Norepinephrine Inhibits a Toxin Resistant Ca\textsuperscript{2+} Current in Carotid Body Glomus Cells: Evidence for a Direct G Protein Mechanism

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Overholt, Jeffrey L. and Nanduri R. Prabhakar. Norepinephrine inhibits a toxin resistant Ca\textsuperscript{2+} current in carotid body glomus cells: evidence for a direct G protein mechanism. J. Neurophysiol. 81: 225–233, 1999. Previous studies have demonstrated that endogenous norepinephrine (NE) inhibits carotid body (CB) sensory discharge, and the cellular actions of NE have been associated with inhibition of Ca\textsuperscript{2+} current in glomus cells. The purpose of the present study was to elucidate the characteristics and mechanism of NE inhibition of whole cell Ca\textsuperscript{2+} current isolated from rabbit CB glomus cells. To determine the type(s) of Ca\textsuperscript{2+} channel involved, NE (10 μM) inhibited 24 ± 2% (SE) of the macroscopic Ca\textsuperscript{2+} current measured at the end of a 25 ms pulse to 0 mV and slowed activation of the current. The α\textsubscript{2} adrenergic receptor antagonist, SK&F 86466, attenuated these effects. Inhibition by NE was fast and voltage-dependent i.e., maximal at −10 mV and then diminished with stronger depolarizations. This is characteristic of G protein βγ subunit interaction with the α\textsubscript{1} subunit of certain Ca\textsuperscript{2+} channels, which can be relieved by depolarizing steps. A depolarizing step (30 ms to +80 mV) significantly increased (14 ± 1%) current in the presence of NE, whereas it had no effect before application of NE (1 ± 1%). To further test for the involvement of G proteins, NE was applied to cells where intracellular GTP was replaced by GDP-βS. NE had little or no effect on Ca\textsuperscript{2+} current in cells dialyzed with GDP-βS. To determine whether NE was inhibiting N- and/or P/Q-type channels, we applied NE in the presence of ω-conotoxin MVIIIC (MVIIIC). In the presence of 2.5 μM MVIIIC, NE was equally potent at inhibiting the Ca\textsuperscript{2+} current (23 ± 4% vs. 23 ± 4% in control), suggesting that NE was not exclusively inhibiting N- or P/Q-type channels. NE was also equally potent (30 ± 2% vs. 26 ± 4% in control) at inhibiting the Ca\textsuperscript{2+} current in the presence of 2 μM nisoldipine, suggesting that NE was not inhibiting L-type channels. Further, NE inhibited significantly larger proportion (47 ± 6%) of the resistant Ca\textsuperscript{2+} current remaining in the presence of NISO and MVIIIC. These results suggest that NE inhibition of Ca\textsuperscript{2+} current in rabbit CB glomus cells is mediated in part by effects on the resistant, non-L-, N-, or P/Q-type channel and involves a direct G protein βγ interaction with this channel.

INTRODUCTION

Carotid bodies (CB) are sensory organs that detect changes in arterial oxygen. Hypoxemia (low arterial O\textsubscript{2}) augments sensory activity of the CB and the ensuing reflexes are vital for maintaining cardio-respiratory homeostasis during hypoxemia. Carotid body tissue is composed of glomus (type 1) and sustentacular (type 2) cells. Glomus cells, of neuronal lineage, are believed to be the initial sites of sensory transduction. It is generally agreed that chemotransduction involves a hypoxia-induced increase in intracellular Ca\textsuperscript{2+} and Ca\textsuperscript{2+}-dependent release of neurotransmitters from glomus cells. Membrane Ca\textsuperscript{2+} channels have been implicated in this response (Montoro et al. 1996; Obeso et al. 1992; Urena et al. 1994). Several lines of evidence suggest that neurotransmitters released from glomus cells act both on the sensory nerve endings and on the glomus cells themselves in a paracrine or autocrine manner. The final expression of the sensory response to hypoxia depends on the actions of the neurotransmitters at both of these sites (for review see Prabhakar 1994). Thus, elucidating the mechanisms of transmitter actions on glomus cells in the CB is important for understanding the hypoxic transduction process.

Norepinephrine (NE) is one of the major neurotransmitters in the CB. NE is synthesized and released from the glomus cells themselves (e.g., Verna et al. 1993). In addition, sympathetic nerve endings innervating glomus cells also release NE (for references see Almaraz et al. 1997). Previous studies from our laboratory have shown that endogenously generated NE, acting on α\textsubscript{2} adrenergic receptors, exhibits a tonic inhibitory influence on CB sensory discharge (Kou et al. 1991; Prabhakar and Kou 1994). Cellular mechanisms of NE action include inhibition of Ca\textsuperscript{2+} current in glomus cells via an α\textsubscript{2} adrenergic pathway (Almaraz et al. 1997). Ca\textsuperscript{2+} current in glomus cells is exclusively of the high-voltage-activated (HVA) type (e Silva and Lewis 1995; Fieber and McCleskey 1993; Overholt and Prabhakar 1997; Peers et al. 1996). We have previously shown that HVA Ca\textsuperscript{2+} current in glomus cells is conducted by multiple types of channels, which include L, N, P/Q, and a resistant channel (Overholt and Prabhakar 1997). However, it is not known which type of Ca\textsuperscript{2+} channel(s) is inhibited by NE in glomus cells.

