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Modulation of Ca^{2+} channel currents by a novel anti-dementia drug FK960 in rat hippocampal neurons

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Modulation of calcium channels by FK960

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ABBREVIATIONS: SST, somatostatin; LTP, long-term potentiation; ACh, acetylcholine; GABA, γ-aminobutyric acid; PTX, pertussis-toxin; PKC, protein kinase C.

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ABSTRACT

FK960 (N-(4-acetyl-1-piperazinyl)-p-fluorobenzamide monohydrate), a novel anti-dementia drug, has been demonstrated to ameliorate memory deficits in various experimental models of dementia. This drug selectively increases somatostatin release from hippocampal slices and augments long-term potentiation (LTP) in the CA3 area of the hippocampus. In the present study, the effects of FK960 on voltage-activated Ca^{2+} channels were investigated in acutely isolated rat hippocampal neurons, using whole-cell patch-clamp technique to clarify the cellular mode of action of FK960. Application of somatostatin significantly reduced Ca^{2+} currents via G-protein-coupled signaling pathways. This inhibitory effect was completely abolished by FK960 when applied in combination. In contrast, FK960 showed only modest inhibition on the reduction in Ca^{2+} currents produced by baclofen, an agonist of $GABA_B$ receptor. Intracellular application of the protein kinase inhibitor H-7 did not alter somatostatin-induced inhibition and had no significant effect on blockade by FK960. In addition, application of FK960 alone produced modest but apparent increases in Ca^{2+} currents without significant changes in the activation kinetics of the channels. The dose-response relationship on calcium current enhancement was bell-shaped with a maximum effect at 0.1 μM FK960, the same concentration as that for increasing on somatostatin release and CA3-LTP. These results show that FK960 reverses G protein-dependent inhibition of Ca^{2+} currents by somatostatin in hippocampal neurons. Enhancement of Ca^{2+} currents by FK960 may be due to its modulatory actions on Ca^{2+} channels, rather than removal of G-protein inhibited tonic currents. Taken together, these mechanisms may be involved in the selective effects of FK960 on somatostatin

release, excitatory transmission and synaptic plasticity in the hippocampus.

Alzheimer's disease is the most common cause of dementia in the aged population, and is accompanied by extensive neuron loss particularly in the hippocampus and cerebral cortex, with concomitant progressive cognitive decline. We have recently discovered a novel anti-dementia drug, FK960, which has been demonstrated to improve memory deficits in various experimental models of dementia such as passive avoidance, water-maze and 8-arm radial maze tasks in rats (Yamazaki et al., 1996) and also in rhesus monkeys (Matsuoka and Aigner, 1997). Furthermore, we have also shown that FK960 selectively increases the release of somatostatin, but not of acetylcholine (ACh), γ-aminobutyric acid (GABA), noradrenaline or serotonin from rat hippocampal slices (Inoue et al., 2001), and enhances long-term potentiation (LTP) in the mossy fiber-CA3 pathway of guinea-pig hippocampal slices (Matsuoka and Satoh, 1998). LTP in the hippocampus is believed to be a component of learning and memory (Bliss and Collingridge, 1993). Several lines of evidence have suggested that brain somatostatin plays a vital role in regulating cognitive functions (Ohno et al., 1993; Epelbaum et al., 1994; Matsuoka et al., 1995). Clinical evidence has also shown that impairments of somatostatin-mediated neurotransmission in the brain are associated with dementia in Alzheimer's disease patients (Davies et al., 1980; Bissette and Myers, 1992). We have therefore suggested that FK960 acts on synaptic plasticity in the hippocampus through activation of somatostatinergic neurotransmission to exert its cognitive facilitating effects. However, the molecular mechanism responsible for the ability of FK960 to enhance somatostatin release is not fully understood.

The important regulatory mechanisms of neuronal Ca^{2+} channels in multiple cellular functions such as transmitter release, synaptic excitability and transmission,

hippocampal LTP and synapse formation have been extensively studied. The activation of voltage-activated Ca^{2+} channels is regulated by neurotransmitters and a variety of intracellular second messenger pathways (Anwyl, 1991). Many neurotransmitters, including somatostatin, are known to inhibit Ca^{2+} channels through G-protein-coupled receptor and membrane-delimited pathways that may lead to autoreceptor-mediated inhibition of exocytotic release from presynaptic terminals (Hille et al., 1995; Dolphin 1995). Somatostatin selectively reduces N-type Ca^{2+} channel currents in hippocampal and other central neurons possibly via the activation of pertussis-toxin (PTX)-sensitive Gi/Go proteins (Ikeda and Schofield, 1989; Ishibashi and Akaike, 1995; Viana and Hille, 1996). Moreover, the inhibitory effect of somatostatin on Ca^{2+} channels results in the presynaptic inhibition of hippocampal synapse transmission (Boehm and Betz 1997).

To further understand the molecular basis for the ability of FK960 to enhance somatostatin release, in the present study, we investigated the effect of FK960 on voltage-dependent Ca^{2+} channels and its interaction with somatostatin using whole-cell patch-clamp recording in acutely isolated rat hippocampal neurons. Here we report that FK960 reverses the somatostatin-induced inhibition of Ca^{2+} currents and that this effect appears to be mediated via a G-protein dependent mechanism. We also further demonstrated that FK960 has facilitatory actions on basal Ca^{2+} channel currents in hippocampal neurons.

Methods

Cell preparation.

Hippocampal neurons were acutely dissociated as described by Kay and Wong (1986) with slight modifications. Briefly, transverse hippocampal slices were prepared from 5- to 14-day-old male Wistar rats (Charles River, Japan Inc.). Slices were incubated at 30°C for 60-90 minutes in an oxygenated (100% O_2) solution containing 0.6-0.8 mg/ml trypsin (Type I, Sigma), 120mM NaCl, 5mM KCl, 1mM $CaCl_2$, 1mM $MgCl_2$, 20mM PIPES (piperazine N, N'-bis-[2-ethanesulfonic acid], 1,4-piperazinediethanesulfonic acid) and 25mM glucose (pH 7.0). After rinsing with trypsin-free solution, slices were incubated at room temperature in an O_2 atmosphere. Before use, cells were triturated mechanically into individual cells with a fire-polished Pasteur pipette and were transferred to the recording chamber in Dulbecco's Modified Eagle medium. Visible pyramidal neurons with clear cellular membranes were chosen for whole-cell recording.

Current recording and analysis.

