Receptor-mediated modulation of recombinant neuronal class E calcium channels

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Abstract **The modulation of a cloned neuronal calcium channel was studied in a human embryonic kidney cell line (HEK293). The HEK293 cells were stably transfected with the α_{1Ed} cDNA, containing the pore forming subunit of a neuronal class E calcium channel. Inward currents of 25 ± 1.9 pA/pF (n = 79) were measured with the cloned α_{1Ed}-subunit. The application of the peptide hormone somatostatin, carbachol, ATP or adenosine reduced the amplitude of Ca^{2+} and Ba^{2+} inward currents and exhibited a slowing of inactivation. This inhibitory effect by somatostatin was significantly impaired after pre-incubating the transfected cell line with pertussis toxin (PTX). Internal perfusion of the cells with the G-protein-inactivating agent GDP-β-S or with the permanently activating agent GTP-γ-S also attenuated the somatostatin effect. The inhibition indicates that modulation of the α_{1Ed}-mediated Ca^{2+} current involves pertussis toxin-sensitive G-proteins. The block of Ca^{2+} and Ba^{2+} inward currents by somatostatin is also found in cells expressing a truncated α_{1Ed}-subunit which lacks a 129-bp fragment in the C-terminus. This fragment corresponds to the major structural difference between two native human α_{1E} splice variants. As somatostatin inhibits inward currents through both, the cloned α_{1Ed}- and the truncated $\alpha_{1Ed-DEL}$-subunit, the hormone-mediated modulation is independent from the presence of the 129-bp insertion in the C-terminus.**

Key words: Cloned Ca^{2+} channel; α_{1E}-Subunit; HEK293 cell; G-protein; Ionic current; Modulation; Somatostatin; Adenosine; ATP; Carbachol; Electrophysiology

1. Introduction

In neurons as well as in many non-exitable cells, the stimulation of multiple types of neurotransmitter and hormone receptors activates heterotrimeric GTP-binding proteins (G-proteins) which subsequently influence the activity of voltage-gated Ca^{2+} channels [1–6]. The regulation of Ca^{2+} channels is crucial as Ca^{2+} is one of the main intracellular messengers that triggers neurotransmitter release, contraction, secretion, and gene expression. Voltage-activated Ca^{2+} channels in skeletal muscle, heart and brain exist as heterooligomeric proteins composed of at least three different subunits, α_1, β, and $\alpha_2\delta$. Additional tissue-specific subunits, like a γ-subunit, are found e.g. in skeletal muscle (for review, see [7–10]).

The α_1-subunit determines the major biophysical and pharmacological properties of the channel complex. Molecular cloning and expression studies have demonstrated that the α_1-subunit is sufficient to form functional channels without the co-injection or co-transfection of auxiliary subunits [11]. The expression of the human α_{1B} subunit, however, might be dependent on auxiliary subunits [12].

So far, six different mammalian α_1-subunits have been described (α_{1A} to α_{1E}, and α_{1S}). The α_1-subunit of the class E (α_{1E}) has been cloned from human brain [11,13]. Channels formed by this type are resistant to common Ca^{2+} channel blockers and seem to underlie the Ca^{2+} currents predominantly found in cerebellar granule cells [31]. Immunocytochemical staining showed that the α_{1E}-subunit is distributed throughout the brain, and it is most prominently expressed in deep midline structures, such as caudate-putamen, thalamus, hypothalamus, amygdala, cerebellum, and a variety of nuclei in the ventral midbrain and brainstem [13–15]. Hormonal modulation of E type Ca^{2+} channel currents has been reported with opposite results [26,28]. The rabbit α_{1E-3}-subunit is inhibited by somatostatin (SST) [28] while the human homolog is insensitive when expressed in HEK293 cells [26]. In this study, we show that E type Ca^{2+} channel currents recorded from stable transfectants expressing human full-length α_{1E} (α_{1Ed}) or a truncated α_{1E}-subunit ($\alpha_{1Ed-DEL}$) in HEK293 cells are reduced by activation of somatostatin receptors. A similar reduction was achieved by stimulating purin-receptors and muscarinic receptors. This in vitro hormonal inhibition of Ca^{2+} inward currents is in accordance with the presence of structural motifs for Gβγ binding in cloned α_{1E}-subunits (for review, [37]).

2. Materials and methods

2.1. Construction and transfection of the expression plasmids

HEK293 cells were transfected with the longer human α_{1E} splice variant [11], defined as α_{1Ed} now. The *Sma*I fragment of the pHBE239 vector [11] which carried a full-length human α_{1Ed} cDNA was subcloned into the *Eco*RV site of the pcDNA3 vector (Invitrogen). In addition, a second expression plasmid was constructed (= $\alpha_{1Ed-DEL}$) in which 129 bp of the longer human α_{1Ed} splice variant [11] were deleted (bp 5896–6024, accession number in Genbank L27745). This insertion is not present in the human α_{1E-3} splice variant used in the study of Toth et al. [26] and represents the major difference between the two cloned human α_{1E} splice variants [11,13]. The cDNA of $\alpha_{1Ed-DEL}$ in the second expression plasmid was mutated by overlap extension using PCR-amplification with appropriate primer [35]. The amplified cDNA fragments of $\alpha_{1Ed-DEL}$ were completely sequenced. The successful deletion of 129 bp was confirmed, and the rest of the sequence was found to be identical with the wild-type α_{1Ed} cDNA.

HEK293 cells were transfected using standard calcium-phosphate precipitation technique (Stratagene Kit). Positive cell clones were iso-

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lated after growth in the presence of geneticin (1 mg/ml) and cloning by limiting dilution.

2.2. Culture of the HEK293 cells

The stably transfected HEK293 cells were grown in DMEM supplemented with 15% fetal calf serum, non-essential amino acids, antibiotics (penicillin, 50 IU/ml, streptomycin, 50 mg/ml, and geneticin, 1 mg/ml).

2.3. Electrophysiological recordings

Ca^{2+} and Ba^{2+} currents were measured using the whole-cell variant of the patch-clamp method [33] at 37°C. For data aquisition, an Axoclamp 2000 amplifier (Axon Instruments, USA) connected to a PC equipped with the ISO-2 acquisition package (Friedrich, Cologne, Germany) was used.

Prior to recording, cells were rinsed in normal Tyrode's solution. The coverslip with the cells was transferred to the recording chamber which was warmed to 37°C. All drugs were applied using a rapid perfusion system consisting of a multibarrelled ejection pipette. The cells were continuously superfused by standard extracellular solution or by the drug-containing solution. The extracellular solution contained (in mM): $CaCl_2$ or $BaCl_2$ 15, NMG 154, $MgCl_2$ 1, glucose 5, HEPES 10. The pH was adjusted to 7.4. The patch pipettes were filled with an internal solution containing (in mM): CsCl 112, $MgCl_2$ 1, HEPES 5, MgATP 1, EGTA 10 (pH 7.4). Part of the experiments were carried out using the perforated-patch method with amphotericin-B added to the pipette solution.