The effects of neurotransmitters on HVA Ca\textsuperscript{2+} channels involves second messenger systems that are coupled to a specific type of Ca\textsuperscript{2+} channel. In general, neurotransmitter modulation of Ca\textsuperscript{2+} channel activity in neurons is inhibitory and mediated via activation of G proteins (for reviews see Dolphin 1998; Hille 1994; Jones and Elmslie 1997). In many cases this inhibition is voltage-dependent and specific for N-type Ca\textsuperscript{2+} current, sparing the L-type Ca\textsuperscript{2+} current (e.g., Plummer et al. 1989, 1991). However, L-type Ca\textsuperscript{2+} current has also been shown to be inhibited by neurotransmitters in other neuronal preparations (e.g., Chavis et al. 1994). Many of the inhibitory effects of neurotransmitters on N- and P/Q-type channels involve G protein βγ subunits that bind directly to the α\textsubscript{1} subunit comprising N-, P/Q-, and perhaps E-type Ca\textsuperscript{2+} channels (De Waard et al. 1997; Herlitze et al. 1996; Ikeda 1996; Qin et al. 1997; Zhang et al. 1996). One hallmark of direct inhibition of Ca\textsuperscript{2+} channels by G protein βγ subunits is that inhibition is voltage-dependent and can be reversed by depolarizing steps (Jones and Elmslie 1997).
Relatively little is known about the second messenger systems in glomus cells and their effects on ion channels. Identifying the characteristics and mechanism of channel modulation in glomus cells is important to understand the physiological implications. Therefore, the purpose of the present study was to determine the characteristics and mechanism of NE inhibition of Ca\(^{2+}\) current in glomus cells and the Ca\(^{2+}\) channels involved.

Our results suggest that NE inhibits both the magnitude and rate of activation of the macroscopic Ca\(^{2+}\) current conducted primarily by a non L-, N- or, P/Q-type resistant channel. This inhibition is voltage-dependent, can be reversed by a depolarizing pulse, and its characteristics suggest that it could be mediated by a direct G protein effect on the channel.

**METHODS**

Experiments were performed on glomus cells freshly isolated from carotid bodies of adult male rabbits killed with CO\(_2\). Individual glomus cells were dissociated enzymatically from carotid bodies (Overholt and Prabhakar 1997). Briefly, cells were incubated at 37°C in a solution that contained trypsin (type II, 2 mg/ml) and collagenase (type IV, 2 mg/ml), and was composed of (in mM) 140 NaCl, 5 KCl, 10 N-2-hydroxyethylpiperazine-N’2-ethanesulfonic acid (HEPES), 5 glucose, pH 7.2. Single cells were obtained by gentle trituration during the incubation. Following the incubation, cells were maintained at 37°C in a CO\(_2\) incubator in medium composed of a 50/50 mixture of Dulbecco’s modified Eagle’s medium (DMEM) and HAM F12 supplemented with antibiotics (penicillin, 100 U/ml; streptomycin, 100 μg/ml; Gibco-BRL), 10% fetal bovine serum and insulin, transferrin and selenium (ITS, Sigma). Experiments were performed at room temperature (≈22°C) and cells were used within 36 h.

Membrane Ca\(^{2+}\) current was monitored using the whole cell configuration of the patch clamp technique (Hamill et al. 1981). Pipettes were made from borosilicate glass capillary tubing, coated with Sylgard (Dow Corning) and had resistances of 4–5 MΩ. Currents were recorded using an Axopatch 200A voltage clamp amplifier; filtered at 5 kHz; and, using an IBM compatible computer with a Digidata 1200 interface and pCLAMP software (Axon Instruments), were sampled at a frequency of 28.5 kHz. For the facilitation protocol, currents were filtered at 5 kHz and sampled at 10 kHz. Currents were not leak subtracted. Current-voltage (I-V) relations were elicited from a holding potential of −90 mV with the use of 25 ms steps (5 s between steps) to test potentials over the range of −50 to +70 mV in 10 mV increments. Current at each potential was measured as the average over a 2.5 ms span at the end of the 25 ms step. Current rundown and drug effects were monitored using a wash protocol (25 ms step to 0 mV, 10 ms between steps). The effects of channel blocking agents were compensated for rundown with the use of a linear regression of the current decrease during the wash protocol in the absence of test compounds. Cells in which rundown was excessive or did not appear linear were not used in this study. For comparison of I-V relations, current at each potential was normalized to the maximum value recorded during the control I-V relation in individual cells (usually 0 mV).