Whole-cell recordings were performed using the conventional patch-clamp techniques (Hamill et al., 1981) with an Axopatch 200A amplifier (Axon Instruments, USA). Patch pipettes were fire-polished and had resistance of 2-4 MΩ when filled with the internal pipette solution containing 100mM CsCl, 5mM $MgCl_2$, 10mM EGTA, 40mM HEPES, 4mM ATP-Tris and 0.2mM GTP-Tris (pH 7.3). The normal external solution for recording Ca^{2+} channel currents consisted of 135mM tetraethylammonium chloride, 10mM $BaCl_2$ and 10mM HEPES (pH 7.3). The recording chamber was

continually perfused with the external solution with or without test drugs through gravity-fed flow pipes at a constant flow rate. Unless indicated otherwise, whole-cell inward currents were elicited every 10 s by depolarization to 0 mV from a holding potential of –80 mV. Currents were four-pole Bessel-filtered and digitized at 10 kHz with DigiData 1200 Interface. Data were acquired and leak subtracted using the P/4 protocol under the control of the pCLAMP (6.0) software (Axon Instruments. U.S.A) using a personal computer. All experiments were carried out at room temperature (21-23°C). All data are presented as mean ± S.E.M, (n = number of cells in parentheses in the figures). Statistical analysis of data was performed using Student's *t* test or Dunnett's multiple comparison test. A *p* value less than 0.05 was considered significant.

Pharmacological materials.

FK960 (*N*-(4-acetyl-1-piperazinyl)-*p*-fluorobenzamide monohydrate) was synthesized by Fujisawa Pharmaceutical Co. Ltd. (Osaka, Japan). A 10mM stock solution of FK960 was prepared daily with distilled water and diluted in the external solution to the desired final concentrations just before use. Somatostatin, baclofen, GTPγS, pertussis toxin (PTX) and H-7 were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Somatostatin was dissolved in distilled water and frozen stored until use. Both GTPγS and H-7 were dissolved in the pipette solution immediately before use. GTP in the standard pipette solution was omitted when GTPγS was included.

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Results

FK960 disrupts somatostatin-induced inhibition of Ca^{2+} currents.

Ba^{2+} was used as the charge carrier for the recording of Ca^{2+} currents in hippocampal neurons. Under whole-cell patch-clamp configuration, high voltage-activated inward Ba^{2+} currents were completely blocked by external application of Cd^{2+}, indicating that they pass through Ca^{2+} channels. Previous results have demonstrated that somatostatin selectively inhibits N-type Ca^{2+} channels in hippocampal neurons since these currents are sensitive to ω–conotoxin GVIA (Ishibashi and Akaike, 1995). Fig. 1 illustrates the effect of FK960 on somatostatin-induced inhibition of Ca^{2+} currents in isolated rat hippocampal neurons. External application of somatostatin (0.1μM) rapidly reduced the peak current amplitude and slowed the activation kinetics of Ca^{2+} currents (Fig. 1A). The inhibition by somatostatin was reversible after removal of somatostatin, and was reproduced by repeated application of somatostatin with slight desensitization (Fig. 1B). In cells perfused with both somatostatin (0.1 μM) and FK960 (0.1 μM) concomitantly, however, the inhibition of Ca^{2+} currents by somatostatin was not observed. FK960 completely blocked the somatostatin-induced inhibition and eliminated the kinetic slowing. In contrast to the mean inhibition of 23.03±1.80% (n=12) produced by somatostatin in control conditions, there was only 2.17±3.91% (n=8) reduction in Ca^{2+} currents in the presence of FK960 (Fig. 1C).

Considering that Ca^{2+} channels are regulated by a variety of neurotransmitters, we further investigated the effect of FK960 on inhibition of Ca^{2+} channels mediated by $GABA_B$ receptor. As shown in Fig.2, application of a $GABA_B$ receptor agonist baclofen

(25 μM) reduced the peak currents with an average 16.33±2.88% (n=7) inhibition in control condition and 11.38±2.63% (n=7) inhibition in the presence of 0.1 μM FK960. While FK960 tended to reduce baclofen-induced inhibition, this effect was partial and was not statistically significant compared to baclofen-alone treated group, in contrast to the combination with somatostatin. These results suggest that FK960 selectively disrupts inhibition of Ca^{2+} currents produced by somatostatin.

G protein-mediated inhibition by somatostatin.

It is well demonstrated that voltage-dependent N-type Ca^{2+} channel activity is regulated by G protein-coupled membrane-delimited pathway (Hille et al., 1995; Dolphin 1995) and the G protein-mediated inhibition of Ca^{2+} currents can be relieved by a large degree of depolarization (Bean 1989; Ikeda 1991). To test the voltage-dependent facilitation of current inhibition, currents were observed following a stronger depolarizing step to +80 mV. Under control conditions, the depolarizing prepulse did not produce characteristic facilitation of the Ca^{2+} currents. This implied the lack of tonic inhibition of Ca^{2+} currents by G proteins in these cells that has been shown in several neuronal systems even in the absence of neurotransmitters (Ikeda, 1991; Kasai, 1991). As shown in Fig. 3A, somatostatin-induced inhibition of Ca^{2+} currents was mostly but not completely relieved and the altered current kinetics was eliminated after the depolarizing prepulse, suggesting that voltage-dependent components reliving in somatostatin action. These was still small portion of currents remained following the prepulse in these cells. This may be due to the voltage protocol used here that resulted in incomplete recovery from somatostatin inhibition. In contrast, the application of FK960

abolished somatostatin inhibition before and after the prepulse applied. Furthermore, FK960 restored the relief of the resting currents following the prepulse in these cells. These results suggest that FK960 modulates voltage-dependent inhibition of Ca^{2+} channels by somatostatin.

Line of evidence has shown that many neurotransmitters including somatostatin represent the inhibition of voltage-activated Ca^{2+} channels through direct activation of inhibitory G proteins (Shapiro and Hille 1993; Hille et al., 1995; Zhang et al., 1996; Herlize et al.1996). In the present study, we employed GTPγS, a nonhydrolyzable analog of GTP to characterize the G proteins-dependent inhibition by somatostatin. When 100 μM of GTPγS was intracellularlly present in the pipette solution, the basal currents were reduced before somatostatin application (data not shown). As shown in Fig. 3B, the mean percent inhibition by somatostatin was 22.49±1.96% (n=9) in the control condition but was largely reduced to 4.51±4.20% (n=4) when GTPγS was added in the pipette solution ($p< 0.01$). For PTX pretreatment, currents were obtained after incubation in culture medium containing high concentration of PTX (25 μg/ml) at 35°C for more than 1h (Beech et al., 1992). Application of somatostatin had an inhibition of 2.18±5.6% (n=3), comparing with 22.73±2.45% (n=7) inhibition obtained without PTX treatment ($p< 0.01$). Taken together, both of GTPγS and PTX pretreatment significantly eliminated somatostatin-induced inhibition of Ca^{2+} currents, suggesting that PTX-sensitive G proteins are involved in somatostatin-induced inhibition of Ca^{2+} channel currents.

Involvement of protein kinases activation.