Recordings were filtered at 3 kHz using a 4-pole Bessel-filter and digitized at 40 kHz. Holding potentials of −80 mV were used to record ionic current data throughout the study. Steady-state inactivation of ionic currents were measured by 2-s pre-pulses and applying the depolarizing protocol from the same membrane potential (−80 mV) each time. Recordings were capacity corrected. From the holding potential, 4 scaled hyperpolarizing pre-pulses were given and the sum of the pre-pulses was subtracted from each recording.

2.4. Materials

Tissue culture media, sera and other supplements were from GIBCO (Eggenstein, Germany). Pertussis toxin and cholera toxin were obtained from RBI (Biotrend Chemikalien, Cologne, Germany). Guanosine 5′-*O*-(2-thiodiphosphate) (GDP-β-S); guanosine-5′-*O*-(3-thiotriphosphate)(GTP-γ-S) and all other substances were of the highest grade and purchased from Sigma (Deisenhofen, Germany). A concentrated stock solution (30 mg/ml) of amphotericine-B was made up in DMSO and diluted to the final concentration of 450 μg/ml in the pipette solution.

2.5. Data analysis

Statistical analysis and curve-fitting were carried out using the ISO-2 software package, Origin (Microcal, Northampton, MA), or SigmaPlot (Jandel). All grouped data are reported as mean ± S.E.M.

3. Results

3.1. Characterization of α_{1Ed} and $\alpha_{1Ed-DEL}$ channels expressed in HEK293 cells

During the time period of our measurements (within 2–4 days after trypsin-dissociation and plating), untransfected HEK293 cells did not express any significant voltage-dependent Ca^{2+} channel currents (see also [16]). The HEK293 cell clone expressing the α_{1Ed}-subunits developed current densities between 10 and 70 pA/pF with a mean of 25 ± 1.9 pA/pF ($n = 79$).

In stably transfected HEK293 cells, Ca^{2+} currents could be elicited in Ca^{2+} (Fig. 1A) or Ba^{2+}-containing solution (Fig. 1B). As a typical high-voltage-activated channel [11], the α_{1Ed} channel shows an activation curve similar to L-type Ca^{2+} channels. The channels activated at about −30 mV and maximal currents were elicited at +20 mV in Ca^{2+}-containing solution (Fig. 2). Inactivation occurred at rather negative holding potentials (Fig. 2C). The midpoint of the steady-state inactivation curve was calculated as −40 mV. With Ba^{2+} instead of Ca^{2+} as charge carrier, the current–voltage curve was shifted by 10 mV to more negative potentials potentials (Fig. 1C). However, kinetics of the Ba^{2+} currents were not different from the currents carried by Ca^{2+} (Fig. 1A). This suggests that the fast inactivation of the currents is not related to a Ca^{2+}-induced inactivation as it is seen with the L-type Ca^{2+} channels.

A second cell clone, which stably expressed the shorter $\alpha_{1Ed-DEL}$ subunit (see Section 2), showed identical basic biophysical properties as the α_{1Ed}-expressing cells. The mean current density of these HEK-$\alpha_{1Ed-DEL}$ cells was 16 ± 6 pA/pF ($n = 6$).

3.2. Somatostatin inhibition of Ca^{2+} currents

SST is known to inhibit the Ca^{2+} influx through voltage-dependent Ca^{2+} channels in various neuronal [17] and neuroendocrine cells [18]. HEK293 cells endogenously express SST receptors of the subtype SSTR2 [19,20]. Application of SST to α_{1Ed}-transfected HEK293 cells produced a rapid, reversible and concentration-dependent reduction in the magnitude of the Ca^{2+} and Ba^{2+} currents (Fig. 3). A concentration that induced a consistent maximal inhibitory effect, i.e. 1 μM SST, was used throughout all experiments (Fig. 3A). In some experiments, desensitization was observed after 20–30 s of SST application (Fig. 7B).

The SST-induced reduction of the Ca^{2+} and Ba^{2+} current amplitude was voltage-dependent. The Ca^{2+} current amplitude was reduced over the voltage range −10 to +40 mV with the greatest reduction occurring at the maximal amplitude (at +20 mV, Fig. 4A). Application of 1 μM SST led to a reduction of 50 ± 3.5% ($n = 25$) when 15 mM Ca^{2+} in the extracellular solution was present as charge carrier. In 15 mM Ba^{2+} solution, the SST effect on the peak current was less (37 ± 4.6%, $n = 6$).

The kinetics of the currents were not significantly changed by 1 μM SST. The time to peak was unaltered (Fig. 4B) and in the presence of SST the inactivating phase of the current was not significantly slower with time constant τ of 18 ± 0.6 and 19 ± 0.8 ms, $n = 45$, for control and SST, respectively, each in 15 mM Ca^{2+}. The current voltage relation and the inactivation characteristics during SST application were almost identical with those under control conditions (Fig. 4A,C).

No SST-induced reduction of the Ca^{2+} current amplitude was reported for the shorter human α_{1E} splice variant [26]. To investigate the hypothesis that the additional 129-bp insertion in α_{1Ed} (Fig. 5A) might be responsible for the contrary results, the 129-bp (bp 5896–6024 [11]) sequence was deleted [35]. The SST-mediated inhibition was also found in cell lines expressing the $\alpha_{1Ed-DEL}$-subunit (Fig. 5B). In 23 experiments with the deletion mutants, 6 cells did not react to SST, 2 showed a weak stimulation and in 15 cells Ca^{2+} inward current was blocked by 1 μM SST. The percentage of inhibition was 30 ± 17% ($n = 15$) showing a higher degree of variation as in the α_{1Ed}-expressing HEK293 cells. The fast decay of inhibition could be mediated by a faster desensitization or by other unknown processes (Fig. 5B). As both cell lines expressing α_{1Ed} or $\alpha_{1Ed-DEL}$, respectively, are reacting to SST, other factors must cause the observed differences with respect to SST modulation of human α_{1E}-subunits. A G-protein-binding site has been identified (Fig. 5C) in the I–II loop of all three non-