Current through Ca\(^{2+}\) channels was isolated by using K\(^+\)- and Na\(^+\)-free intra and extracellular solutions of the following composition (in mM): (intracellular) 115 CsCl, 20 tetraethylammonium (TEA)-Cl, 5 Mg adenosine 5’-triphosphate (ATP), 10 phosphocreatine, 0.2 tris(hydroxymethyl)aminomethane guanosine 5’-triphosphate (TrisGTP), 5 ethylene glycol-bis(β-aminoethoxy) ether)-N,N,N’,N’-tetraacetic acid (EGTA), and 5 HEPES, pH was adjusted to 7.2 with CsOH; (extracellular) 140 N-methyl-D-glucamine-Cl, 5.4 CsCl, 10 BaCl\(_2\), 10 HEPES, and 11 glucose, pH was adjusted to 7.4 with CsOH. The extracellular solution was changed using a fast-flow apparatus consisting of a linear array of borosilicate glass capillary tubes (Overholt et al. 1995). In these experiments, Ba\(^{2+}\) was the charge carrier. For simplicity, Ba\(^{2+}\) current conducted by Ca\(^{2+}\) channels will be referred to as Ca\(^{2+}\) current. Stock solutions of drugs were prepared in water (ω-conotoxin MVIIIC, RBI), PEG-400 (nisoldipine, Miles Laboratories) or 50 mM acetic acid (norepinephrine, RBI; isoproterenol, RBI; and SK&F 86466, gift from Dr. Paul Hieble with Smith, Kline and Beecham) and diluted 1:1000 in the extracellular solution. Guanosine 5’-o-(2-thiodiphosphate) (GDP-βS; Sigma) was dissolved directly in the intracellular solution.

Data analysis and curve fitting were carried out using CLAMPFIT software (Axon Instruments) and exported to Excel (Microsoft) for further analysis. To determine τ, current traces were fit over a limited range (≈1.5–13 ms after the start of the step to 0 mV) of the trace where they were well fit by a single exponential. Appropriate statistical analysis was carried out using SigmaStat (Jandel Scientific). P values <0.05 were considered significant. All values are reported as means ± SE.

**RESULTS**

**NE inhibits Ca\(^{2+}\) current in a concentration-dependent manner**

Figure 1A shows an example of raw Ca\(^{2+}\) current elicited from a representative glomus cell by 25 ms steps from −90 mV to test potentials from −50 to +70 mV in 10 mV incre-
ments. The left panel shows control current under conditions designed to isolate current through Ca$^{2+}$ channels (Na$^+$- and K$^+$-free intra and extracellular solutions). It can be seen that the Ca$^{2+}$ current is exclusively high-voltage-activated (note the absence of rapidly inactivating T-type current). This current is nearly exclusively carried by Ca$^{2+}$ as it is completely blocked by 6 mM Co$^{2+}$ (Overholt and Prabhakar 1997) or 100 μM Cd$^{2+}$ (Fig. 3C). The right panel in Fig. 1A shows raw current from the same cell after a 1.5 min exposure to 10 μM norepinephrine (NE) in the extracellular solution. It is apparent that NE inhibits a significant proportion of the macroscopic Ca$^{2+}$ current over the range of membrane potentials tested (see also Fig. 3D). To test whether 10 μM NE was a maximally effective concentration, we examined the effects of a range of concentrations of NE on the Ca$^{2+}$ current. Figure 1B shows the concentration dependence of NE inhibition. Relative inhibition (Rel Inhibition) was measured as the percentage of the control current inhibited at 0 mV by different concentrations of NE and normalized to the amount of inhibition by 10 μM NE in individual cells. NE effects could be seen with concentrations as low as 10 nM, and maximal effects were achieved with concentrations around 10 μM. The concentration-response data were fit to the logistic equation

$$\text{Rel Inhibition} = \frac{\text{Max} - \text{Min}}{1 + \left(\frac{[\text{NE}]}{K_{1/2}}\right)^N} + \text{Min}$$

where Max and Min are the maximum and minimum inhibition, $K_{1/2}$ is the concentration at which the drug effect is half-maximal, N is the slope of the logistic function, and [NE] is the concentration of NE. The solid line (Fig. 1B) shows that the data are well fit by this function with $K_{1/2} = 0.58$ μM and $N = 1.02$.

