Bacteroides gingivalis was successfully reisolated from the inoculated animals, but bone loss in *B. gingivalis*–associated animals was greater than in uninoculated controls only in the mandible [bone loss, 4.52 ± 0.33 mm (SD) in controls versus 5.10 ± 0.22 mm in experimental animals].

Implantation of Bg^R in a large background of the normal microbiota demonstrated that there was a direct connection between the implanted Bg^R and clinical characteristics of periodontitis. The Bg^R emerged from this complex plaque ecological niche in such a way that it produced a "burst" of bone loss indicating that the emergence of *B. gingivalis* in the subgingival microbiota is capable of inducing progression of periodontitis. *Bacteroides gingivalis* therefore is capable of functioning as a primary pathogen in periodontal disease, and the potential of microbial changes to induce a burst of disease progression is thus confirmed. As a result, factors that allow or promote both the colonization and emergence of this microorganism become of great importance to the control of periodontitis.

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Dominant Role of N-Type Ca^{2+} Channels in Evoked Release of Norepinephrine from Sympathetic Neurons

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Multiple types of calcium channels have been found in neurons, but uncertainty remains about which ones are involved in stimulus-secretion coupling. Two types of calcium channels in rat sympathetic neurons were described, and their relative importance in controlling norepinephrine release was analyzed. N-type and L-type calcium channels differed in voltage dependence, unitary barium conductance, and pharmacology. Nitrendipine inhibited activity of L-type channels but not N-type channels. Potassium-evoked norepinephrine release was markedly reduced by cadmium and the conesnail peptide toxin ω-*Conus geographus* toxin VIA, agents that block both N- and L-type channels, but was little affected by nitrendipine at concentrations that strongly reduce calcium influx, as measured by fura-2. Thus N-type calcium channels play a dominant role in the depolarization-evoked release of norepinephrine.

CALCIUM ENTRY VIA VOLTAGE-gated Ca^{2+} channels is essential for neurotransmitter release (*1*). The existence of multiple types of neuronal Ca^{2+} channels (*2–4*) has raised questions about which channel (or channels) contributes to transmitter release. With notable exceptions [for example, see (*5–7*)], evoked release of neurotransmitters is largely resistant to dihydropyridine (DHP) antagonists in systems such as brain slices (*8*), cultured neurons (*9*), and synaptosomes (*10, 11*), which suggests that transmitter release may be dominated by DHP-resistant Ca^{2+} channels (*4, 9, 11, 12*). To test this hypothesis, we have studied the relation between Ca^{2+} entry and norepinephrine (NE) release in rat sympathetic neurons.

Whole-cell recordings (*13*) in rat sympathetic neurons (*14*) provided evidence for two types of Ca^{2+} channels that differed in their time and voltage dependence of inactivation. Calcium ion channel currents carried by 10 m*M* external Ba^{2+} were recorded with Na^+ and K^+ channel currents blocked (Fig. 1). Depolarizing pulses from different holding potentials (HPs) to a test potential of +10 mV evoked a decaying inward current and a late inward current which showed differing dependence on the HP. The late current amplitude was nearly maximal at HP = −60 mV, while the decaying current grew progressively larger as the HP approached −120 mV. These results are similar to those from chick dorsal root ganglion (DRG) neurons (*3, 4*) and have been attributed to the presence of two populations of Ca^{2+} channels—the L-type Ca^{2+} channel, which contributes maintained currents from depolarized HPs, and the N-type Ca^{2+} channel, which inactivates during maintained depolarizations but requires negative HPs to reprime. The rate of inactivation is much slower in sympathetic neurons than in DRG neurons.

Cell-attached patch recordings of single-channel activity provided evidence for distinct types of Ca^{2+} conductance (Fig. 1B). With 110 m*M* Ba^{2+} as the external charge carrier and isotonic K^+ outside the cell to bring its resting potential to zero, depolarizing pulses from an HP of −80 to −20 mV evoked unitary Ca^{2+} channel openings of roughly 1 and 2 pA in amplitude. The larger unitary currents occasionally appeared grouped in bursts of long-lasting openings (fifth sweep), a characteristic of L-type Ca^{2+} channels in other cells (*15*). The voltage dependence of the smaller and larger unitary currents corresponded to slope conductances of 11 and 27 pS (eight patches total). These slope conductances are close to average values for N- and L-type Ca^{2+} channels in chick DRG neurons, 13 and 25 pS, respectively (*4*). The Ca^{2+} channel with the smaller unitary conductance has the same dependence on holding potential as component N in whole-cell recordings (Fig. 1A). Unitary events seen with depolarizations from an HP of −80 mV (Fig. 1D) largely disappeared when the HP was shifted to −40 mV for 30 seconds or more (Fig. 1E) and reappeared after an HP of −80 mV was restored (Fig. 1F). In cell-attached patches,

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as in whole-cell recordings, the N-type Ca^{2+} channel showed very little inactivation over the course of a 100- to 200-msec pulse.