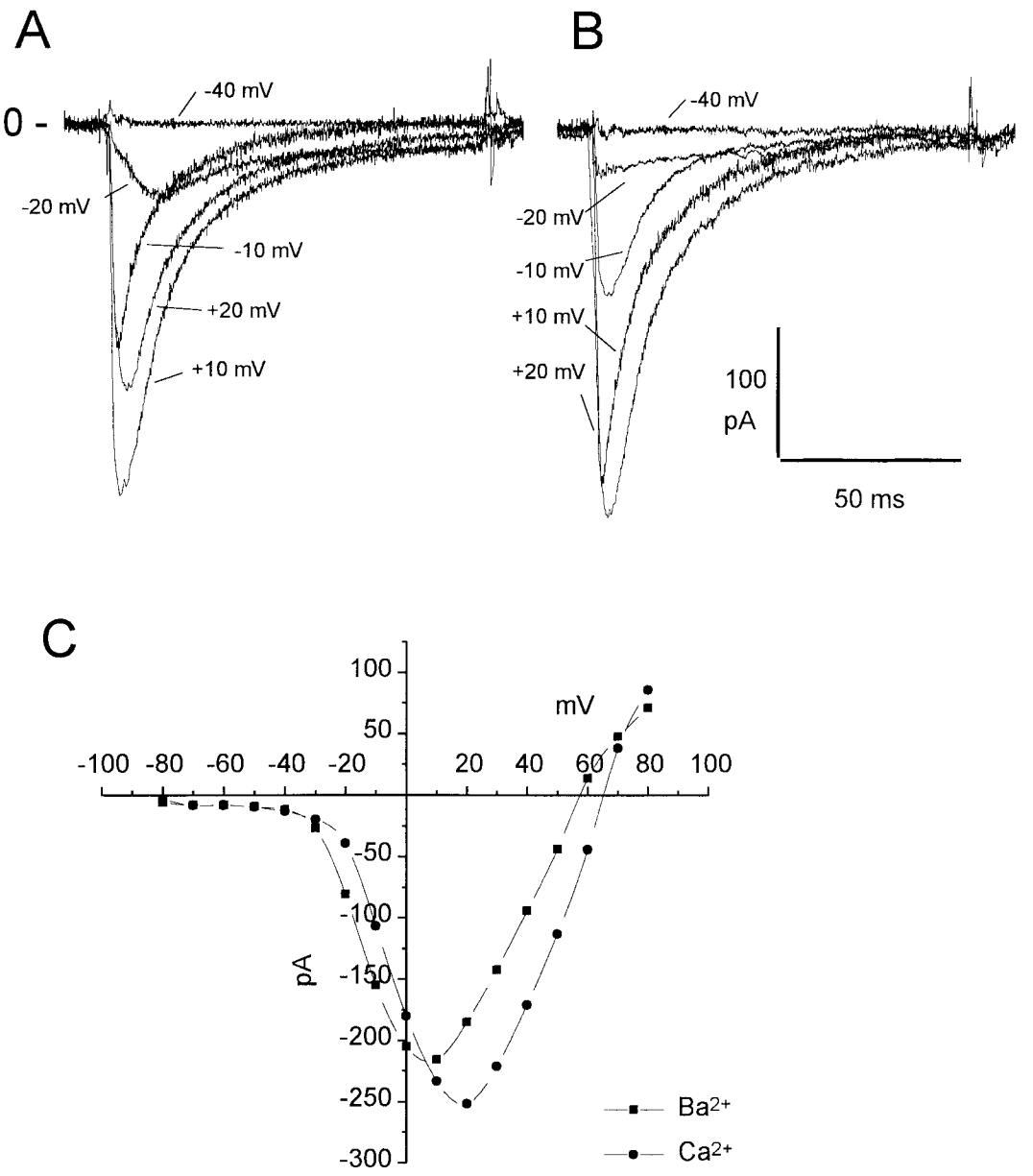


Fig. 1. Voltage-activated calcium currents in HEK293 cells expressing the α_{1Ed}-subunit. A,B: examples of currents, elicited from holding potential −80 mV with pulses as indicated. Traces of currents in solution containing 15 mM Ba^{2+} (A) or Ca^{2+} (B). C: current–voltage relation of the peak currents in Ba^{2+} or Ca^{2+}.

L-type Ca^{2+} channel α_1-subunits [27] which supports the idea that at least the longer [11] and the truncated human α_{1Ed}-subunit are sensitive towards a SST-mediated inhibition of Ca^{2+} inward currents after expression in HEK293 cells.

3.3. *Inactivation of G-proteins by pertussis toxin (PTX) or cholera toxin (CTX)*

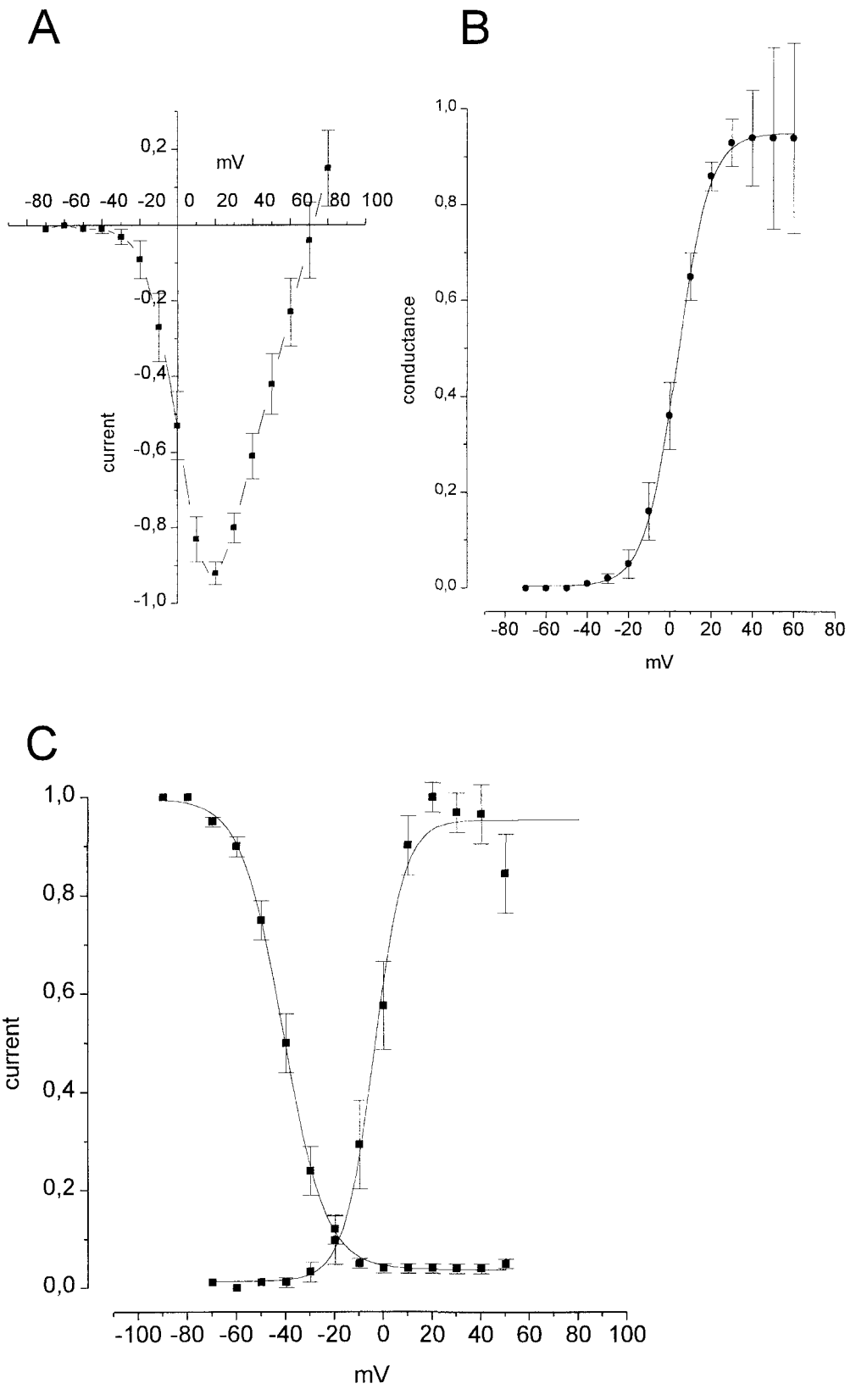
It has been shown that SST-receptors act via G-proteins [18,21]. To investigate whether G-proteins are involved in this regulation of the α_{1Ed} channels, HEK293 cells were incubated with PTX (1 mg/ml) for 12 h to inactivate the PTX-sensitive G_i- and G_o-proteins. The mean Ca^{2+} current density was not changed by PTX incubation (25.6 ± 2.2 pA/pF, $n = 15$). The SST-induced inhibition of the peak current, however, was reduced to 37.6 ± 3.9% ($n = 11$), compared to 50% without PTX, suggesting that part of the effect of SST is due to the activation of a PTX-sensitive G-protein. The incubation of the cells with CTX (12 h, 1 or 2 mg/ml) to inactivate G_s-proteins did not affect the reduction of the peak currents caused by SST. The mean reduction induced by 1 μM SST on the CTX-treated cells was 54 ± 2.9% ($n = 10$) in solution containing 15 mM Ca^{2+}.