NE inhibits Ca$^{2+}$ current via an α$_2$ adrenergic receptor pathway

It has previously been shown that α$_2$ adrenergic receptors are present in carotid body tissue, and endogenously generated NE, acting on α$_2$ adrenergic receptors, inhibits sensory discharge of the CB (Kou et al. 1991; Prabhakar and Kou 1994). To test whether NE inhibition of Ca$^{2+}$ current in glomus cells also involves α$_2$ receptors, we tested the effect of NE in the presence of the α$_2$ receptor antagonist SK&F 86466 (SK&F). Figure 2A shows the time course for changes in Ca$^{2+}$ current elicited by a step to 0 mV as the extracellular solution is switched to and from one containing 1 μM NE, 10 μM SK&F, or NE and SK&F from a representative experiment. It can be seen that NE inhibited a significantly greater portion of the Ca$^{2+}$ current before and after application of SK&F than in its presence. SK&F alone had no effect on the Ca$^{2+}$ current. As a control, in another series of experiments we tested the effect of 10 μM isoproterenol (ISO), a β receptor agonist, on the Ca$^{2+}$ current. Figure 2B shows the time course for changes in Ca$^{2+}$ current elicited by a step to 0 mV as the extracellular solution is switched to and from one containing either 10 μM NE or 10 μM ISO. ISO did not significantly effect the Ca$^{2+}$ current in any of the eight cells tested. These results are summarized in Fig. 2C, which compares the percentage of the current inhibited (% Inhibition) at 0 mV by NE alone (first application), SK&F alone, NE in the presence of SK&F, and ISO

![Image](http://jn.physiology.org/resource/figures/227000/227002/227002.png)

**Fig. 2.** SK&F 86466 (SK&F) attenuates the inhibitory effect of NE on whole cell Ca$^{2+}$ current in rabbit CB glomus cells. A: time course for changes in the Ca$^{2+}$ current elicited by a 25 ms step to 0 mV as the extracellular solution is switched to and from one containing 1 μM NE, 10 μM SK&F, or NE and SK&F. B: time course for changes in the Ca$^{2+}$ current elicited by a 25 ms step to 0 mV as the extracellular solution is switched to and from one containing 10 μM NE or 10 μM isoproterenol (ISO). C: comparison of the average percent of the Ca$^{2+}$ current inhibited by NE alone, SK&F alone, NE in the presence of SK&F, and ISO (n = 8). ** P < 0.01, paired t-test (NE, SK&F, and NE + SK&F from same 5 cells).

where $I_{Con}$ and $I_{NE}$ are the magnitude of the current recorded before and during application of NE, respectively. These results suggest that NE inhibition of the Ca$^{2+}$ current in glomus cells is mediated by α$_2$ adrenergic receptors.

Characteristics of NE inhibition of the Ca$^{2+}$ current in glomus cells

Figure 3 further characterizes the effect of 10 μM NE on glomus cell Ca$^{2+}$ current. Figure 3A shows the time course for changes in current elicited by a step to 0 mV every 10 s before, during, and after 2 successive applications of 10 μM NE in the extracellular solution. This figure shows that inhibition by NE is fast, usually complete in 10–20 s. In addition, it can be seen that the effect is reversible and repeatable. However, the response to NE exhibited some desensitization as repetitive applications of NE resulted in a significant (P < 0.001, paired t-test) decrease in the magnitude of the response. This can be seen more clearly in Fig. 3B, which compares the percentage of the current inhibited at 0 mV by NE in successive applications. Figure 3C shows average current-voltage (I-V) relations before, during, and after washout of 10 μM NE in the extracellular solution. This figure also shows that the inward Ca$^{2+}$ current is completely
different membrane potentials by 10 μM cells. The maximum (usually 0 mV) inward control current recorded in individual cells also shown. Average currents are corrected for rundown and normalized to 

$$I = A_0 + A_1 e^{-t/\tau}$$

where $A_0$ and $A_1$ are the initial and ending amplitude, respectively, and $t$ is time to determine $\tau$, the time constant. The effect of NE on the time constant for activation of the Ca$^{2+}$ current is summarized in Fig. 4C. As can be seen in this figure, NE significantly ($P < 0.001$, paired $t$-test) increased the time constant for activation of the macroscopic Ca$^{2+}$ current in glomus cells.

**NE inhibition of the Ca$^{2+}$ current is mediated via a direct G protein interaction**

The characteristics of NE inhibition, i.e., voltage dependence and attenuation of the magnitude and rate of activation, are like those associated with direct binding of G protein $\beta\gamma$ subunits to the $\alpha_1$ subunit of Ca$^{2+}$ channels found in other cells. The voltage dependence of direct inhibition by $\beta\gamma$ subunits has been associated with reversal of the interaction of $\beta\gamma$ subunits with the Ca$^{2+}$ channel $\alpha_1$ subunit by strong depolarizations (facilitation). Therefore we tested

blocked by 100 μM Cd$^{2+}$ applied in the extracellular solution. Current was normalized to the maximum inward control current (usually 0 mV) recorded in individual cells. It can be seen that NE attenuated the macroscopic Ca$^{2+}$ current at all membrane potentials tested, and the effect was completely reversed on washout after correction for rundown. Figure 3D, which shows the percentage of current inhibited (Eq. 2) by NE at membrane potentials from −20 to +30 mV, more clearly shows the relative inhibitory effect of NE at different membrane potentials. Inhibition by NE was maximal at −10 mV and then diminished with stronger depolarizations. This suggests that there may be some voltage dependence to inhibition by NE, whereby block is relieved at more depolarized voltages.