N- and L-type Ca^{2+} channels in sympathetic neurons could be distinguished by their responsiveness to DHP Ca^{2+} antagonists as in other cells (*3*). Application of 10 μM nifedipine had no effect on the activity of N-type Ca^{2+} channels (Fig. 2, A and B) but strongly suppressed openings of L-type Ca^{2+} channels elicited from a HP of −40 mV (Fig. 2, C and D). Further addition of the DHP agonist compound BayK 8644 (Fig. 2E) overcame the nifedipine inhibition of L-type channels by promoting a pattern of long openings separated by brief closings. These effects of nifedipine and BayK 8644 on the 27-pS channel in sympathetic neurons are characteristic of L-type channels in other cells (*3, 4, 15, 16*). Effects of DHP Ca^{2+} antagonists on whole-cell currents were consistent with those on unitary currents (Fig. 2F). The sustained current component was reduced by 10 μM nitrendipine while the decaying current was not much changed.

Cadmium ions were potent blockers of both the decaying and sustained currents (Fig. 2G). Peak current and late current were both reduced to about 50% by 2 μM Cd^{2+}. The time course and extent of the current decay remained unchanged, indicating that N and L currents are blocked to nearly the same degree (*4*). A peptide toxin, ω-CgTx VIA (*17*), from the snail *Conus geographus* that produces a long-lasting block of N- and L-type Ca^{2+} channels in sensory neurons (*18*) also reduced the peak current and late current to the same extent, by about 70% (Fig. 2H). This was the maximal effect of a transient application of toxin; it remained even after washout of the toxin and could not be increased in a persistent manner by further application of toxin.

