

Literature review

# Ca channel modulation and synaptic plasticity

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# **$\alpha$ -Adrenergic inhibition of sympathetic neurotransmitter release mediated by modulation of N-type calcium-channel gating**

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IN sympathetic neurons, catecholamines interact with prejunctional  $\alpha$ -adrenergic receptors to reduce delivery of transmitter to postjunctional target organs<sup>1–4</sup>. This autoinhibitory feedback is a general phenomenon seen in diverse neurons containing a variety of transmitters<sup>2–4</sup>. The underlying mechanisms of  $\alpha$ -adrenergic inhibition are not clear, although decreases in cyclic AMP and cAMP-mediated phosphorylation have been implicated<sup>1–4</sup> (compare ref. 5). We have studied depolarization-induced catecholamine release and calcium-channel currents in frog sympathetic neurons. Here we show that  $\alpha$ -adrenergic inhibition of transmitter release can be explained by inhibition of  $\text{Ca}^{2+}$ -channel currents and not by modulation of intracellular proteins. Noradrenaline strongly reduces the activity of N-type  $\text{Ca}^{2+}$  channels, the dominant calcium entry pathway triggering sympathetic transmitter release<sup>6</sup>, whereas L-type  $\text{Ca}^{2+}$  channels are not significantly inhibited. The down-modulation of N-type channels involves changes in rapid gating kinetics but not in unitary flux. This is the first detailed description of inhibition of a high-voltage activated neuronal  $\text{Ca}^{2+}$  channel at the single-channel level. The coupling between  $\alpha$ -adrenergic receptors and N-type channels involves a G protein, but not a readily diffusible cytoplasmic messenger or protein kinase C, and may be well suited for rapid and spatially localized feedback-control of transmitter release.

# Noradrenergic inhibition does not depend on mechanisms subsequent to rise of cytosolic calcium, but it is likely to involve modulation of calcium entry