3.4. *G-protein activation or inactivation by GTP-γ-S and GDP-β-S*

To corroborate the role of G-proteins in the signal cascade leading to a reduction of the Ca^{2+} current, we added stable GTP analogs to the pipette solution to directly activate or inactivate G-proteins. The inactivation of all heterotrimeric G-proteins by GDP-β-S did not change the currents under

control conditions. The SST-induced reduction of the current was in some cases almost totally blocked. On average, the peak current was reduced only by 22.7 ± 3.8% ($n = 11$) after GDP-β-S treatment.

To irreversibly activate the G-proteins, we applied the non-hydrolyzable GTP analog GTP-γ-S. GTP-γ-S (1 mM) produced an enhanced rundown of the Ca^{2+} current during the first minute after gaining access to the cell (Fig. 6), whereas under control conditions often a small increase in current during the first 30 s was observed (Fig. 3). This fast rundown



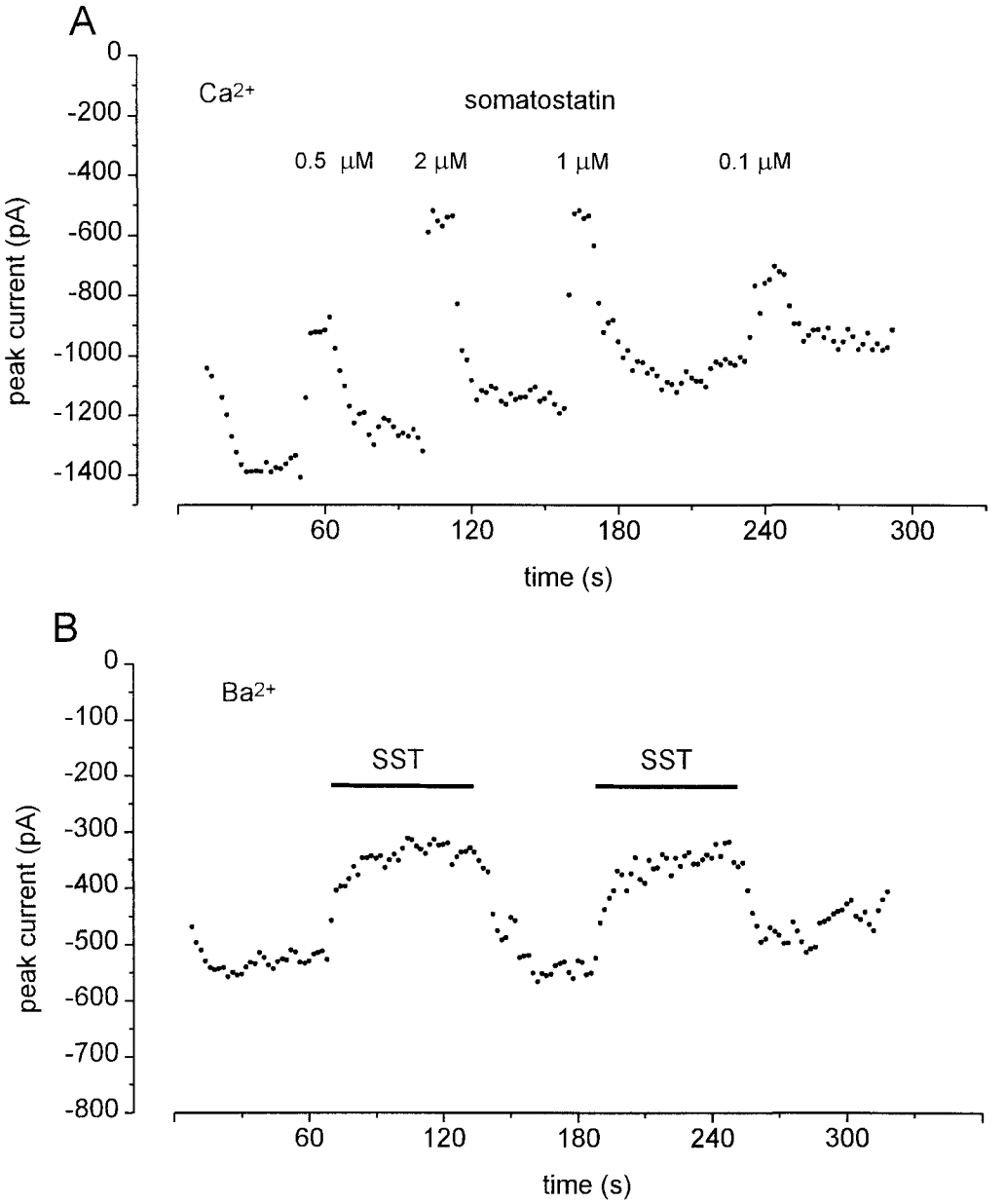


Fig. 3. Suppression of the peak currents by somatostatin. A: currents elicited in 15 mM Ca^{2+} were suppressed by SST in different concentrations. The duration of each SST application was 20 s. B: suppression of the currents in 15 mM Ba^{2+} by 1 μM SST. The duration of the SST application is indicated by bars.

effect might result from the gradual activation of G-proteins due to a gradual equilibration between the GTP-γ-S within the pipette solution and the cytoplasm. If SST was applied after the equilibration period, we observed a much smaller reduction in the peak Ca^{2+} currents compared to the control measurements. The mean SST-induced current reduction under the influence of GTP-γ-S was only 24.7 ± 5% ($n = 7$).

