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Physiology

Antisense oligonucleotides against rat brain α_{1E} DNA and its atrial homologue decrease T-type calcium current in atrial myocytes

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ABSTRACT **Low voltage-activated, or T-type, calcium currents are important regulators of neuronal and muscle excitability, secretion, and possibly cell growth and differentiation. The gene (or genes) coding for the pore-forming subunit of low voltage-activated channel proteins has not been unequivocally identified. We have used reverse transcription–PCR to identify partial clones from rat atrial myocytes that share high homology with a member of the E class of calcium channel genes. Antisense oligonucleotides targeting one of these partial clones (raE1) specifically block the increase in T-current density that normally results when atrial myocytes are treated with insulin-like growth factor 1 (IGF-1). Antisense oligonucleotides targeting portions of the neuronal rat α_{1E} sequence, which are not part of the clones detected in atrial tissue, also block the IGF-1-induced increase in T-current, suggesting that the high homology to α_{1E} seen in the partial clone may be present in the complete atrial sequence. The basal T-current expressed in these cells is also blocked by antisense oligonucleotides, which is consistent with the notion that IGF-1 up-regulates the same gene that encodes the basal current. These results support the hypothesis that a member of the E class of calcium channel genes encodes a low voltage-activated calcium channel in atrial myocytes.**

Voltage-dependent calcium channels are multimeric proteins that control the electrochemical diffusion of calcium ions across cell membranes. The calcium currents gated by these proteins can be differentiated by their biophysical and pharmacological properties. They are involved in a variety of important physiological functions including electrical excitability, muscle activation, secretion, gene expression, and the regulation of cell growth and proliferation. To date, six gene families (designated by the letters A, B, C, D, E, and S) known to encode the pore-forming α subunits of calcium channels have been identified from mammalian tissues (1–6). In addition, as many as six different calcium currents (L, T, N, P/Q, R) have been identified based on their biophysical and pharmacological properties (reviewed refs. 7–11). In most cases, the correlation between particular genes and the calcium currents produced when they are expressed in a cell have been established. For example, expression of the α_{1C} gene produces the high voltage-activated, dihydropyridine-sensitive, or L-type current recorded from cardiac myocytes. Currents with similar properties are encoded by the α_{1S} and α_{1D} genes in skeletal muscle and brain. However, such a clear functional identification has not been made for members of the E gene class. One member of this class, the rat brain α_{1E} (rbEII) gene, produces a calcium current with a voltage dependence characteristic of mid- to low voltage-activated calcium currents like the T-type current (12). It also shows some pharmacological characteristics (high sensitivity to nickel) and selectivity properties (Sr > Ca > Ba) similar to T-type currents (12, 13). The possibility that the E gene class codes for the T-type calcium channel is also supported by the observation that α_{1E} cDNA has been detected by reverse transcription (RT)–PCR in mouse spermatogenic cells, which express only T-type current (14). However, other members of the E class, when expressed in heterologous systems, give rise to currents that are less like the T-type current in both their voltage dependence and pharmacology (15–20). For instance, in the case of the α_{1E} homologue doe-1 found in the marine ray, expression of the gene in *Xenopus* oocytes produces a high-voltage-activated channel that behaves more like the R-type current described in rat cerebellar granule cells (11, 21). Additionally, the presence of α_{1E} cDNA has been reported in rat sympathetic ganglia, which do not express T-type currents (22). Consequently, the identification of the gene(s) encoding members of the low voltage-activated class of calcium currents, including the T-type current, remains problematic.

In atrial myocytes there are two types of voltage-gated calcium currents, a high voltage-activated or L-type current encoded by the $\alpha_{1C/D}$ gene and a low voltage-activated or T-type current encoded by an unidentified gene. The density of cardiac T-type calcium currents is increased during periods of differentiation and growth and under pathological conditions such as hypertension and acromegaly (23, 24). During normal postnatal development atrial T-current density is highest when the serum concentrations of growth hormone and insulin-like growth factor 1 (IGF-1) are elevated (25). Thus, atrial tissue from actively growing animals may provide mRNA enriched in T-channel message, making this a useful preparation for identification of the T-channel gene from cardiac tissue. In addition, expression of T-type calcium currents in atrial myocyte primary cultures can be up-regulated by physiological concentrations of IGF-1 in the culture medium (unpublished data). We report here the isolation of partial cDNA clones from rat atrial tissue that share high homology with rat brain α_{1E} cDNA. We have used antisense oligonucleotides (ON) based on portions of these sequences to directly test the hypothesis that a member of the E class of calcium channel genes encodes the T-type calcium channel expressed in atrial myocytes.