Several protein kinases are implicated in neurotransmitter receptor-mediated modulation of Ca^{2+} channels by phosphorylation of transmitter receptors themselves, the associated G-proteins, and functional domains of Ca^{2+} channel subunits (Alijanian et al., 1991; Swartz, 1993; Stea et al., 1995; Zamponi et al., 1997; Hamid et al., 1999; Cooper et al., 2000). To test whether protein kinase activation is involved in the effects of FK960 and somatostatin, we employed a broad protein kinase inhibitor H-7 by intracellular application. Fig. 4 illustrates the effects on Ca^{2+} currents by somatostatin and FK960 with 50 μM H-7 in the pipette solution. In the intracellular presence of H-7, somatostatin reduced the peak current amplitudes with mean inhibition of 23.29±4.33% (n=8), similar to control conditions. FK960 again significantly abolished the somatostatin-induced inhibition of Ca^{2+} currents in the presence of H-7 treatment, however, the magnitude of the reduction by FK960 (mean somatostatin inhibition of 6.98±2.73%; n=8) was slightly smaller compared with that in control cells (Fig. 1C). Basal Ca^{2+} currents were unchanged by intracellular application of H-7 (data not shown). These results show that blocking of protein kinase activity by H-7 in our cell preparation has no significant effects on somatostatin receptor-mediated inhibition of Ca^{2+} channels.

FK960 enhances the basal Ca^{2+} currents in hippocampal neurons.

In addition to its modulating actions on somatostatin-induced inhibition of Ca^{2+} currents, we found that external application of FK960 reversibly enhances Ca^{2+} currents under the basal condition in some hippocampal neurons. Fig.5 illustrates the increase in the amplitude of peak currents following application of FK960. Application of FK960 did not alter the kinetics of the channels but increased the peak currents at most test

potentials without measurable voltage-dependence (Fig. 5B). Enhancement of Ca^{2+} currents by FK960 displayed a bell-shaped concentration-dependence that is similar to that seen in our previous pharmacological studies (Matsuoka et al., 1998; Inoue et al., 2001). As illustrated in Fig. 5C, application of 0.01 to 1 μM FK960 enhances the basal currents significantly than in the control condition, and the maximal effect was obtained at a concentration of 0.1 μM of FK960. Difference between 0.1 μM to other doses of FK960 was not statistically significant.

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Discussion

Regulation of neuronal voltage-activated Ca^{2+} channels by neurotransmitters and intracellular signaling pathways is important step in the control of neurotransmitter release, synaptic transmission and neuronal plasticity. In the present study, we have determined the effect of a novel anti-dementia drug FK960 on voltage-activated Ca^{2+} channels in isolated rat hippocampal neurons. Our results demonstrate for the first time that FK960 modulates the G protein-mediated inhibitory effect of somatostatin on Ca^{2+} channels and, furthermore, enhances the basal Ca^{2+} currents in hippocampal neurons.

It has been suggested that somatostatin receptors inhibit N-type Ca^{2+} channels via PTX-sensitive G proteins through a direct membrane-delimited model (Shapiro and Hille 1993; Hille et al., 1995; Zhang et al., 1996). Somatostatin-induced inhibition of Ca^{2+} currents in these isolated hippocampal neurons is mediated by activation of G-proteins since PTX as well as GTPγS eliminated this inhibition. In addition, prepulse facilitation relieved most of the current reduction by somatostatin, a characteristic form of inhibition occurring via a direct interaction of the channel and G-protein (Hille et al., 1995). Importantly, FK960 disrupted G-protein-dependent inhibition of Ca^{2+} currents by somatostatin. Inhibitory effect of FK960 was more robust than that induced by depolarizing prepulse which resulted in the recovery from somatostatin inhibition of Ca^{2+} currents. Somatostatin-induced inhibition of Ca^{2+} channels has been suggested to be one of the mechanisms underlying presynaptic inhibitory control of transmitter release (Boehm and Betz, 1997). Therefore, the ability of FK960 to modulate the inhibitory effect of somatostatin on Ca^{2+} channels may contribute to its ability to selectively enhance somatostatin release and somatostatinergic transmission in

hippocampal slices (Inoue et al., 2001).

In the present study, we found that basal Ca^{2+} channel currents in hippocampal neurons were enhanced by FK960. Compared with its obvious effect on somatostatin-induced depression of Ca^{2+} currents, the enhancement of basal Ca^{2+} currents produced by FK960 was more modest, and was not observed in all cells. Although several types of voltage-activated Ca^{2+} channels have been identified in the hippocampal neurons, it has been suggested that N-type channels predominantly contribute to the transmitter-stimulated synaptic transmission (Wheeler et al., 1994). Further study is necessary to determine which type of Ca^{2+} channel currents are enhanced by FK960. The FK960-induced increase in basal Ca^{2+} channel currents is unlikely to be a consequence of removal of G-protein-mediated tonic inhibition, as observed in some neurons (Dolphin, 1995), since the rebound current facilitation by large depolarization pulse was not observed in these cells. The facilitatory effect of FK960 on Ca^{2+} current demonstrated a bell-shaped concentration-response relationship comparable with our previous studies on somatostatin release and CA3-LTP enhancement, indicating the phenomenon might share common cellular mechanisms.

Voltage-activated Ca^{2+} channel activities are regulated by a variety of neurotransmitter and intracellular signaling pathways (Hille et al., 1995; Dolphin 1995). Therefore, the modulation of Ca^{2+} channels by FK960 could be mediated at the level of neurotransmitter receptor, G proteins, Ca^{2+} channels, or other intracellular signaling pathways. Our previous studies have shown that FK960 does not bind to somatostatin receptors or a number of other neurotransmitter receptors (unpublished observations), although the possibility of that an unknown protein component associated to its action could not be ruled out. Our present results demonstrated that FK960 disrupted G

protein-dependent inhibition by somatostatin and enhanced the basal Ca^{2+} current, further suggesting that the modulation of FK960 is not in the level of somatostatin receptors activation and appear to be involved in the direct interaction between G protein and Ca^{2+} channels. Recently, an occluded inhibition of Ca^{2+} channels by activation of two types of transmitter receptor by opioid agonists and somatostatin has been investigated (Polo-Parada and Pilar, 1999).

Protein kinases are important for regulation of neuronal Ca^{2+} channel activity and have been shown to directly phorsphorylate the channel subunit/G protein complex (Swartz, 1993; Hamid et al., 1999; Cooper et al., 2000). In central neurons, an activation of PKC has been found to augment Ca^{2+} currents (Swartz et al., 1993; Stea et al., 1995; Hamid et al., 1999) and disrupt G protein-dependent inhibition of Ca^{2+} channels (Swartz 1993; Zamponi et al., 1997; Barrett and Rittenhouse 2000). Furthermore, PKC activators block the inhibition of Ca^{2+} currents induced by somatostatin in rat hippocampal neurons(Ishibashi and Akaike, 1995). In the present study, intracellular application of protein kinase inhibitor H-7 had no effect on somatostatin-induced inhibition of Ca^{2+} currents. In addition, H-7 slightly reduced FK960's modulation of somatostatin inhibition, but the effect was not significant. Considering that H-7 is known to be relatively more effective on cAMP (or cGMP)-dependent kinase activation than on PKC activation, however, we could not rule out the possibility of PKC-mediated regulation involved in the mechanism of action of FK960. Further studies with highly selective PKC modulators are required to clarify the contribution of intracellular phosphorylation pathways to the mechanism of FK960 action.