These results clearly show that about one-half of the SST-induced depression of the Ca^{2+} inward current is mediated through a G-protein-dependent pathway.

3.5. *Reduction of the currents by carbachol, adenosine and ATP*

To see whether the modulation of α_{1Ed} channels could be

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Fig. 2. Characterization of the calcium currents in the HEK293 cells expressing the α_{1Ed}-subunits. A: current–voltage relation of the peak currents. Mean of 14 normalized curves. B: conductance curve. The conductance (G) was calculated according to $G(V) = I(V)/(V - V_{rev})$ where $I(V)$ is the peak I_{Ca} as a function of the test potential, V_{rev} is the reversal potential for I_{Ca}. The data were fitted by a Boltzmann function: $G/G_{max} = 1/\{1+\exp[-(V_{test} - V_{0.5})/k]\}$, where $V_{0.5}$ is the half-maximal activation and k the slope factor. $V_{0.5}$ was estimated as 3.6 ± 0.27 mV and $k = 7.8 \pm 0.24$. C: Steady state activation and steady state inactivation. The steady state inactivation was determined by examining the amplitude of the current elicited by a test pulse to 10 mV after prepulses to various potentials. Averaged normalized current amplitudes ($n = 12$) were plotted as a function of the prepulse potential. The data were fitted by a Boltzmann function. $I/I_{max} = I/\{1+\exp[(V_{hold} - V_{0.5})/k]\}$. $V_{0.5}$ was estimated as −40 ± 0.2 mV, $k = 8.5 \pm 0.28$ for the inactivation, and for the activation $V_{0.5}$ was −3.8 ± 1 mV, $k = 6.2 \pm 1$.

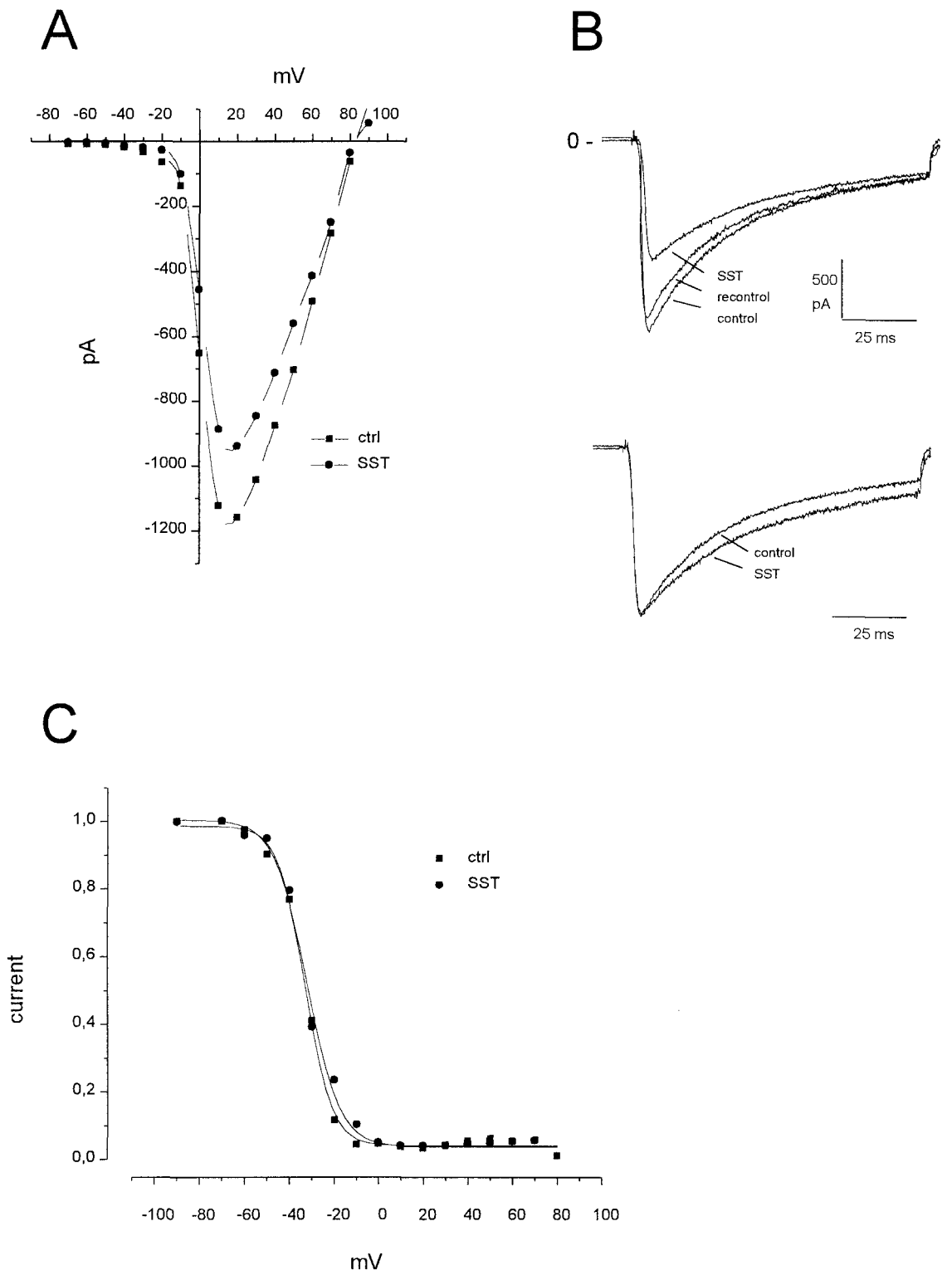
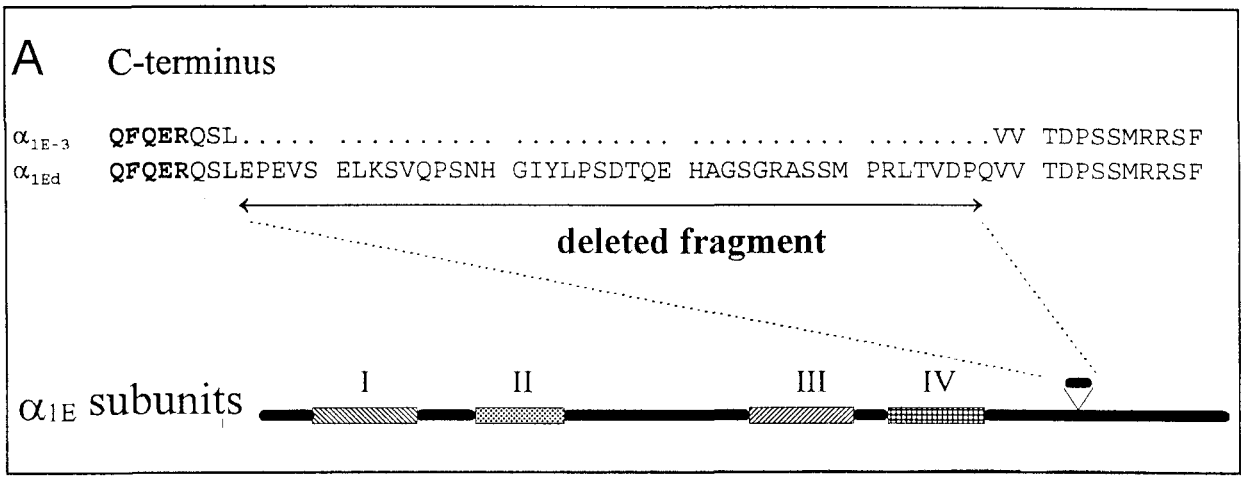
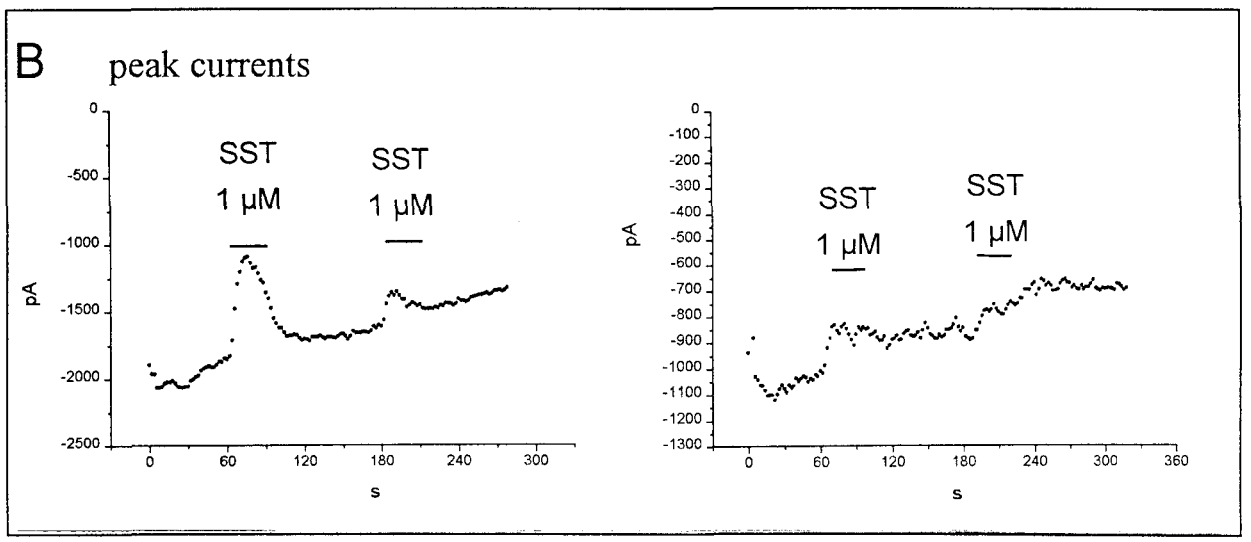