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Abbreviations: RT, reverse transcription; IGF-1, insulin-like growth factor 1; ON, oligonucleotide; DIV, days *in vitro*.
The sequence reported in this paper has been deposited in the GenBank database (accession no. AF033580).
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METHODS

RNA Isolation and cDNA Preparation. Total RNA was isolated from 4.5-week-old rat atria by using Ultraspec reagent (Biotecx, Houston), based on the guanidine/urea/acidic phenol extraction method. First strand atrial cDNA was obtained by using Superscript II reverse transcriptase in the presence of oligo(dT) and gene-specific promoters (Integrated DNA Technologies, Coralville, IA; Life Technologies, Grand Island, NY). Negative controls were run in the absence of RT to detect possible contamination by genomic DNA. Two sets of primers were designed and analyzed with the program GENERUNNER (Hastings Software, NY): Set 1) degenerate primers based on the α_{1E} sequence from nt 5331–5349 (5331D forward: 5′ TAYACNGARATGAYGARATG 3′) and nt 5666–5685 (5666D reverse: 5′ GYTTRTARTCCATDATCATC 3′); and Set 2) specific primers based on the neuronal α_{1E} sequence 4674–4693 (4674 forward: 5′ TTGAACATCTTTGACTTCAT 3′) and a modification of 5666D reverse (5654 reverse: 5′TCCATGATCATTGCTGCATAG 3′). Amplification was carried out by cycling 30 times from 94°C (30 sec), 55°C (1 min), and 72°C (1 min), followed by an 8-min extension step at 72°C. Five initial cycles at 94°C (30 sec), 45°C (1 min), and 72°C (1 min) were added when degenerate primers were used. PCR products were subcloned into a pCRII vector (Invitrogen) and sequenced twice. Sequences were analyzed by using the BLAST program from National Center for Biotechnology Information at the National Institutes of Health web site. Positive controls were generated with the rat α_{1E} clone generously provided by Terry Snutch (University of British Columbia, Canada).

Myocyte Isolation and Cell Culture. Three-week-old Sprague-Dawley rats were anesthetized with a 3.5% halothane/96.5% oxygen mixture. Tracheotomies were performed on the rats, and their breathing was controlled via respirator while 10 ml of cold cardioplegia solution (in mM: 20 Hepes/4 $NaHCO_3$/166.5 glucose/20 KCl/1.1 mg/ml heparin, pH 7.4) was injected into the inferior vena cava to stop the heartbeat. The heart was then removed and perfused retrogradely by aortic cannulation with perfusion solution (in mM: 135 NaCl/5.4 KCl/5 $MgCl_2$/10 Hepes/0.33 NaH_2PO_4, pH 7.3) for 5–10 min followed by an enzyme solution containing 0.3 mg/ml collagenase type B (Boehringer Mannheim) in perfusion solution for 6 min. Atria were separated, trimmed of all fat and connective tissue, and minced in a recovery solution (in mM: 20 taurine/5 creatine/20 glucose/5 pyruvic acid/85 potassium glutamate/5 $MgCl_2$/1 K_2EGTA/2 Tris-ATP/40 K_2HPO_4). Atrial myocytes were dispersed mechanically and kept in recovery solution for 1 h. Cells were collected by centrifugation ($850 \times g$ for 5 min) and resuspended in Tyrode's solution (in mM: 137 NaCl/5.4 KCl/1 $MgCl_2$/0.33 NaH_2PO_4/10 Hepes/2 $CaCl_2$, pH 7.4). Cells were kept in Tyrode's solution at room temperature for 15 min before being plated.

Cells were plated on coverslips precoated with 10 μg/ml rat tail collagen I and 5 μg/ml fibronectin (Sigma) in plating medium (1:1 DMEM/Ham's F-12/4 nM insulin, 2% streptomycin/penicillin/Fungizone/2.5 mg/ml BSA/1 nM selenium/1 nM thyroxine/5 μg/ml transferrin/10 nM testosterone) supplemented with 10% fetal bovine serum (Life Technologies) and kept in 5% CO_2 humidified atmosphere at 37°C. Cells were kept in plating medium for 2 days, after which they were maintained in serum-free medium. In experiments with IGF-1 (recombinant human IGF-1 from Gropep, Adelaide, Australia), cells were rinsed twice with Tyrode's solution and kept in serum-free medium for 24 h before addition of the peptide [4 days *in vitro* (DIV)].