It is conceivable that modulation by FK960 of somatostatin-mediated inhibition of Ca^{2+} channels may be responsible for its enhancement of somatostatin release from

hippocampal slices. Many studies have demonstrated the existence of negative feedback regulation via presynaptic G-protein-coupled receptors (autoreceptors) as well as voltage-activated Ca^{2+} channels, leading to inhibition of transmitter release (Hille et al., 1995; Dolphin 1995; Takahashi et al., 1996). Indeed, somatostatin release from nerve terminals is Ca^{2+}-dependent, and is modulated through activation of an autoreceptor located on presynaptic terminals (Iversen et al., 1978; Fontana et al., 1996; Helboe et al., 1998). Furthermore, somatostatin selectively inhibits the N-type Ca^{2+} channel among diverse subtypes of channels in isolated hippocampal neurons (Ishibashi and Akaike, 1995). It has also been reported that somatostatin inhibits excitatory neurotransmission via presynaptic receptors, by inhibition of downstream of Ca^{2+} entry at rat hippocampal synapses (Boehm and Betz 1997). It will be important to determine whether FK960 acts exclusively on somatostatin-mediated inhibition, or whether it can disrupt the inhibition produced by other transmitters which involve G-protein coupled receptors. In the present study, interestingly, FK960 showed only modest inhibition on the reduction in Ca^{2+} currents produced by an activation of $GABA_B$ receptor, suggesting that FK960's action is not general for G-protein coupled receptors and could be selective for somatostatin receptor over other class of G-protein coupled receptors. Somatostatin-containing neurons are abundant in the hippocampus, and, although they often co-localize or functionally interact with other neurotransmitters such as GABA or ACh, they might regulate Ca^{2+} channels through different G-protein pathways (Shapiro and Hille, 1993; Hille et al., 1995). In our studies on neurotransmitter release in rat hippocampal slices, we found that FK960 had no significant effect on ACh, GABA, noradrenaline and serotonin release (Inoue et al., 2001). We therefore propose that FK960 could exert selective facilitatory actions on somatostatin release from

hippocampal nerve terminals, as a consequence of blockade of the interplay between the somatostatin autoreceptor, inhibitory G protein and (possibly N-type) Ca^{2+} channels.

In conclusion, we have demonstrated for the first time that the novel anti-dementia drug FK960 reverses the inhibitory effect of somatostatin on Ca^{2+} channels, and enhances the basal activity of Ca^{2+} currents in rat hippocampal neurons. These cellular mechanisms may explain the unique mode of action of FK960, and may further provide new insights on the molecular basis for the understanding the control of neuropeptide release from presynaptic terminals.

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FOOTNOTES

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LEGENDS FOR FIGURES

Figure 1. Effect of FK960 on somatostatin-induced inhibition of Ca^{2+} currents in hippocampal neurons. Currents were evoked every 10 s by a depolarization to 0 mV from a holding potential of -80 mV. (A) Superimposed current traces were obtained in control external solution and 0.1 μM somatostatin without or with 0.1 μM FK960. (B) Time course of the peak current in application of somatostatin (0.1 μM) in the presence or absence of 0.1 μM FK960. (C) Pooled results of peak current inhibited by somatostatin in the absence or presence of FK960. *** $p< 0.001$; statistically significant compared to somatostatin alone group (by Student's t-test). SST; somatostatin.

Figure 2. Effect of FK960 on baclofen-induced inhibition of Ca^{2+} currents in hippocampal neurons. (A) Superimposed current traces were obtained in control external solution and 25 μM baclofen without or with 0.1 μM FK960. (B) Pooled results show the peak current inhibition produced by baclofen and concomitant application with FK960. No statistical significant change was observed between two groups (by Student's *t* test).

Figure 3. Involvement of G proteins in the action of somatostatin. (A) Effect of prepulse facilitation on somatostatin-induced inhibition. Currents were evoked by the voltage protocol as shown above. Superimposed current traces were obtained in control external solution and 0.1 μM somatostatin without (top traces) or simultaneously with 0.1 μM FK960 (bottom traces). SST; somatostatin. (B) Effects of intracellular GTPγS and PTX treatment. Peak currents were reduced by the intracellular application of 100 μM GTPγS

or by pretreatment of PTX. Both of them blocked significantly the somatostatin-induced inhibition. Pooled results show the peak current inhibition by somatostatin in the absence or presence of FK960. ** $p< 0.01$; statistically significant compared to somatostatin alone group (by Student's *t* test).

Figure 4. Effect of protein kinase inhibitor H-7 on somatostatin-induced inhibition of Ca^{2+} currents and on the blockade by FK960. (A) Superimposed inward currents were recorded before and after application of 0.1 μM somatostatin in the intracellular presence of 50 μM H-7 (upper trace). Time courses of peak current recorded under application of 0.1 μM somatostatin (bottom trace). (B) Pooled results of peak current inhibited by somatostatin in the absence or presence of FK960. ** $p< 0.01$; statistically significant compared to somatostatin-alone group (by Student's *t* test). SST; somatostatin.

Figure 5. Enhancement of the basal Ca^{2+} currents by FK960. (A) Time course of enhancement of peak currents by 0.1 μM FK960. Insert shows superimposed currents recorded before, during and after FK960 application. (B) Peak current-voltage relationship in the control condition and application of FK960 in the same cell. (C) Concentration-effect relationship for the enhancement of Ca^{2+} currents produced by FK960. Pooled results show the percentage changes of peak currents in the absence (set as 100%) and presence of FK960. ** $p< 0.01$, *** $p< 0.001$; statistically significant compared to control group (by ANOVA followed by Dunnet's comparison test).

