The Journal of Neuroscience, July 1992, 12(7): 2665–2671

Tumor Necrosis Factor Modulates Ca^{2+} Currents in Cultured Sympathetic Neurons

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The effect of recombinant human tumor necrosis factor-alpha (rhTNF) on calcium currents of cultured neurons from neonatal rat superior cervical ganglia (SCG) was studied using whole-cell patch-clamp technique. We found that rhTNF-treated SCG neurons exhibited increased calcium current density without significant alteration in the steady-state parameters of activation and availability. The fraction of the current sensitive to dihydropyridines and ω-conotoxin also remained unchanged. Recovery from slow inactivation of the current, but not recovery from fast inactivation, was prolonged in rhTNF-treated cells when compared to that of control cells.

We conclude that immune peptides such as rhTNF can alter cellular functions of sympathetic neurons via modulating ionic conductances. However, these changes observed in calcium currents of SCG neurons cannot account for the effect of rhTNF on norepinephrine secretion observed in a previous study. It is proposed that rhTNF exerts an additional effect at a later event in the exocytotic process.

The spectrum of cytokine activities extends beyond the regulation of hematopoiesis and immune mechanisms. Many of these cytokines also exert pleiotropic effects on cells of the nervous system, including the sympathetic nervous system (for review, see Bazan, 1991). For example, γ-interferon improves the survival of NGF-deprived sympathetic neurons (Chang et al., 1990), whereas interleukin-2 (IL2) enhances their neurite outgrowth (Haugen and Letourneau, 1990). Regulation of transmitter phenotype expression of sympathetic ganglia (i.e., substance P) by recombinant human interleukin-1β (rhIL1-β) has also been described (Jonakait et al., 1990; Freidin and Kessler, 1991). The effects of these cytokines are important in view of the known sympathetic innervation of lymphoid organs (Felten et al., 1987), allowing locally accumulated cytokines to modulate sympathetic neuronal function directly and allowing immune cells to respond to neurotransmitters released.

Tumor necrosis factor-α (TNF-α), a 17 kDa polypeptide secreted by activated macrophages, has been implicated as one of the mediators of septic shock and in the pathogenesis of some autoimmune disorders (for review, see Fiers, 1991; Vilcek and Lee, 1991). TNF secretion by macrophages often correlates with the disease severity. For instance, catecholamines suppress TNF secretion and also decrease the severity of experimental autoimmune disorders, whereas chemical sympathectomy in mice increases TNF secretion and augments the severity of experimental autoimmune disorders (Chelmicka-Schorr et al., 1988, 1992; Hu et al., 1991). On the other hand, it is not clear whether catecholamine secretion or other biological properties of sympathetic neurons could be directly regulated by TNF.

We have recently shown that cultured sympathetic neurons treated for 4–24 hr with recombinant human TNF-α (rhTNF) exhibited a decreased secretory response to a repeat K^+-induced depolarization given 6 min after the initial depolarization. The effect was observed with rhTNF at concentrations of ≥0.28 nM and was attenuated when rhTNF was preneutralized with anti-TNF antibody. Although the inactivation of secretion was similar between control and rhTNF-treated cells, the recovery from inactivation of secretion was prolonged in rhTNF-treated cells (Soliven and Albert, 1992; see Discussion). The effect of rhTNF on superior cervical ganglion (SCG) neurons could be mediated via modulation of ionic conductances or via regulation of a later event in the stimulus–secretion coupling. Passive depolarization with high K^+–containing solutions bypasses receptors and directly activates Na^+ and Ca^{2+} channels. TTX-sensitive Na^+ channels do not appear to contribute to catecholamine secretion evoked by high K^+ (>15 mM K^+) in either cultured sympathetic neurons or chromaffin cells (Wakade and Wakade, 1982; Marley, 1988). In contrast, regulation of intracellular calcium/calcium fluxes plays a major role in modulating neurotransmitter release (for review, see Smith and Augustine, 1988; Stjärne et al., 1990). Furthermore, there is evidence suggesting that neurosecretory habituation in PC12 cells is the result of decreased intracellular calcium levels in response to repetitive depolarization (Martin and Koshland, 1991). To determine whether rhTNF could exert its effect by regulating calcium entry, we used the whole-cell patch-clamp technique to study the modulation of Ca^{2+} currents from neonatal rat SCG neurons during acute exposure (perfusion experiments) as well as after prolonged exposure to rhTNF and other cytokines.

Materials and Methods

Cell culture. Superior cervical ganglia were dissected from 1–3-d-old Holtzmann rats and cultured by a modification of the method of Wakade and Wakade (1982). The ganglia were enzymatically dissociated by incubation in Ca^{2+}/Mg^{2+}-free phosphate-buffered saline containing collagenase type IA (Sigma, St. Louis, MO) and dispase (Boehringer-Mannheim Biochemicals, Indianapolis, IN) for 20 min followed by treatment with 0.25% trypsin type II (Sigma, St. Louis, MO) for 10–15 min. Trypsinization was stopped with media containing 10% horse serum. Cells were dissociated further by trituration through a Pasteur pipette,

Received Sept. 26, 1991; revised Feb. 7, 1992; accepted Feb. 12, 1992.