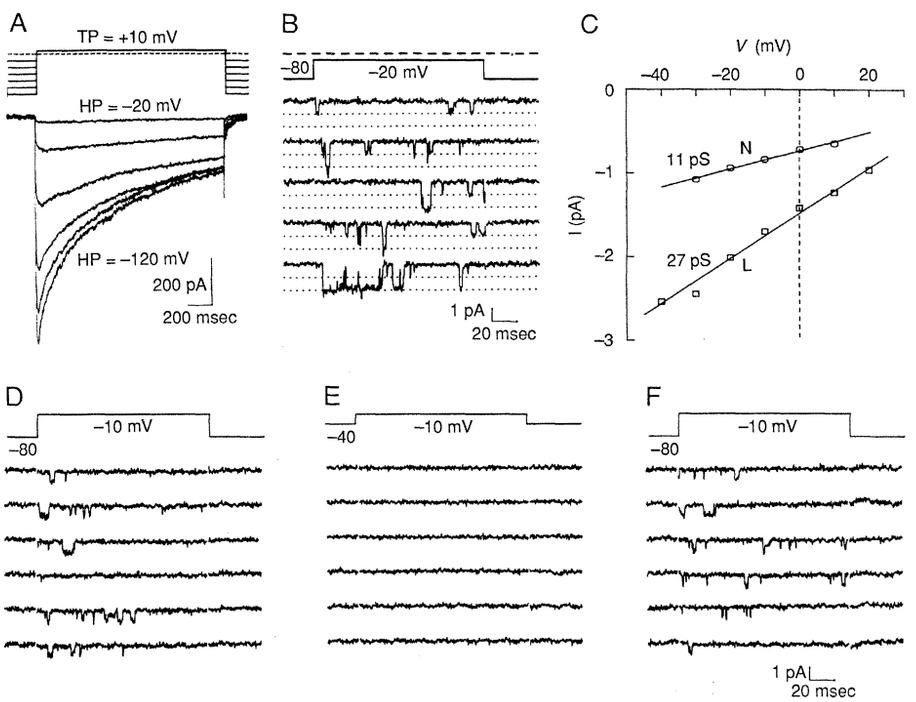

Fig. 1. Two types of Ca^{2+} channels in rat sympathetic neurons studied with whole-cell and cell-attached patch recordings. (**A**) Whole-cell recordings of N and L currents evoked by depolarizations to test potential (TP) of +10 mV from HPs as indicated. Cell L17A. Linear leak and capacitance currents have been subtracted. Current traces are blanked for 1 msec after depolarizing and repolarizing voltage steps. Pipette solution contained 100 m*M* CsCl, 10 m*M* Cs-EGTA, 5 m*M* $MgCl_2$, 2 m*M* adenosine triphosphate, and 40 m*M* Hepes (*p*H 7.3 with CsOH). External solution contained 10 m*M* $BaCl_2$, 135 m*M* TEA-Cl, 10 m*M* Hepes (*p*H 7.3 with TEA-OH), and 200 n*M* TTX. (**B**) Current records elicited by pulses from an HP of −80 mV to a TP of −20 mV, demonstrating unitary openings of both L- and N-type channels. Dashed lines indicate unitary amplitudes of 1.05 and 2.02 pA. Cell L01E. (**C**) Single-channel current-voltage relations showing data collected from eight patches. Standard errors are not shown because they are generally no larger than the size of the symbols. (**D**) Consecutive sweeps showing N-type channel activity elicited by pulses from a HP of −80 mV to a TP of −10 mV. (**E**) Disappearance of N-type channel activity after HP was changed to −40 mV (L-type Ca^{2+} channel activity remains at this HP; see Fig. 3C). Sweeps recorded ~80 seconds after change in HP. Small amounts of N-type activity remained even after long sojourns at an HP of −40 mV (in single-channel as well as whole-cell recordings). (**F**) N-type channel activity returns upon returning to a HP of −80 mV. Cell L02A. For all single-channel experiments, the cell resting potential was zeroed with an external solution containing 140 m*M* potassium aspartate, 10 m*M* K-EGTA, 1 m*M* $MgCl_2$, 10 m*M* Hepes (*p*H 7.4 with KOH). Pipette solution contained 110 m*M* $BaCl_2$, 10 m*M* Hepes (*p*H 7.4).

A similar degree of block was seen with depolarizations from −50 mV, which evoked sustained currents with very little current decay.

To study transmitter release, we cultured superior cervical ganglia (SCG) neurons on collagen-coated, multi-well tissue culture plates, loaded them with [^{3}H]NE, and depolarized them with elevated external K^+ (*19*). After exposure of cells to 70 m*M* K^+, [^{3}H]NE release proceeded rapidly for the first 45 seconds and more slowly over the next several minutes (Fig. 3A). As expected for a process triggered by opening of voltage-gated Ca^{2+} channels, NE release increased steeply with K^+ concentrations between 30 and 90 m*M* (*6*) or with graded increases in external Ca^{2+} and was abolished by addition of 3 m*M* Co^{2+} or removal of external Ca^{2+} (*6*).

Pharmacological interventions were used to characterize the relative contributions of L- and N-type Ca^{2+} channels to the Ca^{2+} entry underlying transmitter release. Nitrendipine left the time course of K^+-evoked NE release unchanged (Fig. 3A) and produced little reduction in the overall amount of NE release even at concentrations up to 10 μM (Fig. 3B). Likewise, BayK 8644 had little or no effect on the main phase of NE release (Fig. 3A), although it promoted some additional NE release during long-lasting K^+ depolarizations. In contrast, NE release was potently inhibited by ω-CgTx (*20*) or Cd^{2+} (Fig. 3B). The inhibitory effects of these agents appeared to be specific to voltage-gated Ca^{2+} channels. The effect of the toxin was persistent and selective for K^+-evoked NE release, ionomycin-induced release being unaltered (*21*). Furthermore, the dose dependence of Cd^{2+} inhibition (ED_{50} ~10 μM) was in fair agreement with its potent effects on whole-cell Ca^{2+} channel currents (Fig. 2G). Because N-type channels are resistant to being blocked by nitrendipine and other DHPs, but are strongly inhibited by ω-CgTx and Ca^{2+} ions, these results support the idea that N-type Ca^{2+} channels are the dominant pathway for Ca^{2+} entry underlying NE release.

Since N-type Ca^{2+} channels inactivate, they should generate a large Ca^{2+} entry during the first few seconds of a maintained depolarization, but less influx later on. This does not preclude a dominant role in controlling the K^+-evoked NE release: elevation of intracellular free Ca^{2+} and enhancement of NE release should outlast Ca^{2+} influx since the former two depend on restorative processes such as Ca^{2+} buffering or extrusion. To test the idea that Ca^{2+} influx early during the K^+ depolarization is sufficient to promote subsequent NE release, Ca^{2+} channels were blocked by a delayed

application of 0.5 mM Cd^{2+} 5 seconds after the K^+ depolarization. This did not significantly reduce the rate of NE release over 40 seconds (Fig. 3C).