Unmodified ONs were added directly to the serum-free culture medium (at 4 DIV) at a concentration of 1 μM, and recordings were made at 5 DIV.

Electrophysiology. Ca^{2+} currents were recorded by using the whole cell configuration of the patch-clamp technique. Patch pipettes were made from borosilicate glass and had a resistance of 1–2.5 MΩ. The pipette capacitance was compensated electronically following seal formation and cell rupture. Cell capacitance and series resistance (R_{ser}) were determined from the current transient induced by a hyperpolarizing voltage pulse from −80 to −90 mV and compensated electronically. Mean cell capacitance was 17.8 ± 1.1 pF (n = 23), and mean R_{ser} was 8.1 ± 0.7 MΩ (n = 11).

T-currents were obtained by trace subtraction from currents elicited with voltage-step protocols from holding potentials (V_{hold}) of −90 and −50 mV to various test potentials (V_{test}). L-type currents were measured from a V_{hold} of −50 mV. Test pulses were 100 ms long and were repeated every 3 sec. Current traces were corrected for linear capacitive and leak current by using on-line P/−4 trace subtraction after the test pulse. Current signals were sampled at 2.5–10 kHz and filtered at 1 kHz with an Axopatch-1D patch-clamp system (Axon Instruments, Foster City, CA) interfaced to a personal computer. All experiments were performed at room temperature, 22–24°C.

The pipette solution contained (in mM) 140 cesium aspartate, 10 Cs-EGTA, 5 $MgCl_2$, 1 $CaCl_2$, 10 Hepes, 3 Tris-ATP, and 0.3 Na_2GTP, pH 7.4. The bath solution contained 100 *N*-methyl-D-glucamine methanesulfonate, 1 $MgCl_2$, 5 $CaCl_2$, 10 glucose, 10 Hepes, and 40 μM tetrodotoxin, pH 7.4 (adjusted with CsOH).

All data are reported as the mean ± SE. Mean values were tested for significance by using single factor ANOVA. A value of $P < 0.05$ was taken as indicating statistical significance.

RESULTS

Atrial Tissue Contains mRNA with High Homology to Neuronal α_{1E}. Two partial clones with high homology to the rat neuronal α_{1E} gene (rbEII) were isolated from rat atrial tissue by using RT–PCR (shown schematically in Fig. 1*A*). The first, designated raE1, was amplified by using a set of degenerate primers from α_{1E} (Set 1 as described under *Methods*). This clone was slightly shorter than expected (350 vs. 358 bp) but showed high homology (98.5%) to the neuronal α_{1E} sequence at nt 5331–5685, which correspond to the cytoplasmic region 96 bp downstream of the fourth transmembrane domain IVS6. Five nucleotides from raE1 are different from the neuronal clone but do not represent changes at the amino acid level (Fig. 1*B*). raE1 lacks the last 8 bp of the 3′ end of the primer used in the RT reaction. A second clone, raE2, was isolated by using a set of specific primers modified from the degenerate primer set. This second clone (sequence not shown) is 907 bp long with 99% homology to the neuronal α_{1E} sequence at nt 4674–5674. This clone corresponds to part of α_{1E}'s IVS3 domain to downstream of IVS6. raE2 lacks 97 bp corresponding to nucleotides 5318–5415 from the rat neuronal sequence, which cause a frameshift and a stop codon at position 5415. In addition, raE2 contains three putative silent mutations and two mutations that constitute amino acid substitutions (corresponding to amino acids R1518D and M1621T from the neuronal sequence). A clone of the expected size (1007 bp) was also identified with the same primers used to generate raE2.

Antisense ONs Against Portions of Atrial and Neuronal α_{1E} Decrease T-Type Calcium Currents. We used an antisense ON strategy to test whether the atrial partial clones are from sequences that encode the T-type calcium current expressed in atrial cells. Three antisense ON sets (sense and antisense) were designed against portions of the atrial sequences raE1 and raE2 (designated atrium 1, atrium 2, and atrial gap). Two additional sets of ONs targeting portions of the α_{1E} clone were constructed to test the possibility that the high homology observed between the atrial clones and the rat brain α_{1E} sequence is maintained over the entire length of the atrial message. One, designated α_{1E}, targets a cytoplasmic segment at the 5′ end. The other, designated β-binding, targets the