We thank Dr. A. P. Fox for reviewing the manuscript. This work was supported by MS Society Grant RG-2195-A-2, a grant from Brain Research Foundation, NIH Grant PO1 NS24575, and a gift from the Lucille B. Markey Charitable Trust Foundation.

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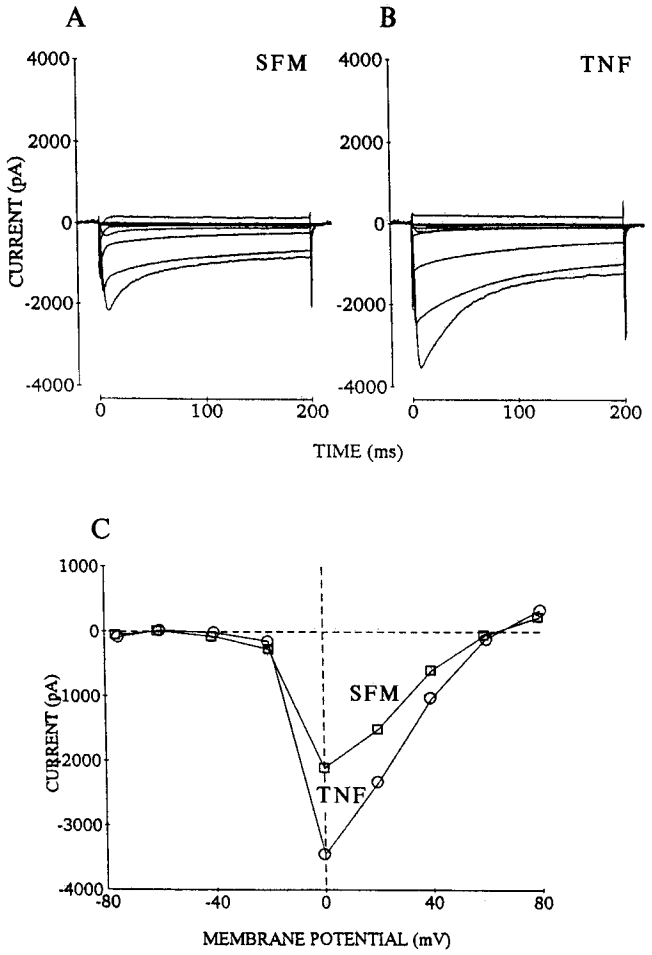

Figure 1. Examples of whole-cell Ca^{2+} currents from SCG neurons with 9 mM Ba^{2+} as the charge carrier. *A*, Control SCG neuron. *B*, rhTNF-treated SCG neuron. Pulses of 200 msec in duration were stepped to various depolarizing potentials in 20 mV increments from a holding potential of −80 mV at 20 sec intervals. *C*, The corresponding current–voltage plots (*I/V* plots) constructed from peak Ca^{2+} currents.

centrifuged, and preplated for 60–90 min to remove non-neuronal cells. Sympathetic neurons were plated on rat tail collagen–coated 35 mm culture dishes at a density of one or two ganglia per plate. The culture medium consisted of Leibowitz L-15 (GIBCO) supplemented with glucose (0.6%), glutamine (2 mM), nerve growth factor 2.5S (20 ng/ml), and penicillin/streptomycin (100 U/ml and 100 μg/ml, respectively). Cells were used for electrophysiology within 32 hr following isolation.

Electrophysiology. Current recordings were obtained using the whole-cell configuration of the patch-clamp technique as previously described (Hamill et al., 1981). The pipette resistance ranged from 1 to 3 MΩ. Cells were studied at room temperature. For recording of Ca^{2+} currents, the bathing solutions consisted of either of the following (in mM): (1) 140 tetraethylammonium (TEA) Cl, 5.4 KCl, 9 $BaCl_2$, 10 HEPES, pH, 7.4; or (2) 100 TEA Cl, 40 NaCl, 5.4 KCl, 9 $BaCl_2$, 10 HEPES, 3 μM TTX, pH, 7.4. Pipette (intracellular) solutions contained (in mM) 140 *N*-methyl-D-glucamine Cl, 2 $MgCl_2$, 11 EGTA, and 10 HEPES, pH, 7.3. Mg-ATP (2 mM) and GTP (0.6 mM) were included in the pipette solution. For recording of I_A currents, bathing solution contained $CdCl_2$ instead of $BaCl_2$ to block Ca^{2+} currents. The pipette solution contained the following (in mM): 140 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 11 EGTA, 10 HEPES, pH, 7.3. Current records were filtered at 2 kHz using an eight-pole Bessel filter and sampled at 5 kHz. Membrane capacitance was measured by integrating the current during either −10 mV or +10 mV voltage step and subtracting the baseline established 10 msec after the step. Where three or more experiments were performed for a given experimental condition, values are reported as either the mean ± SEM or as the range with the number of experiments in parentheses. The statistical significance of the results was assessed by using a Student's *t* test.