Additional experiments extended the finding that NE release is DHP-resistant but ω-CgTx–sensitive. To show that DHP-sensitive L-type channels contribute substantial Ca^{2+} influx under the conditions of the release experiments, we loaded small groups of SCG neurons with fura-2 (*22*) and measured intracellular Ca^{2+} in cells at rest and during exposure to 50 mM K^+ (Fig. 4A). The peak elevation of intracellular Ca^{2+} with high K^+ was reduced by more than 50% in the presence of 1 μM nitrendipine, an inhibition at least as strong as the block produced by 10 nM ω-CgTx. Thus, the DHP responsiveness of the Ca^{2+} transient stands in contrast to the relative DHP-resistance of [^{3}H]NE release under parallel conditions (Fig. 4B).

Depolarizations that inactivate or open L-type Ca^{2+} channels are known to promote effects of DHP antagonists (*5, 23*). In an effort to unmask some contribution of DHP-sensitive channels, we preexposed cells to nitrendipine and high K^+ before initiating release by addition of external Ca^{2+}. Even so, nitrendipine did not reduce release (Fig. 4B, middle). We also monitored NE release with Ca^{2+} reduced to 0.3 mM (Fig. 4B, right), to maximize the effect of changes in Ca^{2+} permeability and to minimize any antagonism between nitrendipine and external Ca^{2+}. Lowering Ca^{2+} reduced NE outflow in the absence of drug, as expected, but nitrendipine still failed to give any inhibition. The toxin ω-CgTx remained effective in inhibiting NE release with either predepolarization or lowered external Ca^{2+}.

These experiments support the hypothesis that N-type Ca^{2+} channels are the dominant type of Ca^{2+} entry pathway controlling NE release in sympathetic neurons. Although L-type Ca^{2+} channels provide at least half the total Ca^{2+} transient, as estimated by DHP inhibition of the fura-2 signal, they seemed to contribute very little to NE release unless their activity was stimulated with BayK