Figure 4 shows that NE also decreases the rate of activation of the macroscopic Ca$^{2+}$ current. Figure 4A shows raw current traces elicited by a 25 ms step from −90 to 0 mV before, during, and after washout of 10 μM NE in the extracellular solution. It can be seen that NE decreases not only the magnitude of the current, but also the rate of activation. The effect of NE on the rate of activation of the Ca$^{2+}$ current is shown more clearly in Fig. 4B. This figure shows the same current traces as in the left panel, but current in the presence of NE and after washout have been scaled to the magnitude of the control current at the end of the step to 0 mV. The attenuating effect of NE on the rate of activation can be clearly seen at the beginning of the step. This figure also shows that the effect of NE on the rate of activation is reversible. To quantify the effect of NE on the rate of activation, current traces were fit to the exponential function

$$I = A_0 + A_1 e^{-t/\tau}$$

**FIG. 3.** NE decreases the magnitude of whole cell Ca$^{2+}$ current in rabbit CB glomus cells. A: time course for changes in the Ca$^{2+}$ current elicited by a 25 ms step to 0 mV as the extracellular solution is switched to and from one containing 10 μM NE and then repeated. B: comparison of the average ($n = 23$) percentage of the Ca$^{2+}$ current inhibited by successive applications of 10 μM NE. C: average ($n = 8$), normalized current-voltage relations before (Con), during (NE), and after (Washout) exposure to 10 μM NE in the extracellular solution. The average effect of 100 μM CdCl$_2$ applied in the extracellular solution in another group of cells ($n = 10$) is also shown. Average currents are corrected for rundown and normalized to the maximum (usually 0 mV), inward control current recorded in individual cells. D: comparison of the percentage of current (Eq. 2) inhibited at different membrane potentials by 10 μM NE.

**FIG. 4.** NE decreases the rate of activation for whole cell Ca$^{2+}$ current in rabbit CB glomus cells. A: raw current trace at 0 mV before (Con), during (NE), and after washout (WO) of 10 μM NE in the extracellular solution. B: raw currents same as in A, but current in the presence and after washout of NE is scaled up to match current at the end of the step during control. C: average ($n = 28$) rates of activation (tau, $\tau$) for macroscopic Ca$^{2+}$ current before (Con) and during exposure to 10 μM NE in the extracellular solution. $\tau$ was determined from fitting Eq. 3 to raw current traces. **P < 0.001, paired t-test.**
The inhibitory effect of NE on the magnitude and rate of activation of Ca$^{2+}$ current can be partially relieved by a depolarizing step. Inset: facilitation protocol that consisted of a 25 ms step to 0 mV before (Pre) and after (Post) a 30 ms step to +80 mV. A: Left: raw current trace before (Con) and during exposure to 10 μM NE in the extracellular solution. Right: raw currents same as in left, but current in the presence of NE is scaled to match the magnitude of the current before the depolarizing step at the end of the step during control (Pre and Post are scaled separately). B: average (n = 28) % facilitation (% Fac, Eq. 4) of current magnitude elicited by the depolarizing step before (Con) and during exposure to 10 μM NE. C: average (n = 28) τ (Eq. 3) in the presence of 10 μM NE before (Pre) and after (Post) the depolarizing step.

Figure 5 shows the effect of a 30 ms step to +80 mV on NE inhibition of Ca$^{2+}$ current in glomus cells. Figure 5A shows raw current traces elicited by 25 ms steps from -90 to 0 mV before and after the depolarizing step to +80 mV (inset). Before application of NE, the depolarizing step had no effect on the Ca$^{2+}$ current (Con, left panel). However, in the presence of NE, the depolarizing step partially reversed the attenuation of the magnitude of the Ca$^{2+}$ current (NE, left panel). The depolarizing step also reversed the effect of NE on the rate of activation of the Ca$^{2+}$ current. This can be seen more clearly in the right panel of Fig. 5A, which shows the same current traces as the left panel, but the magnitude of the current in the presence of NE is scaled to match the magnitude of the control current at the end of the steps to 0 mV. Figure 5B summarizes the effect of the depolarizing step on the magnitude of the current before and during application of NE. This figure shows the percentage facilitation (% Fac) before and during application of NE

$$\text{% Fac} = \frac{I_{\text{post}}}{I_{\text{pre}}} - 1 \times 100$$  (4)

where $I_{\text{pre}}$ and $I_{\text{post}}$ are the current magnitudes recorded at 0 mV before and after the depolarizing step, respectively. Figure 5C summarizes the effect of the depolarizing step on the time constant for activation of the Ca$^{2+}$ current. Current traces before and after the depolarizing step were fit to equation 3 to determine τ. It can be seen that the depolarizing step significantly ($P < 0.01$, paired t-test) decreased τ (increased the rate of activation) in the presence of NE, but not in the absence of NE (data not shown). These results provide further evidence that NE could be acting through a direct effect of G protein βγ subunits on Ca$^{2+}$ channels.