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Fig. 1

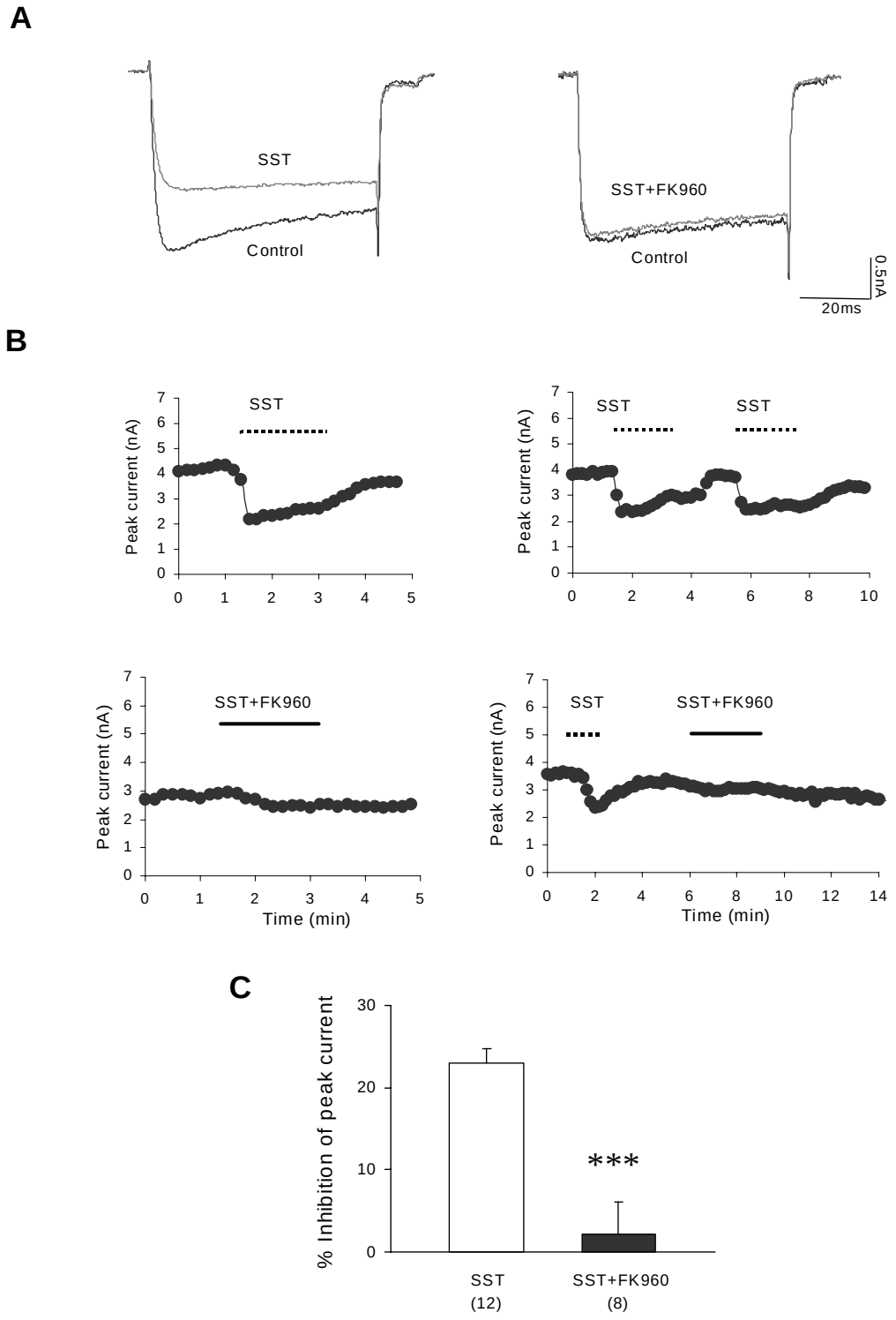

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Fig. 2

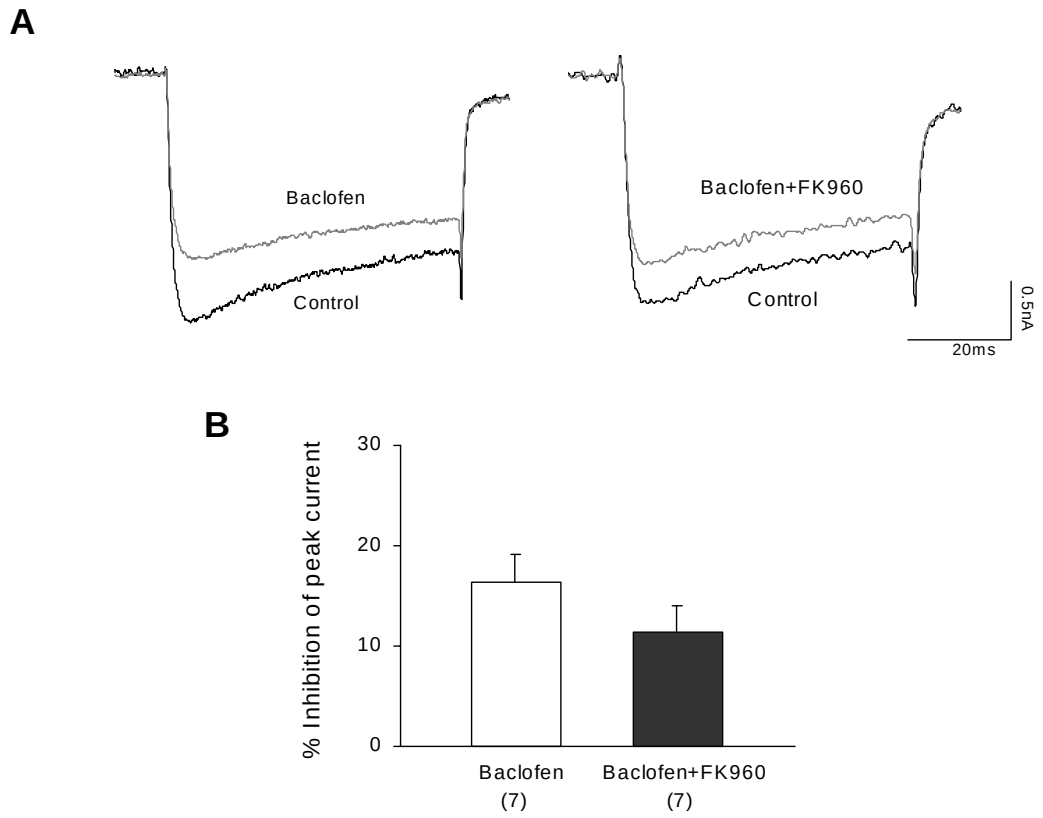

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Fig. 3