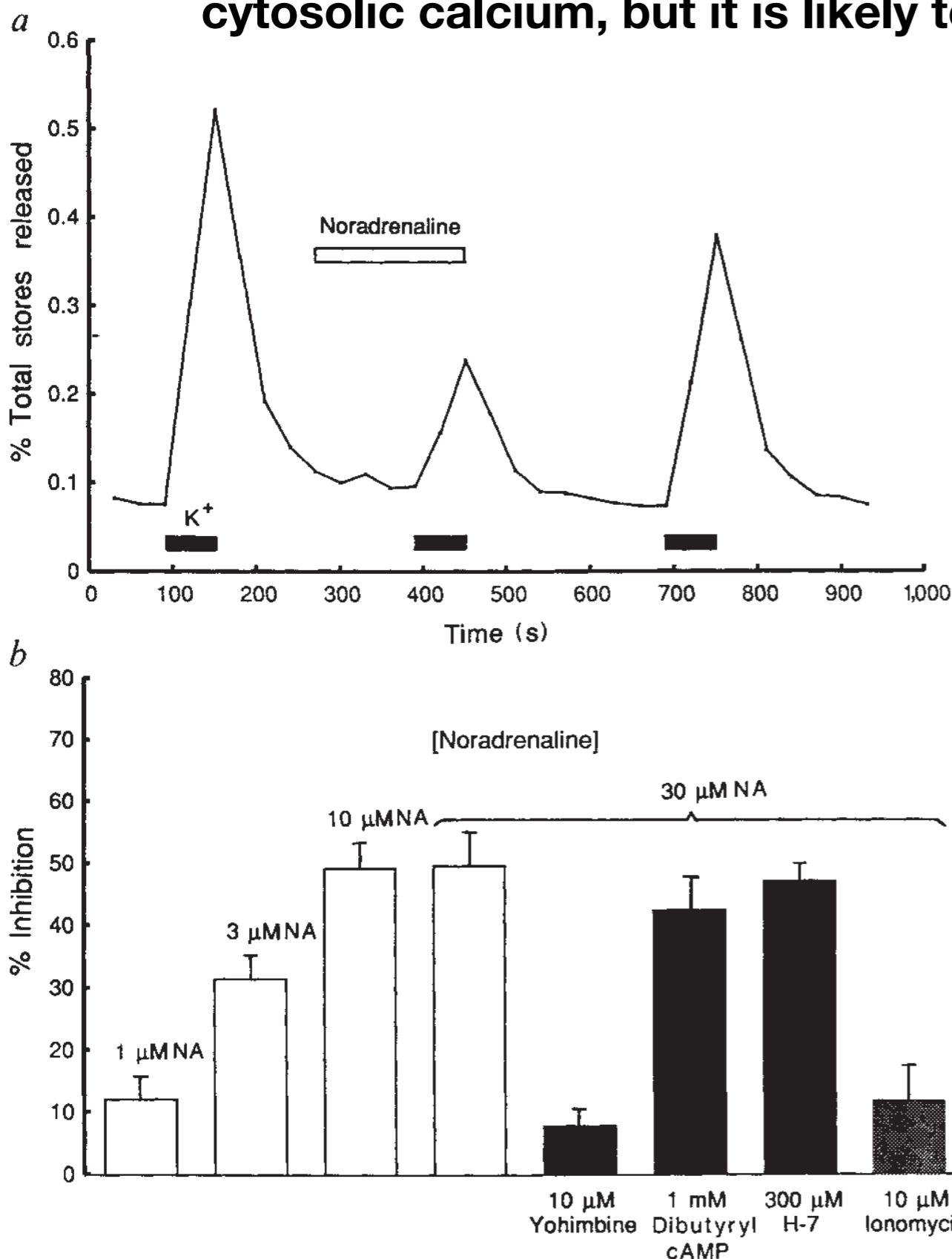
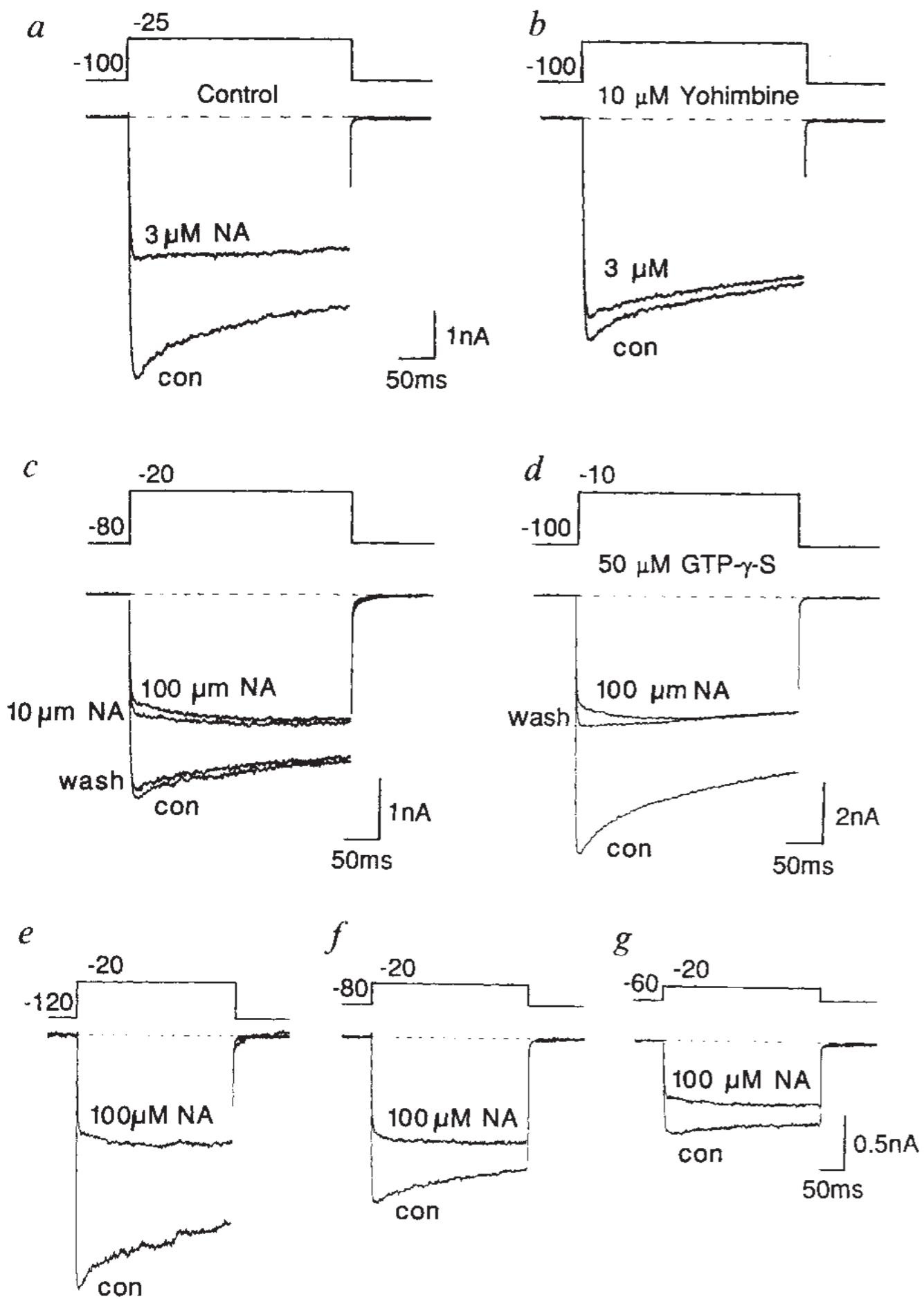