The drugs used in this study were obtained from the following sources: norepinephrine, nifedipine (Sigma, St. Louis, MO); ω-conotoxin GVIA (ω-CgTx) (Peninsula, Belmont, CA); recombinant human TNF-α; recombinant human IL1-β (Genzyme, Boston, MA); rhTNF had a specific activity of 2×10^7 U/mg. (−)Bay K 8644 was a gift from Dr. A. P. Fox (University of Chicago, IL).

Results

Whole-cell Ca^{2+} currents were recorded from neonatal rat SCG neurons using 9 mM Ba^{2+} as the charge carrier. To ensure adequate space clamp, experiments were performed on SCG neurons plated at low density within 32 hr of their isolation. Only recordings obtained from cells with processes less than twice the soma diameter were included in the analysis. Figure 1 illustrates a family of Ca^{2+} currents evoked by depolarizations in 20 mV increments from a holding potential of −80 mV. The current–voltage relationship usually peaks at 0 mV, but the threshold of activation varies from −40 mV to 0 mV.

Perfusion with rhTNF (1–2.8 nM), rhIL1-β (0.1 nM) had no significant effect on current amplitudes or kinetics ($n = 6$ for rhTNF experiments; $n = 10$ for rhIL1-β experiments). However, incubation of SCG neurons with rhTNF (0.56–2.8 nM) for 12–24 hr from the time of dissociation resulted in an increase in the Ca^{2+} current (I_{Ca}) density with peak current amplitudes measured at 0 mV when compared to cells grown in serum-free medium (SFM) [TNF, 71.2 ± 8.4 pA/pF ($n = 43$); SFM, 46.9 ± 6.0 pA/pF ($n = 44$); $p < 0.02$] (Fig. 2). Cells grown in the presence of serum-containing medium exhibited slightly greater I_{Ca} density [57 ± 10 pA/pF ($n = 20$)] compared to cells grown in SFM, but the difference was not significant. No effect on I_{Ca} density was observed when SCG neurons were incubated with rhTNF for 15 min [I_{Ca} density, 42 ± 3.7 pA/pF ($n = 11$)] or for 4 hr [I_{Ca} density, 45.4 ± 3.8 pA/pF ($n = 12$)].

In spite of an increase in I_{Ca} density induced by rhTNF, there was no difference in the current–voltage relationship or in the kinetics of current activation/inactivation between control cells grown in SFM and cells treated with rhTNF for 12–24 hr. The activation time estimated as time to peak current was 6.2 ± 0.7 msec ($N = 10$) in control cells and 4.9 ± 0.4 msec ($N = 10$) in rhTNF-treated cells ($p > 0.05$). The time constant of current inactivation was 107.4 ± 11.8 msec ($N = 10$) in control cells and 89.6 ± 11.6 msec in rhTNF-treated cells ($p > 0.05$).

The steady-state parameters of activation and availability of Ca^{2+} currents from rhTNF-treated cells did not differ significantly from those obtained from control cells (Fig. 3), although the activation and inactivation curves from rhTNF-treated cells tend to be slightly less steep. The values of $V_{½}$ and slope factor derived from the experiments stated below can only be considered as estimates since no attempt was made to separate the calcium channel types that may contribute to the whole-cell current. It has recently been shown that dihydropyridine (DHP)-sensitive current, ω-CgTx–sensitive current, and ω-CgTx–resistant current in dorsal root ganglia (DRG) neurons all had similar midpoints of activation, although the DHP-sensitive component was slightly less steep than the other components (Regan et al., 1991). Values of activation parameters were relative tail current amplitudes at −40 mV following repolarization from various test potentials, and normalized to the value obtained following repolarization from +60 mV. Values for inactivation parameters were determined by normalizing peak currents recorded at a constant test potential (0 mV) when stepped from