GDPCS is a nonhydrolyzable analog of GDP that inhibits G protein dependent responses in other cell types (e.g., Shekter et al. 1997). To further test for the involvement of a direct G protein mechanism in the response to NE, we replaced GTP with GDP-βS in the intracellular solution. Figure 6 shows the effect of NE on Ca$^{2+}$ current in cells dialyzed with GDP-βS. Figure 6A shows the time course for changes in current elicited by a 25 ms step from -90 to 0 mV every 10 s before, during, and after application of an extracellular solution containing 10 μM NE. It can be seen that, under these conditions, NE had no effect on the Ca$^{2+}$ current even after a 6 min exposure. Figure 6B shows that, when compared with control (24 ± 2% inhibition), NE had relatively little effect on Ca$^{2+}$ current in cells dialyzed with GDP-βS (only 2 ± 2% inhibition, n = 7). Ca$^{2+}$ current in cells dialyzed with GDP-βS also exhibited some unexpected behavior. Figure 6C shows that these cells exhibited spontaneous facilitation (Eq. 4) even in the absence of NE.
facilitation was tested shortly after break in, the depolarizing step had no effect ( <4 min.). However, as dialysis continued past 5 min a depolarizing step began to augment the current. This effect was not seen in control cells dialyzed with GTP, and in cells dialyzed with GDP-βS facilitation was not enhanced by NE. It is difficult to explain this effect as it would be expected from dialysis with GTP-γS, not GDP-βS. However, the finding that GDP-βS prevented NE inhibition of Ca²⁺ current is consistent with GDP-βS inhibition of G protein activation and supports our hypothesis that NE is working through a G protein dependent mechanism.

**NE inhibits the resistant, non L- or N- or P/Q-type Ca²⁺ current in glomus cells**

In other systems, binding of G protein βγ subunits is thought to be specific for the α₁ subunits of N- and P/Q-type channels (see Introduction for references). To test whether NE inhibition of the macroscopic Ca²⁺ current was specifically mediated by effects on N- and/or P/Q-type channels, we tested the effect of NE on Ca²⁺ current in the presence of ω-conotoxin MVIIC (MVIIC), a specific blocker of N- and P/Q-type channels. To verify a response to NE in cells before application of toxin, NE was first applied alone and washed out, then applied in the presence of the toxin. The successive responses to NE in individual cells were then compared. Figure 7A shows the time course for changes in current elicited by a 25 ms step from −90 to 0 mV every 10 s as the extracellular solution is changed to one containing 2.5 μM MVIIC, one containing MVIIC and 10 μM NE, and after washout of these substances. On average, MVIIC blocked 51 ± 2% (n = 5) of the macroscopic Ca²⁺ current, confirming the presence of N- and P/Q-type channels. Surprisingly, in the presence of MVIIC, NE was equally potent at inhibiting the magnitude of the Ca²⁺ current, suggesting that NE was affecting current through other channels. This is summarized in Fig. 7B, which compares the average percentage block (Eq. 2) of the Ca²⁺ current by NE before and during application of MVIIC. However, it should be noted that the response to NE in the presence of MVIIC may be underestimated due to desensitization (see Fig. 3B and Discussion). Likewise, current in the presence of MVIIC and NE showed a similar degree of facilitation as in NE alone (data not shown). These results show that the effect of NE on Ca²⁺ current is similar in the presence and absence of MVIIC, suggesting that NE is affecting current through channels other than N- and/or P/Q-type.

A portion of the Ca²⁺ current in rabbit glomus cells is also conducted by L-type channels. To determine whether NE was specifically attenuating Ca²⁺ current conducted by L-type channels, we tested the effect of NE on Ca²⁺ current in the presence of nisoldipine (NISO), a specific blocker of L-type channels. Experiments were performed similarly to those with MVIIC. Figure 7C shows the time course for changes in current elicited by a 25 ms step from −90 to 0 mV every 10 s as the extracellular solution is changed to one containing 2 μM NISO, one containing NISO and 10 μM NE, and after washout of these substances. On average, NISO blocked 19 ± 5% (n = 5) of the macroscopic Ca²⁺ current, confirming the presence of L-type channels. In the presence of NISO, NE tended to inhibit a greater portion of the Ca²⁺ current. This is summarized in Fig. 7D, which compares the average percentage block (Eq. 2) of the Ca²⁺ current by NE before and during application of NISO. Likewise, current in the presence of NISO and NE showed a similar degree of facilitation as in NE alone (data not shown). These results suggest that NE is not specifically affecting current through L-type channels.