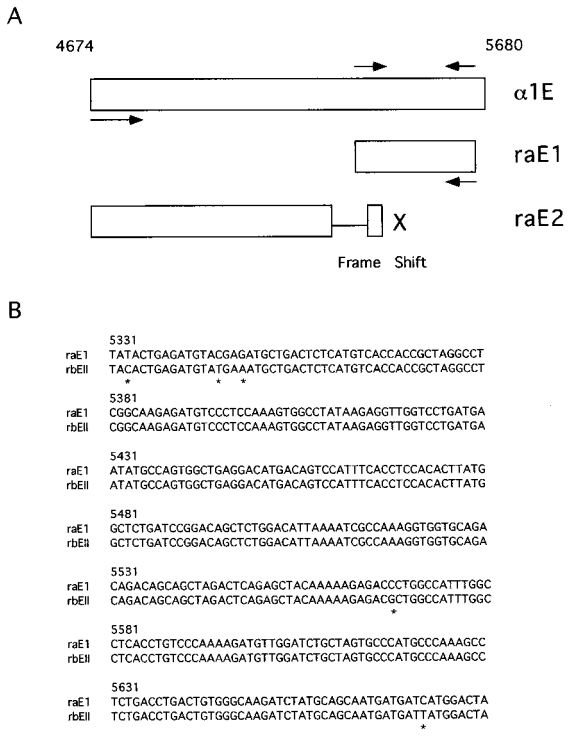

FIG. 1. Rat atrial cDNA contains regions with high homology to the neuronal calcium channel α_{1E}. (*A*) Schematic diagram showing the location of two partial clones, raE1 and raE2, detected from rat atrial mRNA by using RT–PCR. Positions are shown relative to the rat neuronal clone α_{1E} (rbEII). (*B*) Sequence of the atrial clone raE1 aligned with that of α_{1E}. The cardiac sequence differs at 5 bp from α_{1E}.

OLIGONUCLEOTIDES USED TO BLOCK T-CURRENT EXPRESSION

	NAME	SEQUENCE	COMMENTS
1	Atrium 1	nt 5619-5637	Atrial sequence, 98.5% homologous to neuronal α1E, 0% homologous to α1C.
2	Atrium 2	nt 5654-5677	Atrial sequence, 99% homologous to neuronal α1E, 58% homologous to α1C.
3	α1E	nt 321-341	100% homologous to neuronal α1E.
4	β-binding	nt 1182-1218	Conserved β-binding site, 100% homologous to neuronal α1E, 70% homologous to α1C.
5	Atrial gap	nt 5308-5317 and nt 5415-5424	Atrial sequences flanking nt 5318-5414 from neuronal α1E.
6	α1C	nt 4997-5117	100% homologous to α1C, analogous region to atrium 1 with 0% homology to both neuronal α1E and atrial sequences.

for ON sets 1-5, nt number corresponds to that of α1E (12).
for ON set 6, nt number corresponds to that of α1C (3).

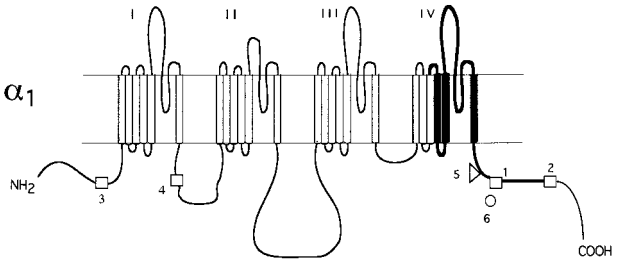

FIG. 2. Characteristics and locations of antisense ONs targeting portions of the atrial clones raE1 and raE2 and the neuronal clone α_{1E}. Dark lines indicate portions of domain IV and the 3′ cytoplasmic region included in atrial clones raE1 and raE2 (not drawn to scale). Numbered symbols indicate location of targeted sequences.

conserved region in the I–II loop that is involved in the interaction with the β subunit (27). The sequence numbering of these ONs and a schematic representation of their location on the α subunit are given in Fig. 2.