Fig. 4. Effects of somatostatin (1 μM) on calcium currents. A: current voltage relation under control condition and after application of SST. B: original traces of currents elicited by test pulses to +10 mV. In the lower part the SST current was scaled up to the peak value of the control current. C: steady-state inactivation curves under control condition and after application of SST. The fitted curves were done according to the Boltzmann equation (see Fig. 2). The half-maximal inactivation $V_{0.5}$ was estimated as -34 ± 0.5 mV in the control and -32 ± 0.2 mV in the presence 1 μM SST.

achieved by other receptors than the SST receptor, we used carbachol to stimulate muscarinic acetylcholine receptors, ATP and adenosine the agonists of purinoceptors. In a similar manner as SST, carbachol produced an inhibition of the Ca^{2+} or Ba^{2+} inward currents (Fig. 7A). With carbachol (1 μM), a reduction of $54 \pm 7\%$ (in 15 mM Ba^{2+}, $n = 6$) was achieved. Both, ATP and adenosine, also decreased the Ca^{2+} and Ba^{2+} currents (Fig. 7B,C).





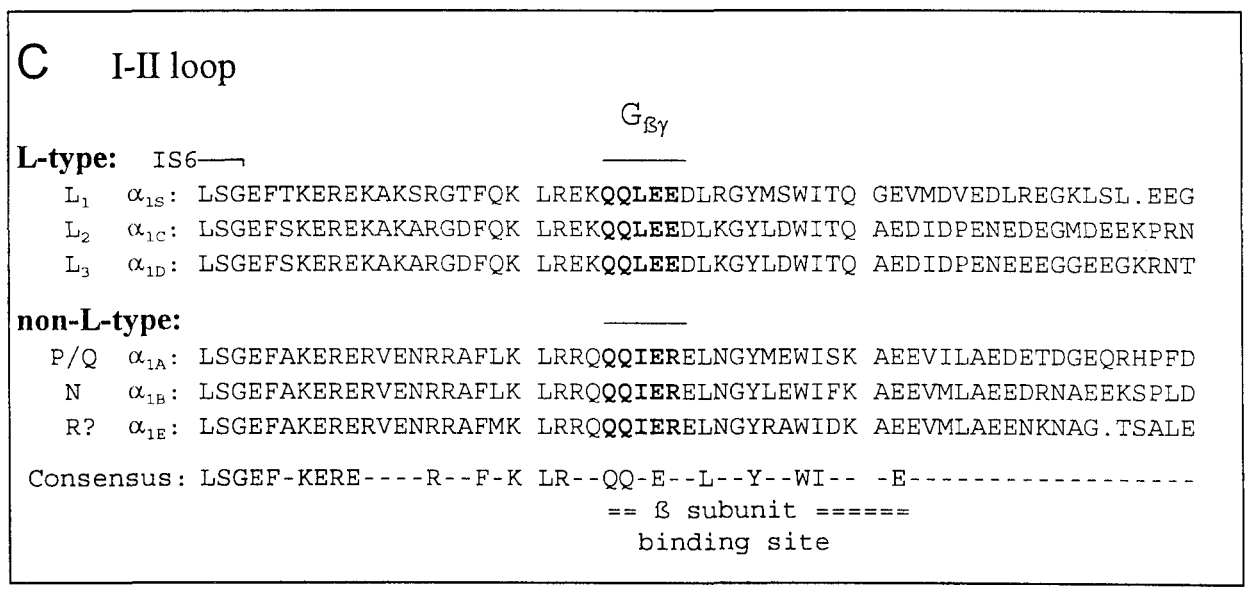


Fig. 5. Inhibition of voltage-induced Ca^{2+} currents by somatostatin (1 µM) in an α_{1Ed} deletion mutant. A: using appropriate primer, a 129-bp fragment was deleted from the cloned human α_{1Ed} cDNA [11]. The deleted fragment is absent in α_{1E-3} [13], another cloned human α_{1E} splice variant. B: suppression of the peak currents by 1 µM SST in $\alpha_{1Ed-DEL}$-expressing HEK293 cells. Desensitization is seen after the second application of SST. Two traces with high and low effects are shown. C: non-L-type Ca^{2+} channel α_1-subunits, but not L-type α_1-subunits share a common Gβγ-binding motif, QXXER, with other G-protein effectors. This motif is localized in the I–II loop at the Ca^{2+} channel β-subunit interaction domain. The end of transmembrane domain IS6 and the region with the homologous sequences (see beyond Gβγ) are marked.