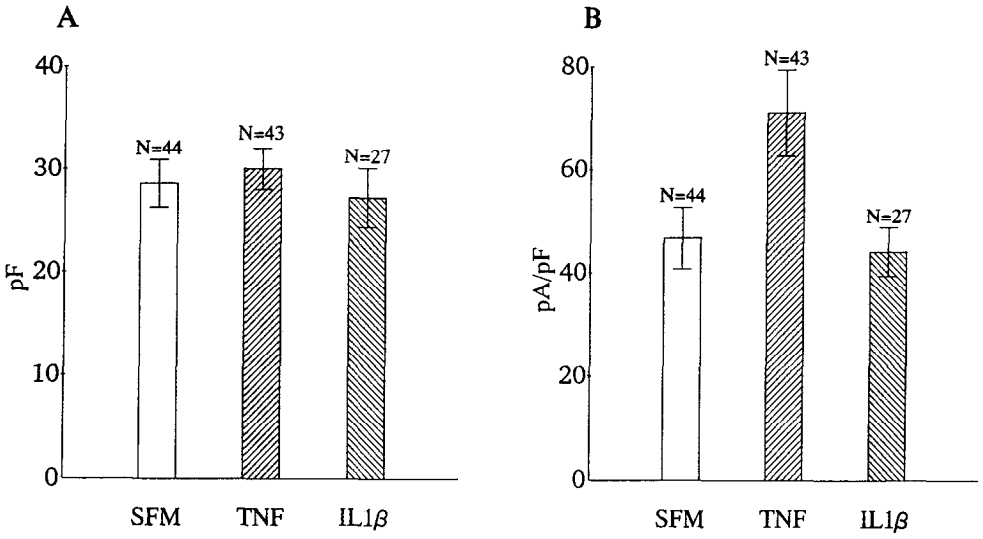

Figure 2. Capacitance expressed in pF (*A*) and Ca^{2+} current density expressed in pA/pF (*B*) in SCG neurons. Ca^{2+} current density with peak current amplitudes measured at 0 mV was increased in rhTNF-treated SCG neurons (*TNF*), as compared to control cells in *SFM*, and rhIL1-β–treated cells (*IL1β*). Holding potential, −80 mV. Total membrane capacitance was not significantly different among the three groups. *Error bars* in this and subsequent figures represent SEM.

various holding potentials. Data points shown in Figure 3 represent average of five to eight experiments and are fitted with a Boltzmann function described in the caption. In control cells, $V_{½}$ of activation was −7.3 mV with a slope factor of −9.5; V_j of inactivation was −60 mV with a slope factor of 10.5. In rhTNF-treated cells, $V_{½}$ of activation was −6.4 mV with a slope factor of −10.5; V_j of inactivation was −58 mV with a slope factor of 11.5. Since nearly full inactivation occurs after a few minutes at −40 mV, it appears that there is little L-type Ca^{2+} current in rat SCG neurons. Alternatively, the L-type Ca^{2+} channels present in rat SGN also exhibit a significant inactivation process.

To clarify further whether rhTNF increases the expression of one or both types of Ca^{2+} channels, whole-cell recordings were obtained before and after perfusion with the following: (1) nifedipine, a DHP antagonist; (2) (−)Bay K 8644, a DHP agonist; (3) ω-CgTx, a snail toxin. At a holding potential of −80 mV, nifedipine (5 μM) had only a minor effect on whole-cell calcium currents of SCG neurons. As shown in Figure 4, the fraction of the current presumed to be carried by DHP-sensitive, L-type Ca^{2+} channels was not significantly different between control cells and rhTNF-treated cells. Nifedipine decreased the amplitudes of peak Ca^{2+} currents measured at 0 mV by 17.5 ± 2.6% in control cells and by 21.6 ± 5.1% in rhTNF-treated cells. The response to (−)Bay K 8644 (1 μM) was also similar in control cells versus rhTNF-treated cells. The increase in tail current amplitudes measured at 0.5–1.5 msec following the termination of voltage steps to 0 mV was 64 ± 9% (n = 5) in control cells and 62 ± 11% (n = 6) in rhTNF-treated cells. Since Bay K 8644 caused a slight leftward shift in the current–voltage curve, changes in peak current amplitudes at a less depolarized test potential, −30 mV, were also measured. The increase in peak current amplitudes was 105 ± 5% (n = 4) in control cells and 126 ± 15% (n = 4) in rhTNF-treated cells. Addition of ω-CgTx (1 μM) decreased the peak current at 0 mV to 20.9 ± 4.1% (n = 6) of the initial current in control cells, and 19.8 ± 2.8% (n = 8) in rhTNF-treated cells, confirming previous reports that the major component of the whole-cell Ca^{2+} current in SCG neurons is carried by ω-CgTx–sensitive N-type Ca^{2+} channels (Plummer et al., 1989; Regan et al., 1991).

The extent of inactivation of Ca^{2+} currents during a pulse to 0 mV (i.e., fast inactivation) varies from cell to cell and is