To determine the effects of antisense ONs on the expression of T-current we used a functional assay based on the observation that IGF-1 causes a significant increase in T-current density in cultured atrial myocytes. That is, we determined the ability of these ONs to inhibit the IGF-1-dependent increase in T-current in atrial cells. Cultured atrial myocytes exposed to IGF-1 for 24 h show a marked increase in the amplitude of T-type calcium current compared with untreated control cells (Fig. 3*A*). This effect is exemplified by the current traces shown in Fig. 3*A* labeled control and IGF-1. The traces shown are the average of 3–4 current records obtained for each experimental condition. Average peak current is −0.71 pA/pF in untreated controls and −1.75 pA/pF in the IGF-1-treated cells. The effect of IGF-1 can also be seen as a downward shift (increase in inward current) in the current/voltage (*I–V*) relationships for the T-current (Fig. 3*B*, filled squares) as compared with untreated cells (Fig. 3B, empty circles).

Addition of antisense ONs to the culture medium produced a marked inhibition of the IGF-1-induced increase in T-current at all voltages as compared with IGF-1-treated cells not exposed to antisense ONs. This is shown in the current traces obtained from cells targeted with the atrial-derived ON atrium 1 (mean peak current = −1.1 pA/pF) and the neuronal sequence β-binding (−0.6 pA/pF) in Fig. 3*A*. The effect of the antisense ONs can also be seen in the *I–V* relationships obtained from cells treated with IGF-1 and ONs atrium 1 and atrium 2 and the neuronal sequences α_{1E} and β-binding (Fig. 3*B*).

The longer atrial clone we sequenced, raE2, is notable because it lacks a 97-bp region that is present in the neuronal clone rbEII. The deletion seen in raE2 could represent a splice variant from α_{1E} but could also be an artifact of the PCR amplification. To determine whether raE2 is expressed in the cultured cells, we designed an ON set, designated atrial gap, from atrial sequences flanking the 97-bp region missing from the clone raE2 (Fig. 2). If the truncated clone was not physiologically expressed, the antisense probe should have no effect on the IGF-1-dependent increase in T-current. However, if the truncated region was part of an expressed sequence, the antisense probe should inhibit the IGF-1 effect. The *I–V* relationship shown in Fig. 3*B* demonstrates that this antisense ON had no effect on T-current expression. It is therefore likely that the partial clone raE2 does not represent a message that encodes the IGF-1-inducible T-current.

The effects of all the ONs tested on the IGF-1-dependent increase in T-current are summarized in Fig. 4 *A* (antisense ONs) and *B* (sense ONs). The inhibition of the IGF-1-induced increase in T-current was significant and specific for the antisense probes. Control ONs did not inhibit the IGF-1-dependent induction of current. The statistical significance of the effect was determined by comparing the average, peak current amplitude at $V_{test} = -30$ mV recorded from cells treated with IGF-1 or IGF-1 plus antisense ON to control (untreated) cells. Data in Fig. 4*A* shows that IGF-1 alone caused a significant increase in T-current. However, peak T-current was not altered significantly in the presence of IGF-1 plus the antisense ONs atrium 1, atrium 2, α_{1E}, or β-binding. Neither the corresponding sense ON controls for atrium 1, atrium 2, and α_{1E} antisense ONs nor a scrambled (SC) version of atrium 1 prevented the IGF-1 induction of T-currents (Fig. 4*B*). This suggests that the effect of the antisense ONs results from specific binding to mRNA that is normally regulated during IGF-1 stimulation. These results support the idea that a gene highly homologous with rbEII over much of its length encodes atrial T-type channels.

We also investigated the effects of the antisense ON β-binding on the expression of basal T-currents from cells not stimulated by IGF-1. This particular ON had the largest inhibitory effect on the IGF-1 induction of T-current. Cells exposed to the antisense ON β-binding showed a significant decrease in basal T-current density as compared with