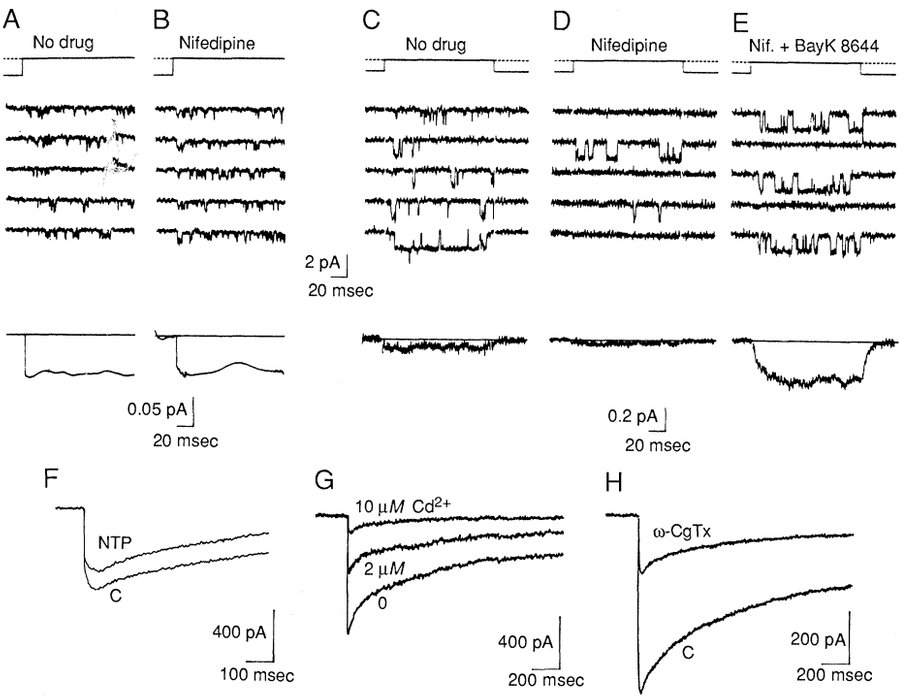

Fig. 2. Nifedipine or nitrendipine block L-type Ca^{2+} channels selectively while Cd^{2+} and ω-CgTx inhibit both L- and N-type Ca^{2+} channels. (**A** and **B**) Lack of response of N-type channel activity to 5 μM nifedipine. Openings with pulses from an HP of −60 mV to a TP of 0 mV before (A) and after (B) the addition of nifedipine to the bathing solution. Average currents shown at bottom were digitally smoothed. Cell L19E. (**C** through **E**) Effects of nifedipine and BayK 8644 on L-type channel activity evoked by depolarizations from an HP of −40 mV to a TP of 0 mV. Records taken before (C) and after (D) addition of 5 μM nifedipine; (E) after further addition of 10 μM BayK 8644 in the continued presence of nifedipine. Average currents shown at bottom. Cell L02F. Solutions as in Fig. 1. (**F**) Whole-cell records showing L and N currents in the absence of drug (C) and after addition of 10 μM nitrendipine. Currents evoked with pulses from an HP of −60 mV to a TP of +10 mV. Solutions similar to those in Fig. 1 but with 2 mM $CaCl_2$ instead of 10 mM $BaCl_2$. Cell L18G. (**G**) Blocking effect of Cd^{2+} ions. L and N currents elicited with pulses from an HP of −90 mV to a TP of +10 mV with 0, 2, or 10 μM Cd^{2+} in the bath. Solutions as in Fig. 1. (**H**) Block of N and L currents produced by a brief application of ω-CgTx VIA from a puffer pipette containing 10 μM ω-CgTx VIA and lysozyme (1 mg/ml) dissolved in external solution. Records were taken just before and several minutes after applying the toxin by means of a brief pulse of pressure to a puffer pipette. External and recording pipette solutions as in Fig. 1. Currents evoked with pulses from an HP of −90 mV to a TP of 0 mV.

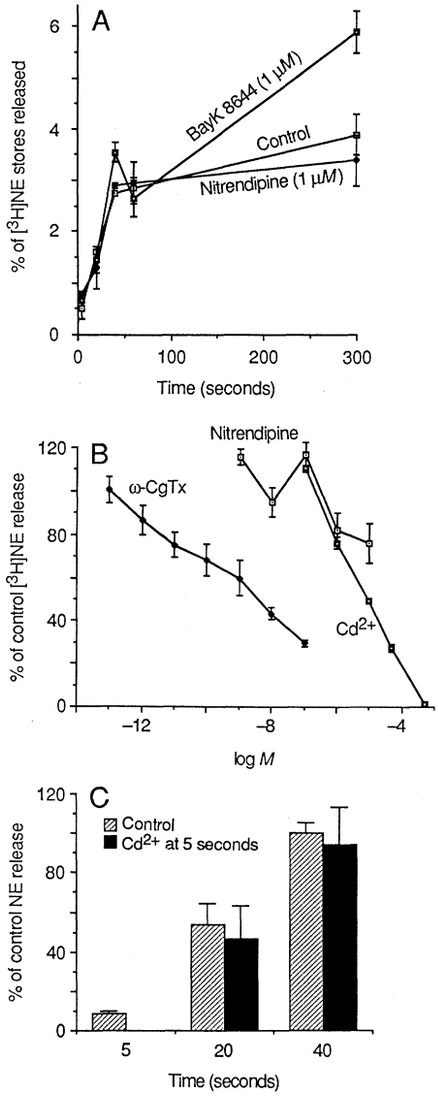

Fig. 3. Pharmacology of Ca^{2+} channel blockers of K^+-evoked [^{3}H]NE release indicates that N-type Ca^{2+} channels are the dominant pathway. (**A**) Time course of [^{3}H]NE release in the absence of drug (control) or in the presence of nitrendipine or BayK 8644. Drugs were administered 10 minutes before application of 70 mM K^+. (**B**) Dose-dependent effects of Ca^{2+} channel blockers on [^{3}H]NE release, expressed as a percentage of release with no drug present. A 5-minute exposure to 70 mM K^+ was preceded by a 10-minute exposure to nitrendipine, a 1-hour exposure to ω-CgTx, or a 10-minute exposure to Cd^{2+}, at the concentrations on the abscissa. (**C**) Evidence that NE release is largely controlled by Ca^{2+} entering the cell within 5 seconds after K^+ depolarization; Cd^{2+} (0.5 mM), a dose sufficient to block all Ca^{2+} channels (*4*), was administered 5 seconds after stimulation with 70 mM K^+. Release of [^{3}H]NE was assayed at the indicated times for both control and Cd^{2+}-exposed cells. Data are displayed as mean ± SEM (n = 4 to 8 per point).