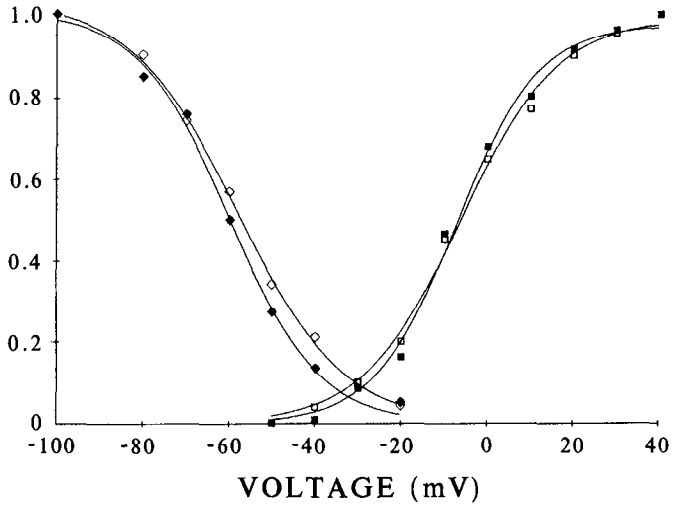

Figure 3. Steady-state parameters of activation and availability of Ca^{2+} currents recorded from control SCG neurons (*solid symbols*) and rhTNF-treated SCG neurons (*open symbols*). To determine the voltage dependence of activation of Ca^{2+} currents, tail current amplitudes were measured at 0.5–1.5 msec after the termination of depolarization at a membrane potential of −40 mV to minimize the contamination by residual capacity transients. Currents were corrected for leakage current determined using a step from −80 mV to −50 mV. The values of the tail currents obtained were then normalized to the value after the step to +60 mV, averaged from six to eight experiments and fitted with a Boltzmann function with the form $y = I_{max}/[1 + e^{(V-V_{½})/k}]$. The half-maximal activation voltage ($V_{½}$) of the currents from control experiments was −7.3 mV, with a slope factor (k_j) of −9.5. In rhTNF-treated cells, $V_{½}$ of the currents was −6.4 mV and the k_j was −10.5. The steady-state parameters of availability of Ca^{2+} currents were determined by varying the holding potential for 2 min prior to recording the current as a constant test potential of 0 mV. The normalized peak currents were averaged from seven experiments in control cells, five experiments in r^hTNF-treated cells, and fitted with a Boltzmann function $j = I_{max}/[1 + e^{(V-V_j)/k_j}]$, where V_j is the voltage at which the current is half-inactivated, and k_j, the slope factor. V_j was −60 mV and k_j was 10.5 in controls, while V_j was −58 mV and k_j was 11.5 in rhTNF-treated cells.

reflected in a corresponding decrease in the amplitude of the currents during a subsequent pulse to 0 mV given after a brief interval. From a holding potential of −80 mV, the fraction of inactivation for pulses of 0.5 sec separated by a 20 msec interval was 24.6 ± 1.9% (n = 10) in untreated cells and 29.2 ± 2.2%

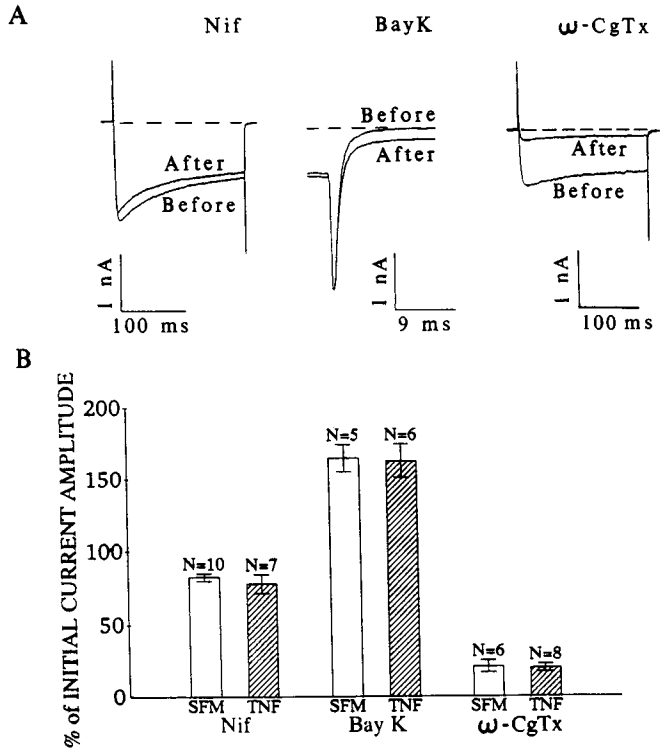

Figure 4. *A*, Examples of current traces recorded from rhTNF-treated cells before and after perfusion with nifedipine (*Nif*; 5 μM), (−)Bay K 8644 (*Bay K*; 1 μM), and ω-*CgTx* (1 μM). For nifedipine and ω-CgTx experiments, pulses of 200 msec in duration were stepped from a holding potential of −80 mV to 0 mV at 30 sec intervals. Only two traces were shown for simplification. For (−)Bay K 8644 experiments, only tail currents were shown. Pulses of 15 msec in duration were stepped from a holding potential of −80 mV to 0 mV followed by repolarization to −40 mV. Tail current amplitudes were measured at 0.5–1.5 msec after the voltage step from 0 mV. *B*, Summary of the results of perfusion with nifedipine, (−)Bay K 8644, and ω-CgTx. Values shown were from peak currents for nifedipine and ω-CgTx experiments, and from tail currents for (−)Bay K 8644 experiments. The sensitivity of Ca^{2+} currents to DHPs and ω-CgTx did not differ between control cells incubated in *SFM* and rhTNF-treated (*TNF*) cells.

($n = 12$) in rhTNF-treated cells. Recovery from fast inactivation of Ca^{2+} currents was determined by applying pairs of identical depolarizing steps to 0 mV separated by various intervals from a holding potential of −80 mV. Recovery time constant for pulses of 0.5 sec duration was similar for control cells and rhTNF-treated cells [3.90 ± 0.3 sec ($n = 6$) in control cells; 3.7 ± 0.5 sec ($n = 6$) in rhTNF-treated cells]. When pulses lasting 0.5 sec were given to 0 mV and 60 mV alternately, followed by post-pulses to 0 mV after a 15 msec delay, the inactivation resulting from the prepulse to 60 mV was less than the inactivation resulting from the prepulse to 0 mV. Under control conditions, Ca^{2+} currents of neonatal SCG neurons exhibit little facilitation current (18%), which is defined as the increase in current following a strong depolarization. Currents recorded from TNF-treated cells did not exhibit greater facilitation current compared to controls.