The persistence of NE inhibition in the presence of MVIIC or NISO alone suggests that NE may also be affecting the resistant, non L-, N- or P/Q-type current in glomus cells. To more directly test this idea, we tested the effect of NE on the resistant Ca²⁺ current elicited in the presence of NISO and MVIIC together in three additional cells. Figure 8 shows the effect of applying NE in the presence of NISO and MVIIC before any prior exposure to NE alone. Figure 8A shows the time course for changes in current elicited by a 25 ms step from −90 to 0 mV every 10 s as the extracellular solution is changed to one containing 2 μM NISO and 2.5 μM MVIIC; one containing NISO, MVIIC, and 10 μM NE; and after washout of NE in NISO and MVIIC. On average, NISO and MVIIC together blocked 57 ± 15% of the macroscopic Ca²⁺ current. Most importantly, when NE was first applied in the presence of NISO and MVIIC (i.e., when no desensitization is expected), it inhibited a significantly larger
CA$^{2+}$ CURRENT IN RABBIT GLOMUS CELLS

**Discussion**

The purpose of the present study was to elucidate the characteristics and mechanism of NE inhibition of glomus cell Ca$^{2+}$ current and to identify the channels involved. The results show that NE inhibits both the magnitude and the rate of activation of macroscopic Ca$^{2+}$ current in rabbit CB glomus cells in a voltage-dependent manner. Further, the effect of NE on Ca$^{2+}$ current was fast, could be partially reversed by a brief depolarizing step, and was prevented by dialyzing cells with GDP-βS, suggesting that NE is working through a direct G protein βγ subunit interaction with the α1 subunit of N- and/or P/Q-type Ca$^{2+}$ channels. However, NE inhibition of the macroscopic Ca$^{2+}$ current was not attenuated by the specific HVA Ca$^{2+}$ channel blockers MVIIIC (N- and P/Q-type) or NISO (L-type) alone, but was augmented in the presence of NISO and MVIIIC together. These results suggest that the main effect of NE is not on L-, N-, or P/Q-type Ca$^{2+}$ channels in glomus cells. Rather, NE appears to mainly inhibit a portion of the macroscopic Ca$^{2+}$ current conducted by a resistant (non-L-, N-, or P/Q-) type of Ca$^{2+}$ channel(s).

**NE inhibits the magnitude and the rate of activation of the Ca$^{2+}$ current**

Our results agree with and extend the observations of Almaraz et al. (1997) that NE inhibits Ca$^{2+}$ current in glomus cells via an α2 adrenergic receptor mediated pathway. One important new finding of the present study is that NE inhibited the rate of activation of the Ca$^{2+}$ current in glomus cells as well as the magnitude. One result of this is that inhibition of the Ca$^{2+}$ current is greater at the beginning than at the end of the 25 ms step. This is important because action potentials are generally very brief, suggesting that the most significant effects of neurotransmitters are those at the beginning of the pulse (Penington et al. 1992). In addition, facilitation was also greater when measured at the beginning than at the end of the pulse (data not shown). Another new finding of the present study is that the effect of NE appears to mainly involve a toxin-resistant current. The function and molecular basis of this current are presently unknown. However, the study by Almaraz et al. (1997) also showed that NE inhibits neurotransmitter release from glomus cells. This, taken together with our finding that NE inhibits the resistant type of HVA Ca$^{2+}$ channel, suggests that the resistant type of channel may be involved in hypoxia induced neurotransmitter release from glomus cells.

**NE inhibition of the Ca$^{2+}$ current involves a direct G protein mechanism**

In most cases neurotransmitter modulation of neuronal Ca$^{2+}$ channels is inhibitory and many different neurotransmitters have been identified in this response. Inhibition may also be transduced by different G proteins (most commonly pertussis toxin sensitive), but these pathways converge at the level of the Ca$^{2+}$ channel. Recent evidence has suggested that one reason for this convergence is that it is the βγ subunit of the activated G protein that interacts with the Ca$^{2+}$ channel protein (de Waard et al. 1997; Herlitze et al. 1996; Ikeda 1996; Qin et al. 1997; Zhang et al. 1996). There is also evidence that this interaction is specific for N- and P/Q-type, perhaps E-type (Qin et al. 1997; Shekter et al. 1997), but not L-type channels (Zhang et al. 1996). In most cases, inhibition shares a number of characteristics. Inhibition causes a decrease in the rate of activation and of the steady state current because of a shift of activation to more positive voltages. The channels have been described as entering a “reluctant” gating mode that can be reversed by depolarization (Bean 1989). This confers the signature characteristic on this type of inhibition: inhibition of the rate of activation and the steady state current can be reversed by steps to depolarizing voltages, i.e., facilitation. The data in
Figs. 3, 4, and 5 show that NE inhibition of Ca\(^{2+}\) current in glomus cells exhibits all of these characteristics and suggest that NE inhibition of Ca\(^{2+}\) current in glomus cells is mediated via a direct G protein effect.