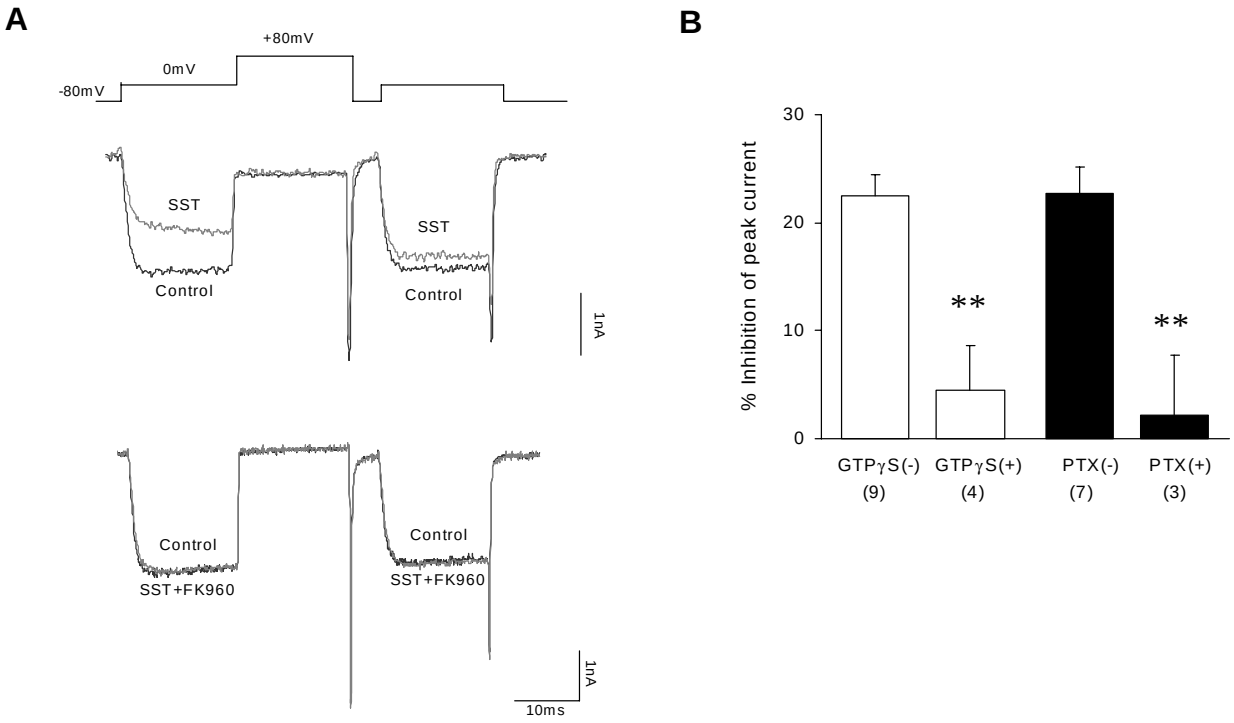

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Fig. 4

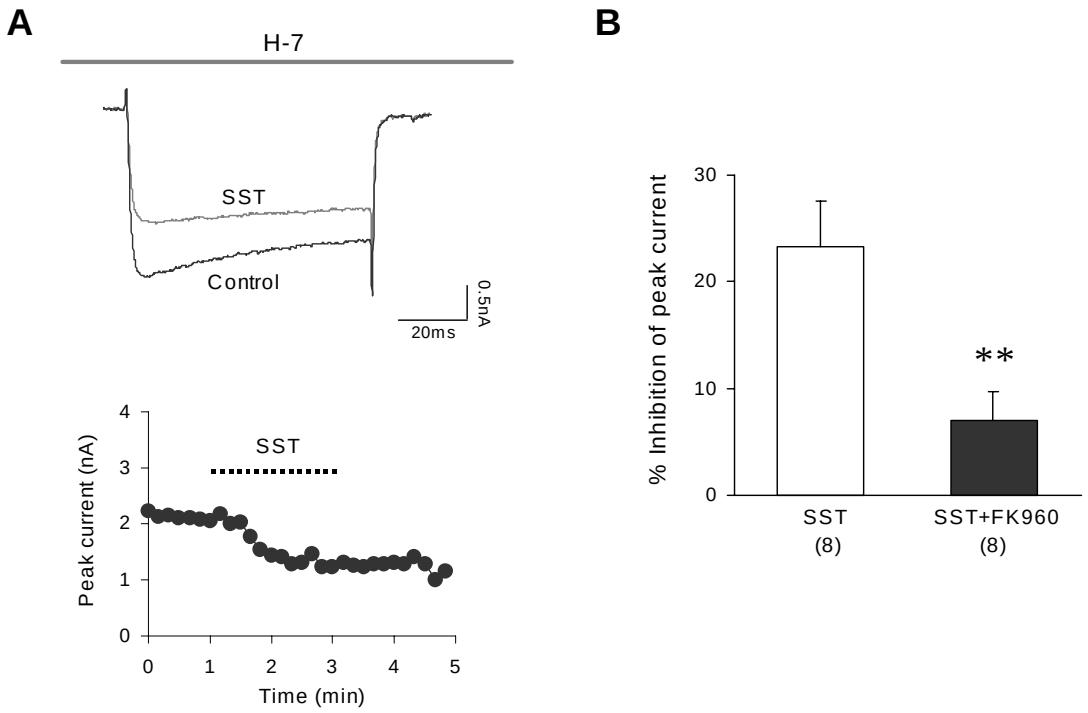

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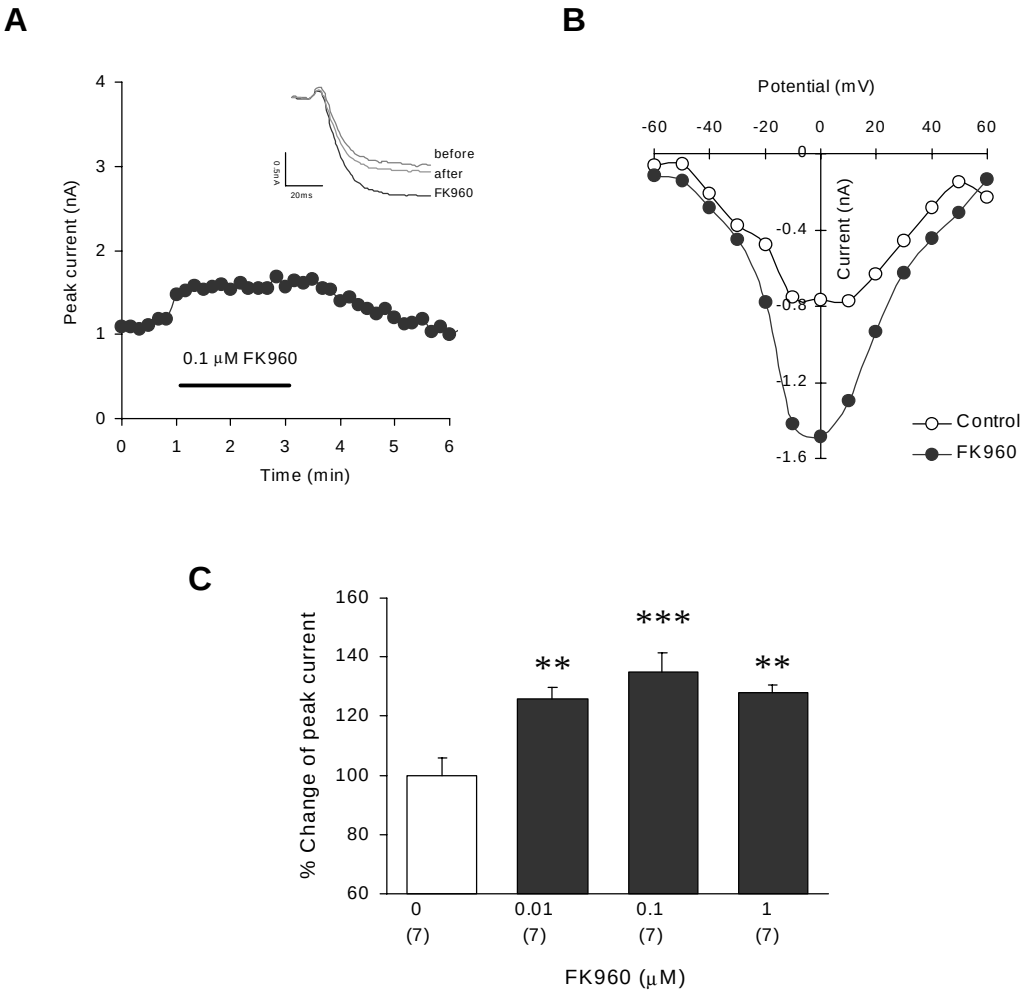
Fig. 5