8644 over a period of several minutes (Fig. 3A) (*24*). The relatively minor effect of DHP antagonists on NE release was all the more striking because we used sustained high K^+ depolarizations, known to promote DHP blockade (*5, 23*), rather than electrical field stimulation. It is not clear why Ca^{2+} influx through L-type channels is relatively ineffective in initiating NE release. Gross differences in the distribution of L- and N-type channels over the surface of the cell cannot be completely excluded, although patch-clamp and fura-2 measurements demonstrate the coexistence of both types of channels on soma, processes, and growth cones of sympathetic neurons (*25*). It seems more likely that the channels differ somehow in their microscopic localization or their relation to the cellular machinery for release (*26*).

Unlike NE release from sympathetic neurons, release of substance P from DRG neurons (*5, 6*) or catecholamine release from adrenal chromaffin cells (*27*) is strongly inhibited by DHP antagonists, consistent with a major participation of L-type channels. One possibility is that Ca^{2+} entry via N-type channels may dominate the release of small vesicles containing only classical transmitters (as in this study), whereas Ca^{2+} influx through L-type channels may favor the release of large dense core vesicles containing peptides (and sometimes "classical" transmitters like catecholamines, as in adrenal chromaffin cells). Control of release by different Ca^{2+} channels would go along with other known differences between these two pathways for secretion (*28*). The differing kinetic properties of N- and L-type Ca^{2+} channels might account for known correlations between various patterns of electrical stimulation and exocytosis of small and large vesicles (*29*). Evenly spaced action potentials would allow repriming and activation of N-type channels and promote release from small vesicles, while intermittent bursts of impulses at high frequency might favor activation of L-type channels and exocytosis from large dense core vesicles.

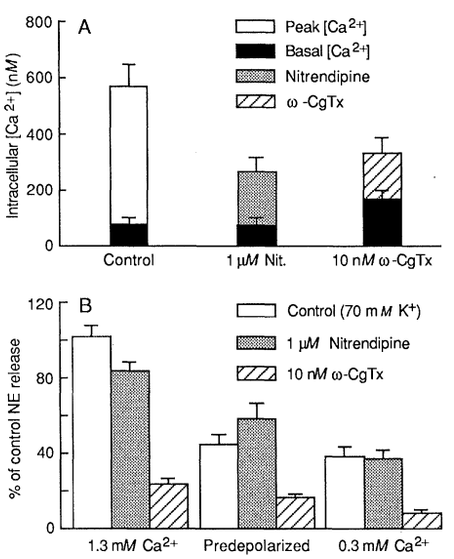

Fig. 4. Effects of Ca^{2+} channel blockers on Ca^{2+} transients and [^{3}H]NE release. (**A**) Intracellular Ca^{2+} levels measured in sympathetic neuron cell bodies by microspectrofluorometry with fura-2 (*23*). Steady Ca^{2+} levels in unstimulated cells (filled bars) and peak response in cells depolarized with 50 m*M* K^+ (open bars); nitrendipine and ω-CgTx reduced the peak Ca^{2+} transient to a similar extent. (**B**) Differential sensitivity of NE release to nitrendipine and ω-CgTx under various conditions for evoking release. (Left) Release with 1.3 m*M* Ca^{2+}, as in (A). Note that nitrendipine inhibits release much less than ω-CgTx, in contrast to (A). (Middle) Release evoked by addition of 1.3 m*M* Ca^{2+} for 1 minute, following a 1-minute predepolarization with 70 m*M* K^+ in the absence of external Ca^{2+}. (Right) Effects of nitrendipine and ω-CgTx on K^+-evoked release with 0.3 m*M* external Ca^{2+}. Norepinephrine release in the absence of the blocking agents is considerably smaller with 0.3 m*M* Ca^{2+} than with 1.3 m*M* Ca^{2+} (left).