GDP-βS is a nonhydrolyzable analogue of GDP and has been used in numerous other preparations to inhibit G protein-dependent processes (Shekter et al. 1997). It is expected that once incorporated into the heterotrimeric G protein complex, it should lock G proteins in the inactive state. The data in Fig. 6A and B are consistent with this idea and support our hypothesis that NE is working through a G protein dependent pathway. However, the data in Fig. 6C are hard to reconcile with this scheme, i.e., GDP-βS is acting like GTP-γS and persistently activating G proteins. We know of no other pathways that should be affected by GDP-βS. One plausible explanation for this effect may be that we are getting transphosphorylation of GDP-βS to GTP-βS under our conditions. The presence of GTP-βS in our intracellular solutions would then lock the G proteins in the activated state and cause constitutive inhibition of the Ca\(^{2+}\) current in these experiments. This could explain the increased rate of rundown in cells dialyzed with GDP-βS, the lack of effect of NE, and facilitation of the Ca\(^{2+}\) current in the absence of NE (Fig. 6C). The absence of facilitation shortly after break in and gradual increase in the magnitude of facilitation with time could be explained by a relatively slow basal nucleotide turnover in unstimulated G proteins. Most importantly, even though the results in Fig. 6C were unexpected, they are consistent with our hypothesis that NE is working through a G protein mediated pathway.

**NE inhibits the toxin resistant Ca\(^{2+}\) current in glomus cells**

The experiments represented in Fig. 7 were undertaken with the hypothesis that if the effect of NE was specific for a certain type of channel, then block of that channel should eliminate the effect of NE. Figure 7 shows that NE is equally potent at inhibiting the Ca\(^{2+}\) current in the presence and absence of NISO or MVIIC. However, the amount of block by NE in the presence of NISO or MVIIC is probably underestimated because the second response to NE was decreased by ~30% in control cells (Fig. 3B). These results are consistent with other findings that neurotransmitter inhibition is little affected after dihydropyridine block of L-type channels in other neurons (e.g., Plummer et al. 1989, 1991; Viana and Hille 1996), and that reconstituted G protein βγ subunits do not interact with the α1 subunit of L-type channels (e.g., Zhang et al. 1996). However, our results differ from other studies in that block of N- and P/Q-type channels has been shown to significantly attenuate neurotransmitter inhibition of Ca\(^{2+}\) current (e.g., Viana and Hille 1996), and G protein βγ subunits are widely known to inhibit the α1 subunit comprising N- and P/Q-type channels (see above for references). The results of the experiments shown in Fig. 7 suggest that the effect of NE is not specific for L-, N- or P/Q-type channels. The resistant current is carried by Ca\(^{2+}\) because it is blocked by 6 mM Co\(^{2+}\) (Overholt and Prabhakar 1997) and 100 μM CdCl\(_2\) (Fig. 3C). However, because the molecular basis for the resistant Ca\(^{2+}\) current is unknown, we have no specific blocker for it. Because the resistant current is always present, it is impossible to isolate the effects of NE on L-, N- or P/Q-type channels, and hence to determine the percentage inhibition of these currents. More importantly, Fig. 8 shows that, under conditions that should isolate the resistant current, NE inhibited a significantly larger percentage of the macroscopic Ca\(^{2+}\) current. Therefore we believe that the inhibitory effect of NE on the Ca\(^{2+}\) current in glomus cells mainly involves the resistant type current.

**Physiological implications**

As in neurons, the most critical role for Ca\(^{2+}\) channels in glomus cells may be that of stimulus secretion coupling. How could the characteristics of NE inhibition of Ca\(^{2+}\) current identified in the present study fit into the mechanism of CB function? As pointed out above, inhibition is not simply a reduction in the number of active channels. Instead, the channels enter a reluctant gating mode that can be overcome by increased electrical activity in the cell. The function of the resistant Ca\(^{2+}\) channel(s) in glomus cells is presently unknown. However, as mentioned above, the findings of the present study and those by Almaraz et al. (1997) suggest that it may be involved in neurotransmitter release from glomus cells. Therefore it would be expected that inhibition of this Ca\(^{2+}\) current would decrease neurotransmitter release and attenuate carotid sinus nerve activity. Based on an idea by Krammer (1978), we postulated that the mechanism of CB oxygen sensing may involve ‘‘disinhibition’’ of an inhibitory input (Prabhakar et al. 1993). It is interesting to speculate that NE, released under physiological conditions, may help to maintain a low level of CB activity. In the rabbit, hypoxia increases spiking activity (analogous to facilitation) in glomus cells (Montoro et al. 1996) and may partially reverse (disinhibit) NE inhibition of Ca\(^{2+}\) current, which has been shown in other preparations (Brody et al. 1997; Williams et al. 1997), and help to sustain the increase in CB chemosensory activity.

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