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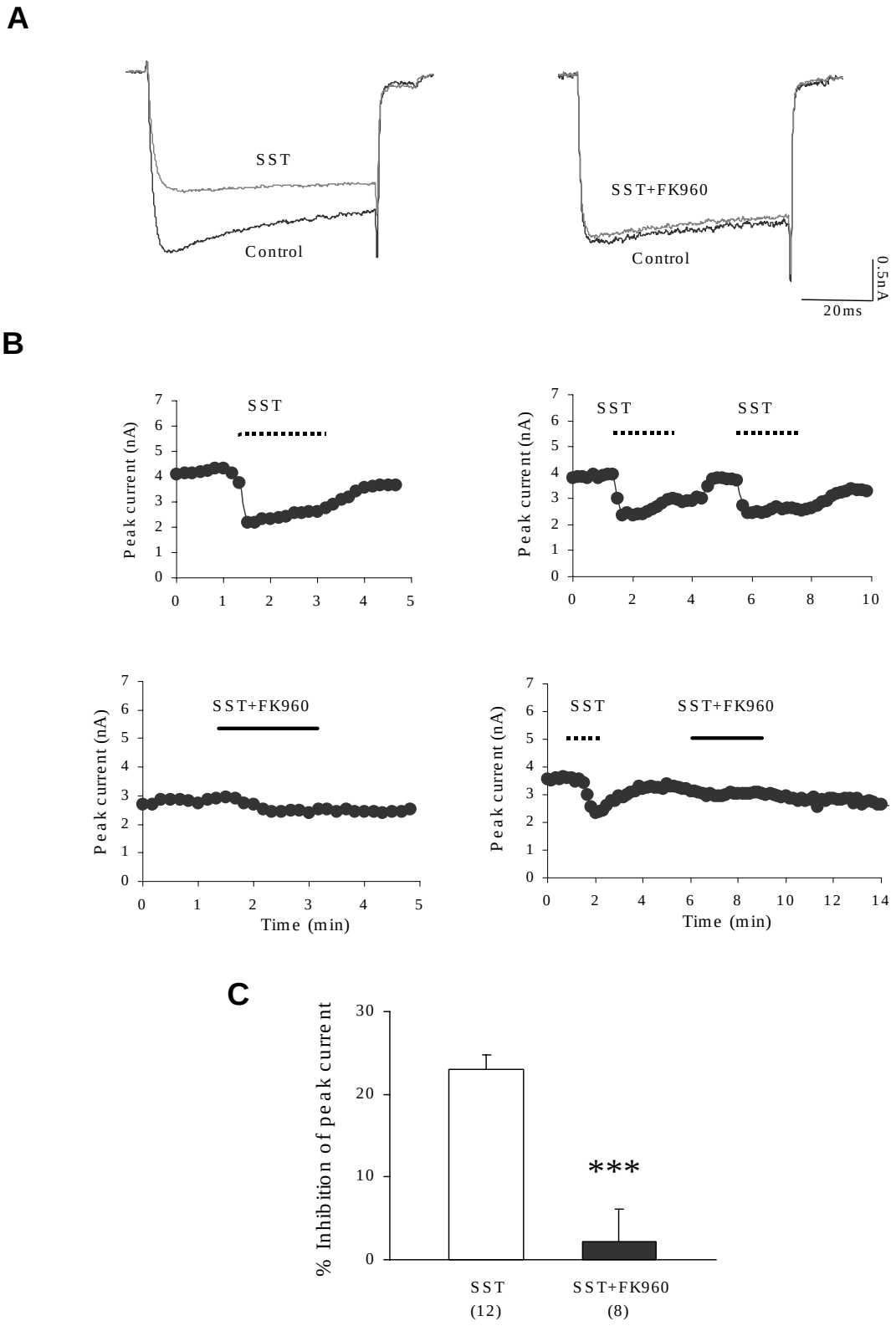

