

Localization of K$_{Ca}$ α subunit proteins in human ovarian sections (5 μm) was examined according to standard procedures [34,40]. Antisera were purchased from Alomone Labs (Jerusalem, Israel) and utilized in the denoted dilutions: anti-K$_{Ca}$ 3.1 (IK; rabbit anti-human; 1:200), anti-SK1 (rabbit anti-rat; 1:200), anti-SK2 (rabbit anti-rat; 1:200), anti-SK3N (rabbit anti-human; 1:500), and anti-SK3C (rabbit anti-human; 1:500). The deparaffinized sections were subjected to an additional microwave treatment for improved antigen retrieval [40] and treated with 3% H$_2$O$_2$ in methanol to block endogenous peroxidase. Thereafter slices were incubated at 4°C overnight with the respective antisera (containing 5% normal goat serum) and finally with goat anti-rabbit antibody (1:500). Immunoreactivity was visualized by the ABC-diaminobenzidine staining reaction ( Vectastain Elite Kit, Vector Laboratories,
Table 1: Oligodeoxynucleotide primer pairs used for PCR amplification.

<table>
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<th>Channel subunit</th>
<th>Alternative nomenclature</th>
<th>Primer sequence 5' - 3'</th>
<th>GenBank accession no.</th>
<th>Annealing temperature</th>
<th>Product size</th>
<th>Source</th>
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<td>BK β1</td>
<td>KCNMB1</td>
<td>Sense AAGGTCAGAGGCCAAA TTCCAAG</td>
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<td></td>
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<td>KCNMB2</td>
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<td>59°C</td>
<td>177 bp</td>
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<td>KCNMB3</td>
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<td>451 bp</td>
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<td>134 bp</td>
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</table>

Primer sequences were derived from the PrimerBank (ID: http://pga.mgh.harvard.edu/primerbank; [37], from the literature, or designed using the software Lasergene (DNAStr; see source).
Cytotoxicity assays
Potential cytotoxicity of the channel blockers was evaluated by using commercial non-radioactive cell proliferation assays (CellTiter and CellTiter-Glo, both from Promega, Mannheim, Germany) [11]. Human GCs were cultured in 24- or 48-well plates and treated in triplicates for 24 h on day 3 of culture with the substances applied for progesterone measurements.

Expression of different K<sub>Ca</sub> channel families in human GCs
The presence of mRNAs coding for all known types of SK channels and the IK channel was demonstrated by cDNA arrays (except for SK2) and by RT-PCR followed by sequencing (Figure 2A). In case of SK1 two PCR products were found and sequenced, which both correspond to the respective channel. They differ with regard to presence or absence of exon 9 (the primers match sequences in exon 8 and 10, respectively) and are, thus, most likely yet unknown splicing variants. IK and SK2 α subunit proteins are expressed in GCs of the human ovary (Figure 2). Expression of SK1 and SK3 α subunits cannot be unambiguously appraised since the antisera used for immunohistochemistry produced unspecific (e.g. nuclear) staining (data not shown).

In electrophysiological recordings in the inside-out configuration, a second type of K<sub>Ca</sub> channel besides the BK<sub>Ca</sub> was recorded at [Ca<sup>2+</sup>]<sub>i</sub> > 1 μM (Figure 3). This channel is most likely the IK channel because of its characteristic intermediate single-channel conductance of 67 ± 5 pS and a reversal potential of <i>V</i><sub>0</sub> = 7 mV under symmetrical K<sup>+</sup>-gluconate concentrations (n = 5; Figure 3B), its increasing <i>P</i><sub>o</sub> with rising [Ca<sup>2+</sup>]<sub>i</sub> (data not shown), and because K<sup>+</sup> was the only permeant ion present in sufficiently high concentrations on both sides of the membrane. The maxiCl can be excluded due to the low symmetrical Cl-concentrations of 9 mM. In almost all recordings exhibiting the IK channel, at least one BK<sub>Ca</sub> channel was simultaneously active (Figure 3A).