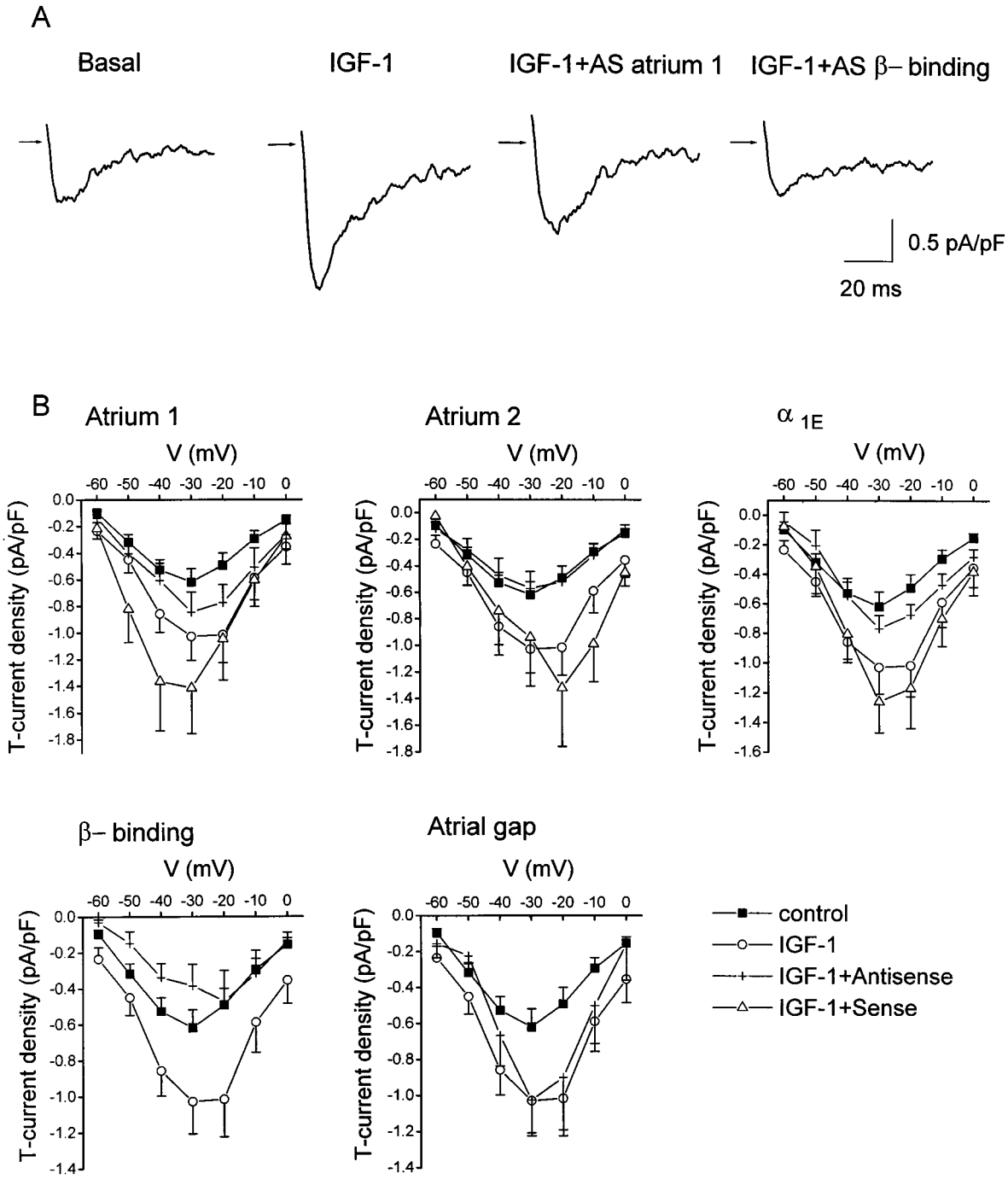

FIG. 3. Antisense ONs inhibit the ability of IGF-1 to increase T-type calcium current. (*A*) Representative T-type calcium current traces from cultured atrial myocytes. Records are the difference traces obtained by subtracting currents elicited at a test potential of −30 mV from holding potentials of −90 and −50 mV in the presence of 5 mM calcium in the recording solution. Each trace represents the average of three to four individual current records recorded from different cells under each condition: serum-free medium (basal), medium containing 52 nM IGF-1, and medium containing 52 nM IGF-1 plus 1 μM of the antisense ON atrium 1 or β-binding. (*B*) T-current density/voltage relationships for cells cultured in the presence of IGF-1 (52 nM, circles), IGF-1 plus antisense (crosses) or sense (triangles) ON, and in serum-free medium (squares). Labels above each graph indicate the ON tested. Number of determinations at each voltage varied from 3 to 9.

controls or with cells exposed to the sense sequence (Fig. 5*A*). This suggests that the basal and IGF-1-induced T-currents are encoded by the same gene in atrial myocytes. This antisense ON also caused a 35% decrease in basal L-current.

As expected, the antisense ONs α_{1E} and atrium 1, which share no homology with the α_{1C} or α_{1D} sequences, did not affect the density of L-type currents (data not shown). To corroborate further that the ONs examined had specific effects we tested an ON against a region of the α_{1C} gene (designated α_{1C}, sequence shown in Fig. 2). As anticipated antisense ON α_{1C} significantly decreased the density of the basal L-type current (Fig. 5*B*) while having no effect on the IGF-1 induction of the T-type current (Fig. 4*A*, bottom). Unexpectedly, the density of the basal T-current was significantly increased by antisense ON α_{1C} (Fig. 5*B*).