FIG. 1 *a*, Effect of 30  $\mu$ M NA (open bar) on radiolabelled transmitter release evoked by exposure to 50 mM  $K^+$  (solid bars). *b*, Characteristics of NA effect. Bars represent means  $\pm$  s.e.m. of percentage of NA-induced inhibition in 3–8 experiments. Open bars, dose-dependence of NA-induced inhibition; solid bars, effect of 30  $\mu$ M NA in the presence of the indicated agents. Dibutyryl cAMP or H-7 (1-(5-isoquinolinylsulphonyl)-2-methylpiperazine) did not significantly affect NA inhibition ( $P > 0.05$ ). Grey bar, NA did not significantly affect transmitter release evoked by  $Ca^{2+}$  in ionomycin ( $P > 0.05$ ). METHODS. Sympathetic chains (3–4 ganglia) from frogs (*Rana pipiens pipiens*) were incubated for 2 h at 22 °C in Ringer's solution containing (mM): NaCl (128), KCl (2), glucose (10), HEPES (10) (pH 7.3 with NaOH); also added were ascorbic acid (antioxidant), (1.0 mM) pargyline (monoamine oxidase inhibitor) (0.1 mM), and [ $^3$ H]-NA (10  $\mu$ Ci ml $^{-1}$   $\approx$  0.2  $\mu$ M) (New England Nuclear). After [ $^3$ H]-NA loading, the ganglia were enclosed in a chamber and perfused at 1.6 ml min $^{-1}$  with Ringer's solution containing 10  $\mu$ M desipramine (reuptake blocker), 10  $\mu$ M propranolol and 2 mM  $CaCl_2$ . Once a stable baseline of  $^3$ H-release was achieved (30–40 min), 30-s fractions were collected continuously. Sympathetic transmitter release (probably a mixture of noradrenaline and adrenaline $^{36}$ , from neuronal somata $^{7,27}$ ) was induced by perfusing ganglia with Ringer's solution containing 50 mM  $K^+$  to directly depolarize the cells, bypassing possible changes in action-potential propagation or duration. Release showed dependence on  $K^+$  (30–80 mM) and extracellular calcium (effector concentration for half-maximum response  $\approx$  0.5 mM), and complete inhibition by cadmium (50% inhibitory concentration  $\approx$  10  $\mu$ M) as expected for exocytosis triggered by voltage-gated  $Ca^{2+}$  influx. The amount of release was calculated as the area ( $A$ ) under each peak after baseline subtraction. The release in the presence of NA ( $A_{NA}$ ) was expressed as a percentage of the average of release evoked before ( $A_{con}$ ) and after washing out NA ( $A_{wash}$ ). Thus, percentage inhibition =  $[1 - (2A_{NA}/A_{con} + A_{wash})] \times 100$ . For ionomycin-induced release, 10  $\mu$ M ionomycin was added to the perfusion solution (external free calcium concentration buffered at 0.1  $\mu$ M with 2 mM EGTA) 10 min before collecting fractions. Release was evoked by exposure to 1 mM free calcium. Peak ionomycin-induced release (0.2–0.4%) was similar to that evoked by 50 mM  $K^+$ .

Yohimbine: Cristaline compound from the yohimbine tree, alpha2 adrenergic blocking agent. Used in the treatment of impotence. Propanolol: beta-adrenergic blocker (no effect). Phentolamine: alpha-adrenergic agonist (prevented inhibition). Clonidine: alpha2 agonist (no effect  $<100\mu$ m). H-7: protein kinase blocker. Ionomycin: Ca ionophore, bypasses Ca entry through voltage-dependent calcium channels (no effect on NA transmission).

Noradrenergic inhibition does not depend on mechanisms subsequent to a rise in cytosolic Ca but it is likely to involve modulation of Ca entry