Ca<sup>2+</sup>- and voltage-dependence of BK<sub>Ca</sub> channels in human GCs
BK<sub>Ca</sub> channels recorded in the inside-out configuration exhibited a characteristic single channel conductance in the range of 200 pS. Channel open probability <i>P</i><sub>o</sub> increased with both elevating [Ca<sup>2+</sup>]<sub>i</sub> and voltage (Figure 4). The Ca<sup>2+</sup>-dependence of the BK<sub>Ca</sub> was evaluated by fitting <i>P</i><sub>o</sub> as a function of [Ca<sup>2+</sup>]<sub>i</sub> with a sigmoidal dose-response curve (Figure 4B). The [Ca<sup>2+</sup>]<sub>i</sub> for half-maximal activation were EC<sub>50</sub> (60 mV) = 20 μM (9 to 41 μM; n = 30), Hill slope = 1.0 μM (0.5 to 1.5 μM), and EC<sub>50</sub> (70 mV) = 12 μM (6 to 24 μM, n = 30), slope = 0.8 μM (0.3 to 1.4 μM). Comparing individual cells, heterogeneity of EC<sub>50</sub> values – even in the same cell preparation – was observed. The values covered a range of 2 to 118 μM (at +60 mV; n = 8) and of 1 to 91 μM (at +70 mV; n = 9), respectively. At high [Ca<sup>2+</sup>]<sub>i</sub> (1 mM) and high positive...
potentials (+70 mV) inactivation of BK\textsubscript{Ca} channels was observed for time periods ranging from several hundred ms to several s (Figure 4C). Periods of inactivation longer than 500 ms were not considered when calculating $P_\text{o}$; only the trace before onset of inactivation was evaluated. The voltage-dependence of the BK\textsubscript{Ca} channel was determined at high [Ca$^{2+}$] because at lower [Ca$^{2+}$] the channel was not active at negative potentials. By fitting the data with a Boltzmann sigmoidal function a potential for half-maximal activation of $V_{50} = -54$ mV (-56 to -51 mV, 95%-C.I.) and a slope factor of +12 mV (+9 to +15 mV) were observed at [Ca$^{2+}$] = 1 mM (n = 4; Figure 4D).

**Expression of BK\textsubscript{Ca}β subunits in human GCs**

By RT-PCR and subsequent sequencing, we identified mRNAs encoding the BK\textsubscript{Ca} subunits β2, β3, and β4 in human GCs (Figure 5A). The β1 subunit was not detected using two different primer pairs, which amplified the β1 subunit in positive control tissues (ovary, prostate, testis, colon, heart, and lung; data not shown). The BK\textsubscript{Ca}β4 subunit protein was also found in endocrine cells of the human CL by means of immunohistochemistry (Figure 5B).