As shown in the steady-state availability curve, a substantial inactivation of the current occurred upon changing the holding potential from −80 mV to −40 mV. Recovery of the current from slow inactivation was studied by keeping the holding potential at −20 mV for 2 min and then changing to a holding potential of −80 mV. Pulses to 0 mV were given every 30 sec after the initial lag time of 15 sec from changing the holding potential to running the protocol. Data points represent normalized currents and are better fitted with two exponentials using the nonlinear least-square method. The fast component of the recovery (τ_1) cannot be assessed accurately because a substantial fraction of currents has recovered at 15 sec (first data point, figure 5A). The slow recovery time constant (τ_2) was prolonged in TNF-treated cells as compared to control cells [SFM $\tau_2 = 71.1 \pm 6.2$ sec ($n = 7$); TNF $\tau_2 = 114.6 \pm 13.0$ sec ($n = 7$); $p < 0.02$]. Figure 5*A* shows an example of recovery of Ca^{2+} currents from slow inactivation, while Figure 5*B* shows the scatter of τ_2 values for control and rhTNF-treated cells.

Although Ca^{2+} currents of rat SCG neurons have been shown to be regulated by norepinephrine (NE) (Galvan and Adams, 1982; Song et al., 1989), a more recent study using neonatal rat SCG neurons reported no significant decrease in Ca^{2+} current in response to NE (Bhave et al., 1990). We examined the sensitivity of Ca^{2+} currents recorded from control and rhTNF-treated cells to perfused NE (1 μM, 100 μM). Perfusion of SCG

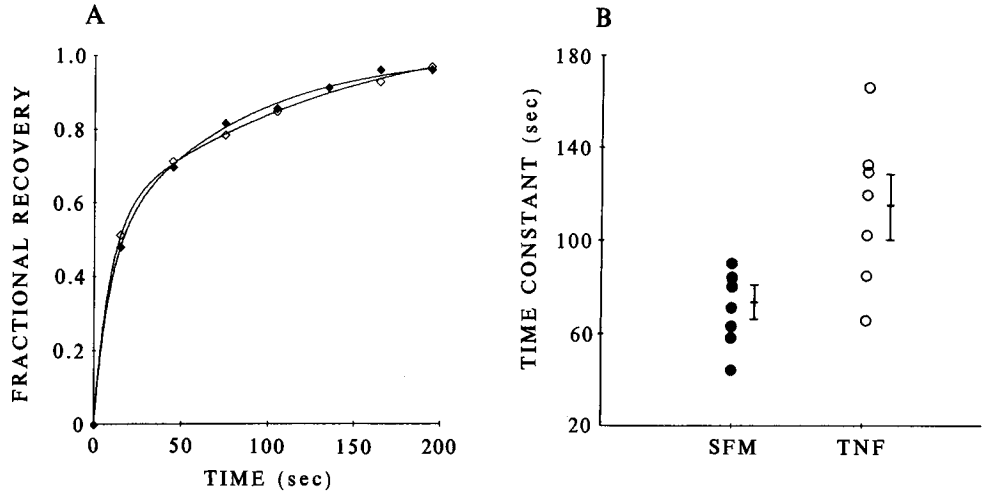

Figure 5. *A*, Examples of recovery of Ca^{2+} currents from slow inactivation in a control SCG neuron (*solid symbols*) and a rhTNF-treated SCG neuron (*open symbols*). Recovery of the currents from slow inactivation was studied by keeping the holding potential at −20 mV for 2 min and then changing to a holding potential of −80 mV. Pulses to 0 mV were given every 30 sec after the initial lag time of 15 sec following changing the holding potential from −20 mV to −80 mV. For these experiments, control $\tau_2 = 73.7$ sec; and TNF $\tau_2 = 129.0$ sec. *B*, Scatter plot of slow recovery time constants (τ_2) in control and rhTNF-treated cells. *Error bars* represent mean ± SEM. Slow time constants of recovery were prolonged in rhTNF-treated cells when compared to control cells (SFM τ_2, 71.1 ± 6.2 sec; TNF τ_2, 114.6 ± 13 sec; $p < 0.02$).