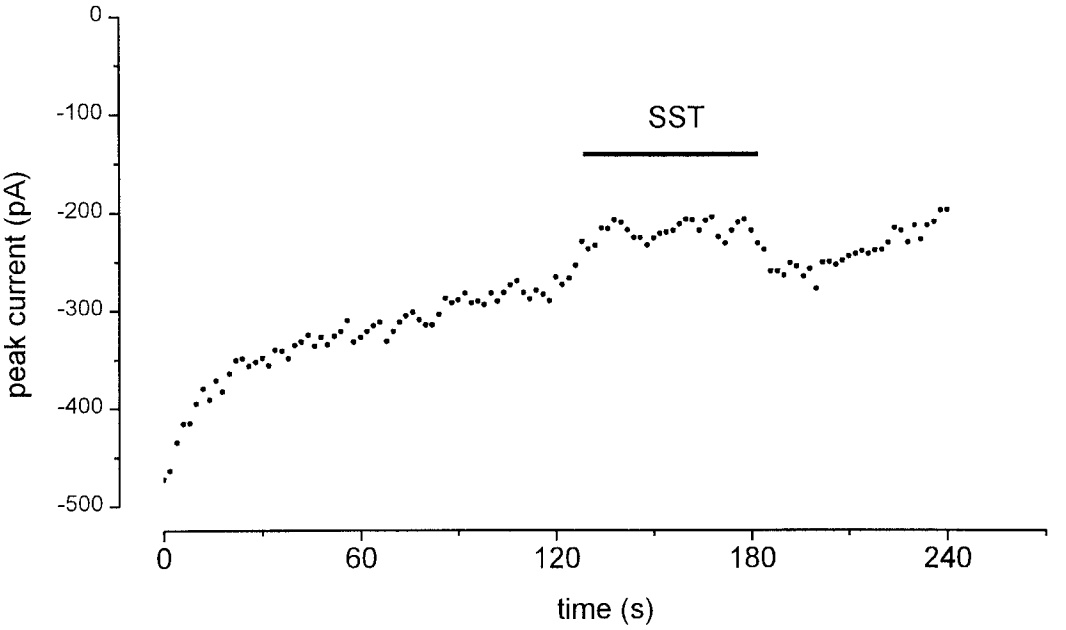


Fig. 6. Reduced inhibition of the voltage-induced Ca^{2+} currents by SST under the influence of GTP-γ-S. Note the fast rundown in the equilibration period during first 30 s.

4. Discussion

The physiological role of the recently cloned voltage-activated calcium channel α_{1Ed} subtype is not clear at all. No selective blockers are known, therefore, it is advantagous if the channels are investigated in an environment devoid of other channel types which might interfere. This can be achieved with some restrictions by expressing the cloned channel subunits in oocytes or in cultured mammalian cells. The HEK293 cell line we used expresses very low levels of endogenous voltage-dependent Ca^{2+} channels (< 0.2 pA/pF; see also [16]). Thus, it was unnecessary to use blockers or special voltage protocols to inactivate interfering channel types as it was the case in a previous study using GH_3 cells [28].

The α_{1Ed} channels expressed in transfected HEK293 cells show high current densities which are 100-fold higher than reported for endogenous currents [16]. They have electrophysiological properties similar to those previously described in an oocyte-expressing system [11,29]. (i) Peak currents were higher than seen with other α_1-subunits and somewhat higher in equimolar Ca^{2+} concentrations than in Ba^{2+}. (ii) The kinetics were not different in Ca^{2+} or Ba^{2+}. (iii) The fast inactivation of the currents was not dependent on the presence of Ca^{2+}. Unlike L-type Ca^{2+} channels, the α_{1E} type does not possess a functional Ca^{2+}-binding motif [30]. (iv) The steady-state inactivation curves gave values in the same range.

The modulation of the activity of voltage-dependent Ca^{2+} channels is an important mechanism by which many hormones, growth factors and neuromodulators exert their action. Thus, in addition to their dependency on the transmembrane voltage, Ca^{2+} channels are regulated by second messengers, phosphorylation, G-proteins and Ca^{2+} itself and of course by combinations of these. The peptide hormone SST, which was described originally as an inhibitor of growth hormone secretion, acts as a neurotransmitter and neuromodulator within the central nervous system. SST receptors couple to distinct cellular effector systems, including adenylyl cyclase, K^+ and Ca^{2+} channels [22,23]. In various cell types, the SST receptors are often linked to the different effector systems by G-proteins. For example, pertussis toxin-sensitive G-proteins of the Go type selectively couple SST receptors to Ca^{2+} channels [24]. Here, we have demonstrated that SST decreases Ca^{2+} currents elicited in α_{1Ed}-transfected HEK293 cells, and that the decrease is in part mediated by a PTX-sensitive G-protein.

It has been shown previously that a cloned somatostatin receptor expressed in CHO cells preferentially associates with $Gi_{\alpha3}$ and $Go_{\alpha2}$ [20]. HEK293 cells express endogenously Gi and Gq and somatostatin receptor subtype SSTR2 ,but no Go [19], and the SST receptor associates with $Gi_{\alpha3}$. It is unlikely that cAMP mediates the effect of SST on Ca^{2+} currents because only the SSTR3 subtype-activating $Gi_{\alpha1}$ is functionally linked to adenylyl cyclase [20]. Similarly, in calcitonin-secreting cells [25] SST inhibition of the calcitonin secretion occurs via suppression of voltage-dependent Ca^{2+} channel currents by a PTX-sensitive mechanism but independent of the cAMP concentration.