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19. [^{3}H]Norepinephrine release was measured from neurons after 5 to 6 days in culture. Cells were preincubated in [^{3}H]NE (1 μCi, 0.25 ml per well) in Hepes-based release media [with essential vitamins (Gibco), MEM amino acids (Gibco), pargyline (19.6 μg/ml), and ascorbic acid (176 μg/ml)] for 1 hour. The excess [^{3}H]NE was removed by four rapid, consecutive washes followed by four additional washes at 5-minute intervals. Wash solutions [containing desmethylimipramine (3.03 μg/ml) to inhibit reuptake] remained on the cells for 5 minutes unless otherwise stated. Evoked NE release was quantitated by liquid scintillation spectroscopy and reported as the percentage of total cellular NE released minus background. Background release was the amount of ^{3}H found in the wash prior to the high K^+ stimulus. The ionic composition of the release solutions was 50 m*M* NaCl, 86.1 m*M* choline chloride, 5 m*M* KCl, 0.44 m*M* KH_2PO_4, 0.34 m*M* Na_2HPO_4, 2.66 m*M* $NaHCO_3$, 1.3 m*M* $CaCl_2$, 0.81 m*M* $MgSO_4$, 5.6 m*M* glucose, and 20 m*M* Hepes. KCl was substituted for choline chloride isoosmotically (up to 70 m*M*) to make the high K^+ media used for depolarization.
20. The broadness of the dose-response relation for ω-CgTx is consistent with that observed for ^{45}Ca uptake by Reynolds *et al.* (*11*). The dose-response curve was obtained with 60-minute exposures to various doses of toxin, when binding was probably not at steady state (*5, 19*). Alternatively, subpopulations of N and L channels may have different affinities for toxin.
21. Ionomycin (10 μg/ml) (Boehringer Mannheim) induced NE release comparable to that evoked by 70 m*M* K^+. Preincubation with ω-CgTx (10 n*M*) did not reduce this release (128% of that found with ionomycin alone).
22. Cytosolic-free Ca^{2+} was determined with the Ca^{2+}-sensitive dye fura-2 [G. Grynkiewicz, M. Poenie, R. Y. Tsien, *J. Biol. Chem.* **260**, 3440 (1985)]. As reported [S. A. Thayer, S. N. Murphy, R. J. Miller, *Mol. Pharmacol.* **30**, 505 (1986)], a microspectrofluorometer [T. M. Perney, R. Dinerstein, R. J. Miller, *Neurosci. Lett.* **51**, 165 (1984)] was used to monitor the fluorescence signal from sympathetic neurons cultured on glass cover slips and loaded with dye by incubation in 2 μ*M* fura-2 actoxymethyl ester for 1 hour at 37°C in Hepes-buffered Hank's balanced salt solution, *p*H 7.45, containing 0.5% bovine serum albumin. After loading, the cells were washed twice in Hepes–Hank's solution and incubated for 30 minutes to allow time for intracellular dye cleavage. The cover slips were then mounted in the experimental chamber of the microspectrofluorometer and maintained at 37°C. Intracellular Ca^{2+} was determined by measuring the ratio of the fura-2 fluorescence detected at 510 nm when excited at either 340 or 380 nm. Ratio values were converted to Ca^{2+} concentrations by comparison with a standard curve determined for the fura-2 pentapotassium salt in buffered salt solutions containing Ca^{2+} and EGTA in ratios calculated to give free Ca^{2+} concentrations ranging from 0 to 1000 n*M*.
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Molecular Crowding on the Cell Surface

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Strong steric interactions among proteins on crowded living cell surfaces were revealed by measurements of the equilibrium spatial distributions of proteins in applied potential gradients. The fraction of accessible surface occupied by mobile surface proteins can be accurately represented by including steric exclusion in the statistical thermodynamic analysis of the data. The analyses revealed enhanced, concentration-dependent activity coefficients, implying unanticipated thermodynamic activity even at typical cell surface receptor concentrations.

THE LATERAL DIFFUSIBILITIES OF most proteins in most cell surfaces have generally been found by fluorescence photobleaching recovery (FPR), postelectrophoresis relaxation (PER), and other techniques to be several orders of magnitude slower than predictable from simple hydrodynamic models, even though these models are appropriate for dilute protein concentrations in nearly pure lipid membranes (*1*). Comparisons with the rapid diffusibilities of proteins reconstituted into nearly pure lipid membranes (*2*) and in disrupted (bleb) membranes (*3*) have suggested that, in cells, interactions with the cytoskeleton, the glycocalix, or other cell surface proteins may constrain molecular diffusibility. Deletions of the cytoplasmic domains of certain cell surface receptors do not enhance their diffusibilities, suggesting that constraints other than direct anchorage to the cytoskeleton must exist (*4*). Interactions among the membrane proteins themselves have been inferred from the strong concentration dependence of the diffusibility of proteins reconstituted into artificial membranes (*2, 5*).