DISCUSSION

Our results show that atrial myocytes contain mRNA with high homology to the rat brain α_{1E} gene, rbEII. We have also

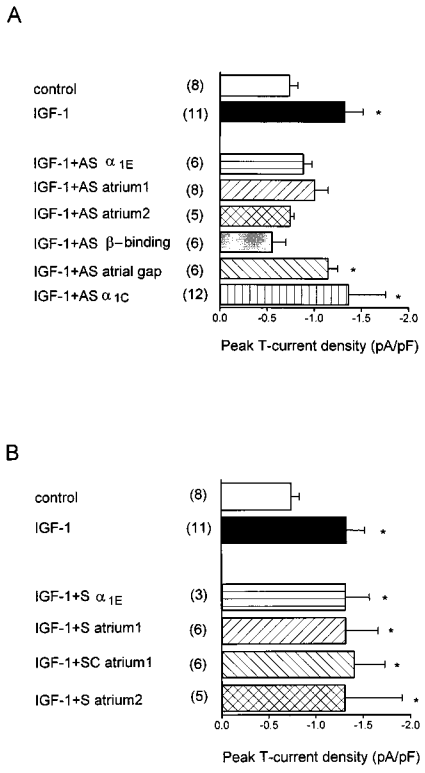

FIG. 4. (*A*) Comparison of peak currents obtained in control, IGF-1, and IGF-1 plus antisense ON-treated cells. All antisense ONs (AS) except "atrial gap" and the negative control "α_{1C}" inhibit the IGF-1 induction of T-type current. The number of determinations is indicated in parentheses to the left of bars. (*B*) sense (S) and scrambled (SC) ONs fail to inhibit the effect of IGF-1. Asterisks denote $P < 0.05$.

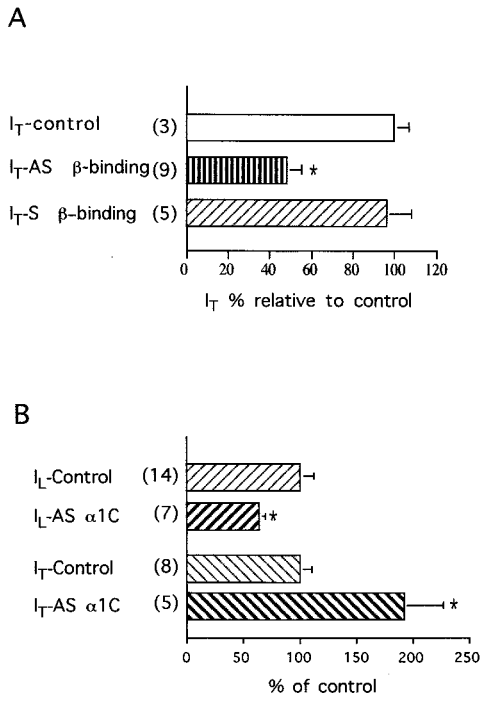

FIG. 5. (*A*) Effect of ONs on the basal expression of calcium currents. Antisense ONs (AS) targeting the β-binding region of α_{1E} significantly decrease the expression of T-current in cells not treated with IGF-1. (*B*) Antisense ONs against the α_{1C} gene inhibit the expression of the L-type current and enhance the expression of the T-type current in cells not treated with IGF-1. Peak L-type currents were measured at $V_{hold} = +20$ mV. Asterisks denote $P < 0.05$.

demonstrated that the presence of specific antisense ONs targeting this message inhibits expression of both the IGF-1-induced and basal T-type calcium currents in atrial myocytes. This result provides strong experimental evidence in support of the idea that an atrial homologue of the α_{1E} gene encodes the cardiac T-channel. This conclusion is consistent with the work of Stephens *et al.* (28), who expressed rbEII in COS-7 cells and investigated the biophysical and pharmacological properties of the resulting calcium current. They demonstrated that when rat brain α_{1E} is expressed in these mammalian cells with appropriate ancillary subunits the resultant channel shares many of the characteristics reported for low voltage-activated channels. Another possible interpretation of our results is that the atrial homologue of α_{1E} regulates the expression of the T-channel gene. However, given the structural similarity of the atrial homologue to a known calcium channel gene we think this possibility is unlikely.