One important finding is that HEK293 cells can be used to investigate interactions between hormone receptors and their effects on G-proteins and ion channels. HEK293 cells express various types of receptors on their surface. They express G_α-subunits of all four major families (α_i, α_s, α_q, and α_{13}) [26]. We could demonstrate effects of SST, carbachol, adenosine and ATP on the human α_{1Ed}-subunit. In vitro studies show that direct binding between recombinant $G\beta_1\gamma_2$ and α_1-subunits from dihydropyridine-insensitive Ca^{2+} channels, like α_{1A}, α_{1B}, and α_{1E}, is possible [27]. A proposed interaction site is conserved only in the non-L-type Ca^{2+} channel α_1-subunits (see Fig. 5C), and it should be expected that Gβγ-subunits mediate SST interaction with α_{1E}-subunits, too. The present study shows that the human α_{1Ed}-subunit is modulated by endogenous hormone receptors of HEK293 cells which are known to interact via Gβγ-subunits. This modulation is not impaired in the truncated $\alpha_{1Ed-DEL}$-subunit which

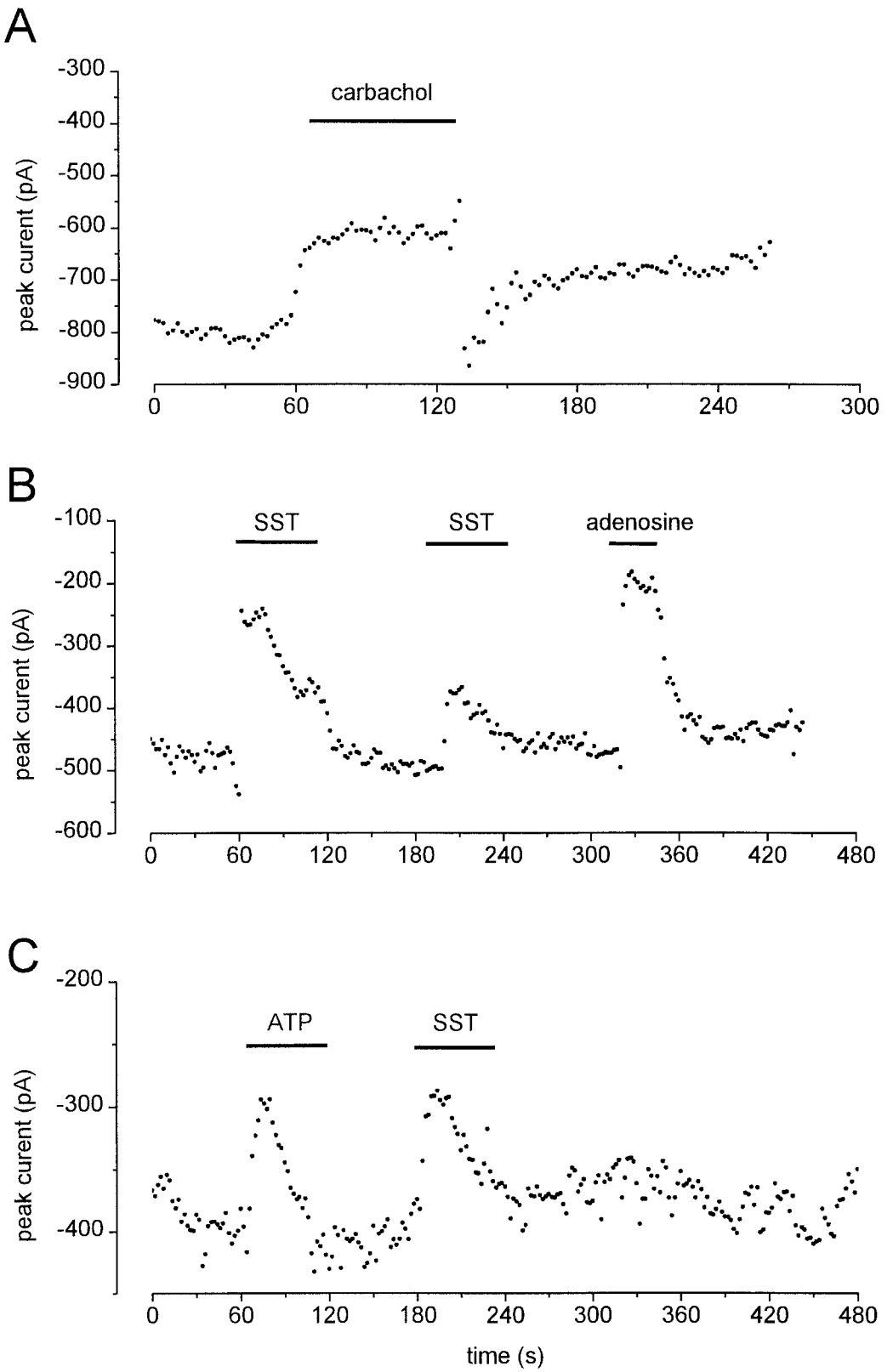


Fig. 7. Reduction of the voltage-induced Ca^{2+} currents by carbachol, SST, ATP, and adenosine. A: decrease of the peak currents by superfusion with 10 μM carbachol. B: first two applications of 1 μM SST showing a desensitization effect, then an application of 2 μM adenosine. C: application of 1 μM ATP followed by 1 μM SST.

shows that the truncated 129 bp of the C-terminus, which differ in two human α_{1E} splice variants [11,26], are not crucial for the hormone-mediated interaction investigated in our study. A similar hormonal modulation is seen in the shorter rabbit α_{1E-3}-subunit [28].

The expression of the shorter cloned human α_{1E-3} variant [13] expressed in HEK293 or COS-7 cells did not lead to SST-sensitive Ca^{2+} currents when co-transfected with β_{1B}- and $\alpha_2\delta$-subunits [26,38]. This could be due either to the different α_{1E} splice variant used or to the fact that co-expressed Ca^{2+} channel β-subunits interfere with the G-protein-mediated inhibition by competing for the same binding site at the α_{1E}-subunit [34]. In this case, it should be expected that the kind of β-subunit used and the level of expression of β achieved might influence the degree of α_{1E} inhibition by SST and other hormones. Preliminary results with co-expressed β_3 still reveal hormone sensitivity for α_{1Ed} (Pereverzev, Mehrke, Hescheler and Schneider, unpublished results) while β_{2a} blocks opioid sensitivity even in the longer α_{1E} splice variant [39]. Future experiments should elucidate these hormononal interactions in the presence of an in vivo co-expressed β-subunit in more detail.

In the α_{1B}-subunit, additional G-protein interaction sites are proposed for the C-terminus [36]. Interestingly, one additional QXXER consensus site for the binding of Gβγ is located in the C-terminus only of human α_{1E-3}-, α_{1Ed}-, and $\alpha_{1Ed-DEL}$-subunit.

In vivo, R type Ca^{2+} currents may be carried at least in part by α_{1E}-containing Ca^{2+} channels [31]. In rat hippocampal neurons, SST inhibits a resistant component of high-voltage-activated Ca^{2+} currents which is seen after applying the known blockers of other Ca^{2+} channel types. The remaining rest component, which is sensitive to 200 μM Cd^{2+}, represents 13% of the whole high-voltage-activated Ca^{2+} current [32]. This indicates that in vivo R type Ca^{2+} currents with a similar SST sensitivity are present which might be mimicked in our expression system. In future, additional studies have to be performed in native systems to elucidate the in vivo function of these hormone-modulated R type Ca^{2+} currents.

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