**Discussion**

In the present communication we report that human GCs possess in addition to the BK\textsubscript{Ca} other functional K\textsubscript{Ca} channels. The detection of mRNAs encoding the intermediate-conductance K\textsubscript{Ca} (IK) as well as all three known types of small-conductance K\textsubscript{Ca} (SK1, SK2, SK3) points at a complex K\textsubscript{Ca} repertoire. The finding of mRNA alone can be misleading as was shown in a study on glioma cells in which mRNAs for...
Figure 4
Electrophysiological characterization of $\text{Ca}^{2+}$- and voltage-dependence of $\text{BK}_{\text{Ca}}$ channels in human GCs (inside-out recordings). (A) $\text{BK}_{\text{Ca}}$ current traces recorded at +60 mV and varying $[\text{Ca}^{2+}]_i$ (right). The closed channel state (c) corresponds to the respective lower current level (o, open state). (B) $\text{Ca}^{2+}$-dependence of the channel open probability, $P_o$, at +70 mV. Data (black circles) represent means ± SEM of recordings from 30 cells and were fitted by a sigmoidal dose-response function with the bottom value fixed to be 0. $\text{EC}_{50} = 12 \mu\text{M}$, Hill-slope = +0.8 $\mu\text{M}$ ($n = 30$). Gray circles and fit curves show data from two individual recordings that represent extrema of $\text{Ca}^{2+}$-dependence of $P_o$. (C) $\text{BK}_{\text{Ca}}$ channel inactivation at $[\text{Ca}^{2+}]_i = 1 \text{mM}$ and +70 mV. (D) Voltage-dependence of $P_o$ at $[\text{Ca}^{2+}]_i = 1 \text{mM}$ ($V_{50} = -54 \text{ mV}$, $n = 4$). Data were fitted by a Boltzmann sigmoidal function with bottom value fixed to be 0.
all $K_{Ca}$ channels were detected, although only $K_{Ca}$ channels were functional [41]. However, in human GCs all three classes of $K_{Ca}$ channels are functional, because specific blockers attenuated hCG-stimulated steroidogenesis. The IK blocker TRAM-34 was recently reported to inhibit nonselective cation channels as well [42], but is still one of the most accepted pharmacological tools to block IK channels. We cannot definitely exclude a role of nonselective cation channels. However, as they were not found in human GCs so far and because we presented further molecular proof of IK presence, we interpret the TRAM-34 action as blockage of IK channels. In addition, during stimulation local rises in $[Ca^{2+}]_i$ in close proximity to the channel might be much higher than the monitored overall cytoplasmic elevation of $[Ca^{2+}]_i$ and thereby $K_{Ca}$ channels can be opened at more physiological voltages. Pronounced differences in alteration of global $[Ca^{2+}]_i$ monitored by standard techniques and of local $[Ca^{2+}]_i$ were described in other cell types [46,47].

The variability of $EC_{50}$ values for $Ca^{2+}$-sensitivity of the $K_{Ca}$ channel over two orders of magnitude could be due to different $\beta$ subunits present since they are known to be important modulators of $Ca^{2+}$- and voltage-sensitivity of the channel [25-32,48]. Similar variations of $EC_{50}$ and half-maximal activation voltages were reported for $K_{Ca}$ in other cell types [13,15,43,45,49-51]. Therefore, we have studied the $K_{Ca}$ $\beta$ subunit expression in human GCs and found mRNAs encoding the accessory subunits $\beta_2$, $\beta_3$, and $\beta_4$, but not $\beta_1$. Reports on the presence of $\beta_2$, $\beta_3$ and $\beta_4$ mRNAs in material from whole human ovaries can now be better interpreted in terms of a GCs contribution to the mRNA findings [29,31].

The consequences of $K_{Ca}$ $\beta$ subunit expression pattern for channel function in human GCs are difficult to compare with studies in cellular model systems expressing only one type of $\beta$ subunit. Nevertheless, the $\beta$ subunit repertoire found on the mRNA level might help to explain our observations. The recorded inactivation of $K_{Ca}$ channels at high positive potentials and at high free $[Ca^{2+}]_i$ was reported to occur in the presence of $\beta_2$ and/or $\beta_3$ subunits [26-28,52,53]. The absence of the $\beta_1$ subunit might explain why oestrogens do not activate the $K_{Ca}$ in human GCs [8]; in contrast to myometrial smooth muscle in which activation by oestrogen was ascribed to the presence of $\beta_1$ [54,55]. The presence of $\beta_4$ should introduce IbTx-resistance to $K_{Ca}$ channels [32]. However, the fact that IbTx blocks both $K_{Ca}$ whole-cell currents and hCG-
stimulated progesterone production points at a more complex picture, i.e. at least a proportion of the \( \beta C_{Ca} \) channels in human GCs is \( \beta C_{Ca} \)-sensitive and, thus, probably contains not only \( \beta 4 \) [8].