In an attempt to understand the nature of protein-protein interactions in cell membranes, we investigated the equilibrium properties of proteins on living cell surfaces. To avoid the complications of the dynamics of diffusion, we measured the equilibrium spatial distribution of fluorescence-labeled receptors on the surface of the nearly spherical rat basophilic leukemia (RBL) cells in an applied electric field. Previous measurements of the time course of PER have provided useful estimates of lateral molecular mobility (*6, 7*). In particular, the measurements of the diffusibilities of the immunoglobulin E receptor complex (IgER) on RBL cells were found to agree in PER and FPR experiments (*8, 9*).

Digital imaging microscopy (DIM) (*10, 11*) provides quantitative fluorescence images from which the distribution of fluorescence-labeled cell surface proteins can be measured accurately. Images of the asymmetric crowding of IgER on the surfaces of RBL cells induced by the electric field were analyzed in order to determine accurate maps of the receptor concentration on the cell surface (Figs. 1 and 2).

In an ideal solution, the equilibrium concentration distributions in a potential gradient can be described by a Boltzmann probability distribution over the potential energies ϵ

$$P(\epsilon) = \exp[-(\epsilon - \mu)/kT] \quad (1)$$

where μ is the chemical potential, k is the Boltzmann constant, and T is absolute temperature. On spherical cells of radius r in a uniform applied electric field E_0 (Fig. 2, inset), the potential energy ϵ of surface particles of effective charge Ze varies with angle θ relative to the applied field as (*12,13*)

$$\epsilon = (3/2)\, Zer \cos\theta \equiv \beta\cos\theta \quad (2)$$

Normalizing the distribution of mobile particles $C(\theta)$ to the initial (and average) concentration of mobile particles C_0 determines the chemical potential, such that the ideal distribution is given by

$$\frac{C(\theta)}{C_0} = \frac{2\beta}{kT} \cdot \frac{\exp[-\beta(1 + \cos\theta)/kT]}{1 - \exp(-2\beta/kT)} \quad (3)$$

Integration of Eq. 3 over all angles gives unity. Any immobile concentration C_{im} must be included in the total concentration $C_T(\theta)$ such that $C_T(\theta) = C(\theta) + C_{im}$ and $C_{0T} = C_0 + C_{im}$, where $C_{im} \equiv C_{0T}I_{im}$ defines an immobile fraction I_{im}. Previous measurements have been fit to a Boltzmann distribution (*7, 12*).

Fluorescence DIM experiments measure the total population $C_T(\theta)$ of labeled mobile and immobile proteins normalized to the initially uniform total concentration C_{0T}. Representative DIM measurements of the equilibrium distribution of fluorescein isothiocyanate (FITC)–labeled IgER on the surface of RBL cells after electrophoresis are shown in Fig. 2. Large discrepancies from the Boltzmann distribution curve are clearly evident (*14, 15*). The curve is forced to fit the data at low concentrations, emphasizing an obvious discrepancy that might be ascribed to saturation at high concentrations.

To account for saturation of the space on the cell membrane occupied by the proteins, we invoked simple steric exclusion. Analo-

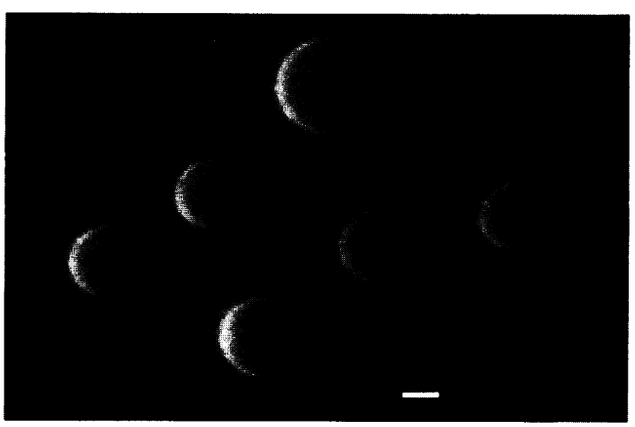

Fig. 1. The distribution of FITC-labeled IgER on the surface of live RBL cells (2H3 subline) after electrophoresis. Cells that were grown, harvested, and labeled with FITC-IgE as described (*8, 23*) were plated on No. 1 glass cover slips, mounted in an electrophoresis chamber (*24*) modified for an inverted microscope, and exposed to an applied electric field. Equilibrium of the surface distribution was verified by monitoring its time course after applying the field. Bar is 5 μm. All experiments were at room temperature unless otherwise noted.

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