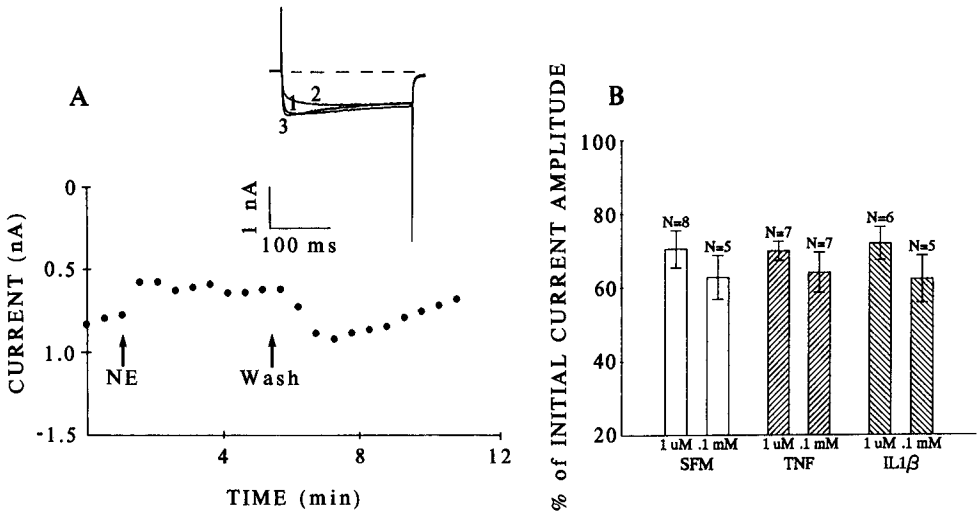

Figure 6. *A*, Reversible effect of NE on Ca^{2+} currents from SCG neurons. The *inset* shows examples of current traces from a rhTNF-treated neuron before 1 μM NE (*1*), after NE (*2*), and upon washout of NE (*3*). Holding potential, −80 mV; test potential, 0 mV. *B*, Summary of the effect of NE (1 μM, 0.1 mM) on Ca^{2+} currents of control SCG neuron incubated in *SFM*, rh-TNF-treated SCG neurons (*TNF*), and IL1-β–treated neurons (*IL1β*).

neurons with NE resulted in a reversible complex response characterized by a reduction of the inactivating current and slowing of activation of the remaining current. An example of such a complex response to NE is depicted in Figure 6*A*. In a few experiments, perfusion of NE resulted in a pure reduction of current amplitudes without changes in kinetics. The reversibility of the effect upon washing out of NE distinguished the latter response from current rundown. Figure 6*B* summarizes the effect of NE on SCG Ca^{2+} currents, including both types of responses. The effect of NE was seen in 12 of 13 control SCG neurons and in 13 of 14 rhTNF-treated SCG neurons. There is no significant difference in the sensitivity of SCG Ca^{2+} currents to NE in control and rhTNF-treated cells.

To determine whether the effect of rhTNF was specific to Ca^{2+} currents, transient outward K^+ currents (I_A) were also recorded from control cells and rhTNF-treated cells. Pulses of 200 msec in duration were stepped from −80 mV to various depolarizing potentials at 20 sec intervals. There was no significant difference in I_A density with peak current amplitudes measured at +60 mV between control cells and rhTNF-treated cells [SFM, 98.5 ± 14.9 (n = 12), vs. TNF, 114.2 ± 15.8 (n = 13); $p > 0.05$]. There was also no difference in the time course of activation estimated as time to peak, and time course of inactivation of I_A between control and rhTNF-treated cells.

Discussion

This study shows that neurons from neonatal SCG treated with rhTNF exhibited an increase in Ca^{2+} current density. Since the fraction of the current sensitive to DHP and ω-CgTx was unaffected, the increase in current density was not due to induction of a new type of Ca^{2+} channel (i.e., P-type Ca^{2+} channel). It would appear that both N- and L-channels are increased in rhTNF-treated cells, although the possibility of a preferential induction of ω-CgTx–sensitive N-type Ca^{2+} channels cannot be totally excluded since a 50% increase in this major component may result in relatively small change in the percentage of the DHP-sensitive component. In addition, the steady-state activation as measured from tail current amplitudes and steady-state availability of the current were not significantly different between rhTNF-treated cells and control cells. Recovery of the current from fast inactivation was similar in both groups, but the recovery from slow inactivation (in particular, τ_2) was prolonged in rhTNF-treated cells. Partial and reversible inhibition of Ca^{2+} currents by NE was observed in both control and rhTNF-treated cells.

Modulation of Ca^{2+} currents in sympathetic neurons by neurotransmitters and hormones is well documented (Galvan and Adams, 1982; Lipscombe et al., 1989; Song et al., 1989; Bley and Tsien, 1990; Elmslie et al., 1990). Whether electrophysiological properties of SCG neurons are also regulated by immune peptides is unknown, although immune peptides have been reported to affect electrophysiological properties of central neurons. Interferons were reported to induce depolarization and to increase $[Ca^{2+}]_i$ in a human colonic cell line (Iwagaki et al., 1988) and to enhance the excitability of neurons in explants of cerebral and cerebellar cortex (Calvet and Gresser, 1979). Application of recombinant IL2 or IL1-β inhibits long-term potentiation in hippocampal slices (Katsuki et al., 1990; Tancredi et al., 1990). Both interleukins also modulate neuronal discharge frequency in hypothalamus (Bindoni et al., 1988; Hori et al., 1988).