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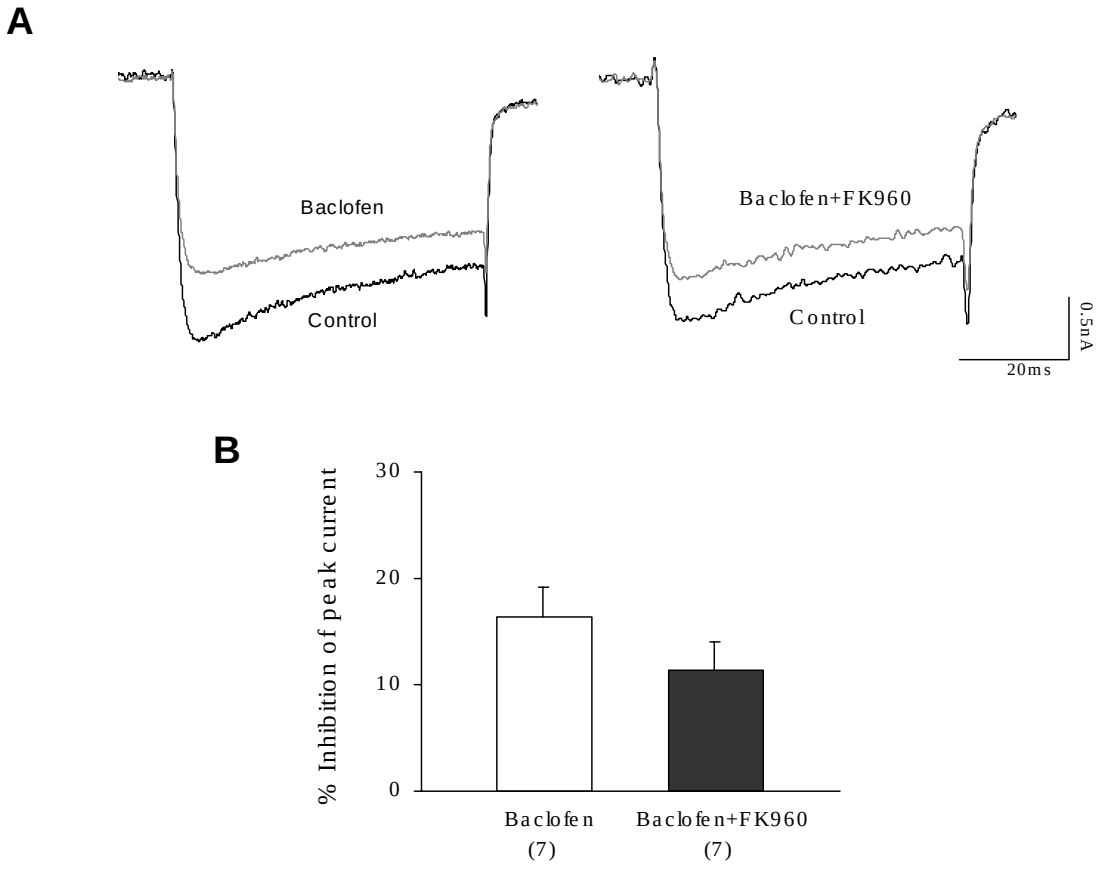

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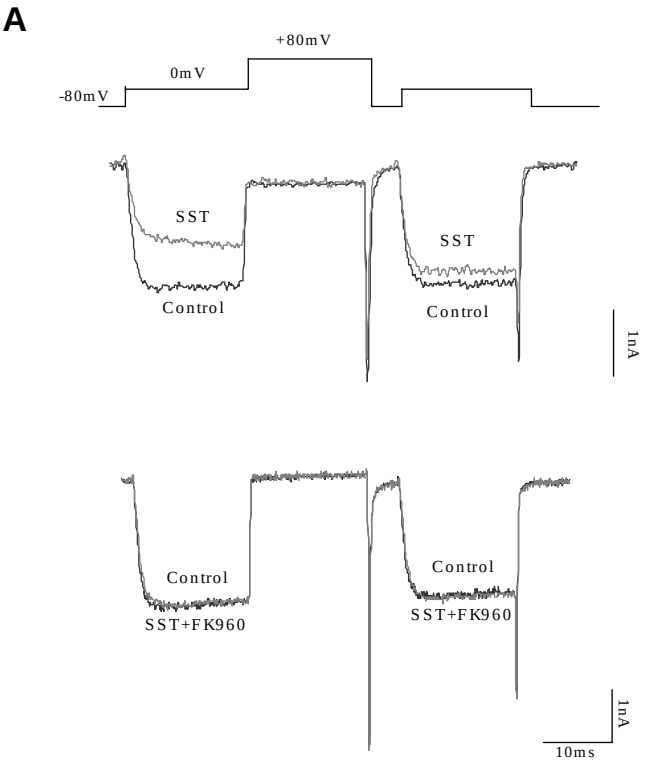

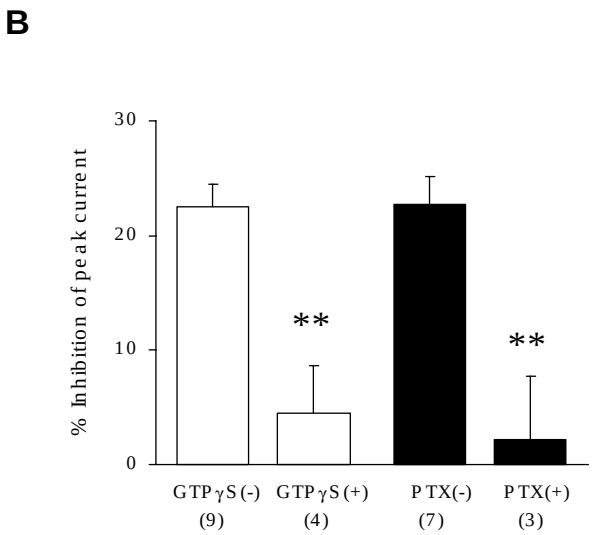

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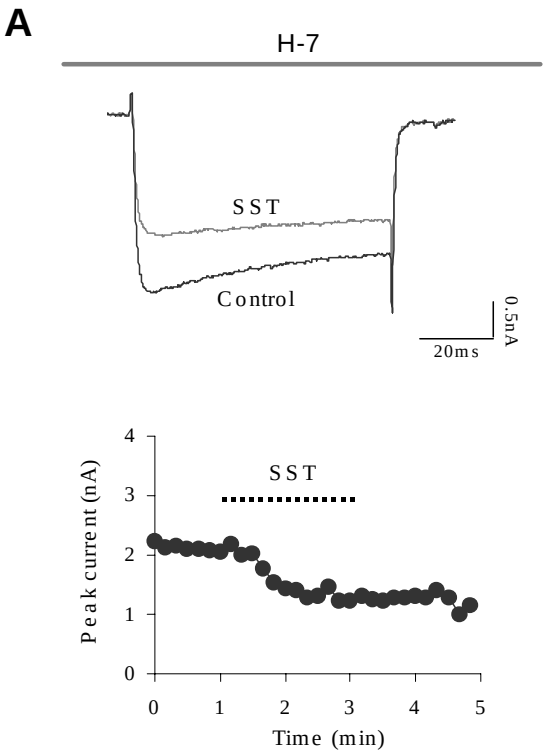

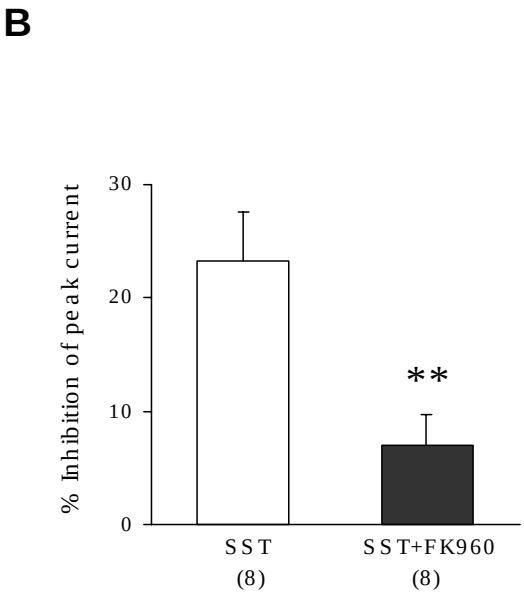

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