The simultaneous presence of different \( \beta C_{Ca} \) types is known from other cell types [18]. But what could be the cellular relevance of the ostensibly redundancy to have different \( \beta C_{Ca} \) channels? They differ regarding regulation, biophysical properties as well as \( Ca^{2+} \) sensitivity with IK and SK channels being activated at lower \( Ca^{2+} \) than the BK \( Ca_{Ca} \) [17,18]. In addition, the \( BK_{Ca} \) is voltage-sensitive in contrast to other \( \beta C_{Ca} \) channels [13,14,16,24], which would allow differentiated responses to the same \( Ca^{2+} \) signals at varying membrane potentials. Concerning the multitude of \( \beta C_{Ca} \) channels in human GCs the question arises whether each individual GC expresses all identified \( \beta 4 \) channel subunits in parallel. The RT-PCR and progesterone production experiments can provide no answers about single cells. But single channel recordings revealed that at least \( BK_{Ca} \) and IK channels can be present in the plasma membrane of the same individual cells. However, it is very likely that individual GCs can exhibit a varying \( \beta 4 \) repertoire and that GC subpopulations might exist regarding the expression of \( \beta \) subunits. Immuno-histochemical results are in favor of such an assumption, since for IK, SK2, and \( \beta 4 \), the degree of immunostaining in GCs of the human CL varies. The variations in \( Ca^{2+} \)-sensitivity observed in single \( BK_{Ca} \) channel recordings might also reflect \( BK_{Ca} \) heterogeneity in single GCs and/or between individual GCs.

**Conclusion**

In summary, we found expression (in vitro, ex vivo) of several classes of \( \beta C_{Ca} \) channels in human GCs, which are all involved in gonadotropin-stimulated sex steroid hormone production. The presence of different \( \beta C_{Ca} \) channels and the observed heterogeneity in \( Ca^{2+} \)-sensitivity of the \( BK_{Ca} \) channel, which is probably due to expression of various \( \beta \) subunits, could allow finely tuned and differentiated cellular responses over a wide \( Ca^{2+} \) range. The question of existence of GCs subpopulations regarding \( \beta C_{Ca} \) channels and BK \( Ca_{Ca} \) \( \beta \) subunits has to be studied in the future to understand cellular processes on the level of individual GCs. The rich instrumentation of \( Ca^{2+} \)-dependent channels might be seen in relation to the abundance of intraovarian signaling molecules (e.g. \( ACh \), ATP, dopamine, oxytocin, relaxin) acting via raised \( Ca^{2+} \) levels. Therefore, we suggest that this channel group has a part in mediating the conjunction between endocrine (hCG, LH) and local ovarian signaling systems.

**Abbreviations**

\( ACh \): acetylcholine; \( BK_{Ca} \): big conductance \( \beta C_{Ca} \) \( Ca^{2+} \); intracellular \( Ca^{2+} \) concentration; C.I.: confidence interval; CL: Corpus luteum; EC\(_{50} \): \( Ca^{2+} \) for half-maximal activation; GC: granulosa cell; \( g_{sc} \): single-channel conductance; hCG: human chorionic gonadotropin; \( Ib/fx \): iberiotoxin; IK: intermediate conductance \( \beta C_{Ca} \); IVF: in vitro-fertilization; \( K_{Ca} \): \( Ca^{2+} \)-activated \( K^{+} \) channel; \( P_o \): channel open probability; SK: small conductance \( \beta C_{Ca} \); TRAM-34: 1-\{2-Chlorophenyl\}diphenylmethyl]-11-1- pyrazole; \( V_{50} \): potential for half-maximal activation.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

MHT performed most of the experiments, was involved in conception of the study, contributed to analysis and interpretation of the data and to writing of the manuscript. DB and UB provided human GCs and were involved in study design. AM conceived of the study and contributed to interpretation of the data and to writing of the manuscript. LK conceived of the study, coordinated the experiments, contributed to analysis and interpretation of the data and to writing of the manuscript. All authors read and approved the final manuscript.

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**References**


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