We were not able to demonstrate any acute effects of rhTNF-α or rhIL1-β on Ca^{2+} currents recorded from SCG neurons. Chronic treatment (24 hr) of SCG neurons with rhTNF, however, resulted in an increase in Ca^{2+} current density, but had no effect on current density of I_A channels. Modulation of ionic currents by rhTNF has been reported in other cell types. Treatment of cultured adult ovine oligodendrocytes with rhTNF for 24–72 hr resulted in a decrease in both inwardly rectifying K^+ current and outward K^+ current (Soliven et al., 1991). A decrease in K^+ conductance was also reportedly induced by brief application of rhTNF onto an identified neuron of *Aplysia* (Sawada et al., 1990). Another cytokine, TGF-β1 (Caffrey et al., 1989), has been shown to suppress the expression of Ca^{2+} and Na^+ channels in the developing skeletal myoblast. In addition to regulating channel expression, inhibition of responsiveness of cardiac myocyte to β-adrenergic agonists by cytokines has been reported (Gulick et al., 1989). In our study, calcium currents from rhTNF-treated cells did not appear to exhibit altered sensitivity to regulation by NE.

The effect of rhTNF on Ca^{2+} current expression is interesting but cannot explain its modulatory effects on neurotransmitter release (Soliven and Albert, 1992). There are limitations in at-

tempting to correlate electrophysiological findings with results of secretion experiments since the experimental conditions may not be exactly the same. Electrophysiologic experiments were done on neurons within 32 hr of their isolation, whereas release experiments were performed in older cultures. Pulse protocols used in electrophysiology may not reflect accurately membrane potential changes during release experiments. Nevertheless, modulation of somatic Ca^{2+} currents by peptides in sympathetic neurons has been reported by other investigators (Bley and Tsien, 1990) to correlate with their effect of transmitter release in cultured neurons. We have recently shown that the baseline spontaneous release and initial ^{3}H-NE release evoked by a 2 min K^+-induced depolarization (70 mM K^+/1.3 mM Ca^{2+}) were not significantly different between control SCG neurons and SCG neurons treated with rhTNF for 4–24 hr. However, rhTNF-treated SCG neurons, but not IL1-β–treated SCG neurons, exhibited a diminished secretory response to a repeat 2 min K^+ stimulus given 6 min following the initial K^+ stimulus. The percentage of inactivation of secretion between control cells and rhTNF-treated cells was similar when the second K^+ challenge was given immediately after the initial one, but the recovery from inactivation of secretion was prolonged in rhTNF-treated cells as compared to control SCG cells when the time interval between K^+ challenges was varied. Recovery from inactivation of secretion had occurred by 6 min in control cells, whereas it only occurred when the time interval between K^+ challenges was increased to 10 min in rhTNF-treated cells (Soliven and Albert, 1992).

There are some discrepancies between the electrophysiologic findings of this study and the results of ^{3}H-NE secretion experiments summarized above. First, the initial depolarization-induced ^{3}H-NE release in rhTNF-treated SCG neurons was not significantly different from controls in spite of the increase in Ca^{2+} current density. Perhaps the increase in Ca^{2+} current density was blunted by spike shortening due to an increased Ca^{2+}-activated K^+ current. However, this possibility should have been circumvented since high-K^+ solution (70 mM K^+) was used as the stimulus for secretion. Another explanation is that the main sites of neurotransmitter release are extrasomatic, and therefore the increase in current density in the soma may not have a dramatic effect on transmitter release. This is supported by the work of Wakade et al. (1990) demonstrating that Ca^{2+}-sensitive stores in sympathetic neurons are located in the soma but are not coupled to ^{3}H-NE release. Although both DHP-sensitive and DHP-insensitive Ca^{2+} channels mediate the cytosolic Ca^{2+} increase following depolarization in the soma and in the processes (Thayer et al., 1987), it is possible that ion channels or ionic transport in the processes are differentially regulated. Second, the effect of rhTNF on recovery of Ca^{2+} currents from slow inactivation (τ_2 = 114 sec) does not explain the inactivation of secretion of NE that was observed even when the interstimulus interval was 6 min. The effect of rhTNF on recovery of the current from slow inactivation cannot be explained on the basis of induction of a new type of Ca^{2+} channels with different kinetics; therefore, one has to postulate that rhTNF exerts multiple independent effects on Ca^{2+} currents of SCG neurons.

A plausible explanation for the effect of rhTNF on secretory response would be that rhTNF also affects a later event in the exocytotic process. This is suggested by our preliminary data from photometric measurements of $[Ca^{2+}]_i$ in single processes or bundles of processes emanating from the soma of SCG neurons loaded with Ca^{2+}-indicator dye fura-2 acetoxymethyl ester. Sequential depolarizing stimuli (70 mM K^+/1.3 mM Ca^{2+}) following a 5–6 min time interval in normal K^+-containing solution (to mimick the experimental conditions of secretion experiments) produced similar increase in 340:380 fluorescence intensity ratio in control (N = 3) and rhTNF-treated cells (n = 4), with minimal decrease of the second response when compared to the initial response.

We conclude that chronic treatment of neonatal rat SCG neurons with rhTNF regulates the expression of somatic Ca^{2+} channels. The changes observed cannot account totally for the effect of rhTNF on neurotransmitter release, but could be related to other calcium-dependent processes such as neuronal survival and neurite extension (Connor, 1986; Mattson and Kater, 1987; Koike et al., 1989).

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