T-type calcium currents with distinct pharmacological profiles (29, 30) can be observed in different cell types and may also co-exist within the same cell (31, 32). Multiple genes or splice variants of a single gene might explain the diversity of biophysical and pharmacological properties reported for these low voltage-activated currents much as they do for the different L-type currents (33, 34). Whereas it seems likely from our results that a member of the E class of calcium channel genes encodes T-channels in atrial cells, this conclusion does not rule out the possibility that other members of the α_{1E} gene class may encode other types of voltage-activated channels in different tissues.

Antisense strategies have been successfully used to identify the pore-forming subunit of P-type calcium channels (35) and the role of β subunits in the modulation of calcium channels (36, 37). Here, we show that antisense probes targeting α_{1E}-like cardiac sequences and neuronal α_{1E} sequences (not included in the atrial sequences) block expression of T-type currents. Whereas this supports the idea that the atrial gene is highly similar over its entire length to α_{1E}, cloning of the complete atrial sequence is required before the extent of this similarity is known. The observation that both the basal and the IGF-1-induced T-type currents were decreased by antisense ONs suggests that both currents are encoded by the same gene in atrial cells.

In general, the antisense ONs against α_{1E} and α_{1C} we have used produced the predicted inhibitory effects on the density of the targeted currents. Differences in the degree of block observed with the various antisense ONs tested against T-currents may reflect differences in the accessibility of the target sequence, such as secondary structure of RNA, or it could be because of variations in the turnover of each particular ON sequence. We also cannot exclude the possibility that more than one sequence is involved in the expression of T-type currents given the fact that none of the antisense ONs tested totally eliminated T-current expression. The antisense ON against α_{1C} inhibited the constitutively expressed L-type calcium current as expected but did not affect the IGF-1-induced T-type current. Although this ON was less effective in decreasing the expression of L-type current compared with the antisense ONs targeting the T-currents, this could be explained if the L channel protein has a longer half-life compared with the T-type channel protein. Alternatively, the efficacy of the ONs targeting L channels could be lower.

An unanticipated result involved the effect of α_{1C} on the basal T-type currents. The antisense ON α_{1C} significantly increased the density of the T-type calcium current in cells not treated with IGF-1. Whereas we have no precise explanation for this result, one possible mechanism could involve the role played by ancillary subunits in the formation of the functional channel protein. β and $\alpha_2\delta$ subunits have been shown to increase the level of expression of calcium currents by enhanc-

ing the efficiency of assembly and/or protein trafficking to the surface membrane (refs. 26 and 38 but see ref. 37 for a negative result). It is conceivable that α_{1E} and $\alpha_{1C/D}$ subunits in atrial tissue bind to common β and/or $\alpha_2\delta$ subunits. A decrease in the amount of the α_{1C} subunit evoked by the addition of antisense ON might enhance the formation of multimeric α_{1E} complexes and thus increase T-current density. The expression of T-current should be particularly sensitive to this effect, because its density is about an order of magnitude less than that of the L-current in cardiac myocytes. It should be noted that exposure of the cells to both IGF-1 and antisense α_{1C} resulted in an increase in T-current density that was not different from exposing cells to each agent individually (Fig. 4*A* and Fig. 5*A*). This could be explained if IGF-1 stimulation resulted in a maximal amount of the α subunit being produced by the cell. This explanation assumes that the functional cardiac T-channel is multimeric and contains at least an α1 and β subunit. Our finding that an antisense ON targeting the consensus β-binding site of α_{1E} inhibits expression of atrial T-current supports this assumption and is consistent with the multimeric nature of other classes of calcium channel proteins. However, in contrast with our results, a recent study with neuronal tissue concluded that the T-channel pore-forming subunit might not associate with a β subunit in nodosus ganglion cells (37). These conflicting results reinforce the idea that the known heterogeneity of low voltage-activated calcium currents might result from the expression of different genes.

In summary, we have identified a partial DNA clone from rat atrial myocytes with high homology to a member of the E class of calcium channel genes. Furthermore, we have used antisense ONs to block the IGF-1 induction of T-current as well as the basal T-current in atrial myocytes. Our results provide strong support for the idea that a member of the class E of calcium channel genes codes for the cardiac T-type calcium channel. The results also demonstrate that altered gene expression is likely to be involved in the increase of T-type calcium current density stimulated by IGF-1.

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