cell bodies (Fig. 2). Noradrenaline strongly and reversibly reduced transmitter outflow evoked by exposure to K<sup>+</sup> (Fig. 1a). The inhibition reached a maximum of ~50% at 10–100 μM NA (Fig. 1b). Inhibition was unaffected by the β-adrenergic blocker, propranolol, but was prevented by phentolamine (10 μM), a general α-adrenergic antagonist, or by yohimbine (10 μM), an α<sub>2</sub>-adrenergic antagonist (Fig. 1b). Application of the α<sub>2</sub>-adrenergic agonist clonidine (at concentrations ≤100 μM) had no effect. These pharmacological properties are characteristic of a distinct subtype of α<sub>2</sub>-adrenergic receptor described in other neurons<sup>8,9</sup>. It has been suggested<sup>1–4</sup> that NA autoinhibition might involve altered phosphorylation of Ca<sup>2+</sup> channels or of intracellular proteins, such as synapsin I (ref. 10), by cyclic AMP- or Ca<sup>2+</sup>-dependent protein kinases. We found, however, that the inhibition by 30 μM NA remained unchanged in 1 mM dibutyryl cAMP, or in H-7 (a protein kinase blocker) at a concentration (300 μM) that inhibits several protein kinases<sup>11</sup> (Fig. 1b). Transmitter release induced by adding Ca<sup>2+</sup> in the presence of the Ca<sup>2+</sup> ionophore ionomycin, bypassing calcium entry through voltage-gated Ca<sup>2+</sup> channels, was not affected by NA (Fig. 1b; ref. 12). This indicates that noradrenergic inhibition does not depend on mechanisms subsequent to a rise in cytosolic Ca<sup>2+</sup> but is likely to involve modulation of Ca<sup>2+</sup> entry.



**FIG. 2** Noradrenaline inhibition of whole-cell  $\text{Ca}^{2+}$ -channel currents in sympathetic neurons. *a, b*, Effect of 3  $\mu\text{M}$  NA in the absence and presence of 10  $\mu\text{M}$  yohimbine. Yohimbine block was reversible (data not shown). *c*, Incomplete inhibition of  $\text{Ca}^{2+}$ -channel currents at maximally effective NA concentrations. Four records were taken: before (con) and after exposure to 10  $\mu\text{M}$  NA, ~30 s after NA removal (wash), and after exposure to 100  $\mu\text{M}$  NA; 10  $\mu\text{M}$  and 100  $\mu\text{M}$  NA were equally effective. Note reversibility of NA effect with standard internal solution (300  $\mu\text{M}$  GTP). *d*, With 50  $\mu\text{M}$  GTP- $\gamma$ -S instead of internal GTP, the inhibitory effects of NA were irreversible over 9 min of washing in drug-free solution. *e–g*, Recordings of  $\text{Ca}^{2+}$ -channel currents evoked by step depolarizations to  $-20$  mV from different holding potentials in the absence and presence of NA (100  $\mu\text{M}$ ). Same cell as in *c*, 2 mM external  $\text{Ba}^{2+}$ . The dependence of the NA effect on holding potential is consistent with inhibition of N-type  $\text{Ca}^{2+}$ -channel current. Under these recording conditions, L-type channels contribute a relatively sustained current, whereas N-type channels show a greater but not complete degree of inactivation with depolarization<sup>6,25–27</sup>. In other experiments with 2–10 mM external  $\text{Ca}^{2+}$ , NA had little or no effect on sustained currents (presumably L-type channel current) evoked by depolarization from  $-40$  or  $-30$  mV (ref. 37).

**METHODS.** Neurons were isolated from sympathetic ganglia of adult frogs by a combination of enzymatic and mechanical dissociation (see, for example, ref. 24). Currents carried by voltage-gated  $\text{Ca}^{2+}$  channels were recorded with the whole-cell patch-clamp method. To minimize  $\text{Ca}^{2+}$ -dependent inactivation and to block residual outward  $\text{K}^+$  currents,  $\text{Ba}^{2+}$  was usually used as the permeant divalent cation but similar results have been obtained with 2–10 mM  $\text{Ca}^{2+}$ . The standard internal (pipette) solution contained (mM): CsCl (100), EGTA (10), Na-ATP (2), MgCl (5), GTP (0.3), HEPES (40) ( $\text{pH}$  7.2 with CsOH). Recording pipettes had resistances  $<1 \text{ M}\Omega$ . The external bathing solution contained (mM): tetraethylammonium (TEA) (130), CsCl (5), Glucose (10),  $\text{BaCl}_2$  (2), tetrodotoxin (TTX) (1  $\mu\text{M}$ ), propranolol (10  $\mu\text{M}$ ) and HEPES (5,  $\text{pH}$  7.4 with TEA-OH). Cells were continually superfused at  $1\text{--}2 \text{ ml min}^{-1}$  with NA-free or NA-containing solutions. A computer (PDP 11/23) controlled the command voltage, and digitized and stored the filtered current signals (−3 dB at 1 kHz). Step depolarizations lasting 320 ms were applied every 10–12 s. All current records were leak-subtracted and are shown together with the voltage protocol. All experiments were carried out at room temperature ( $\sim 22^\circ\text{C}$ ).

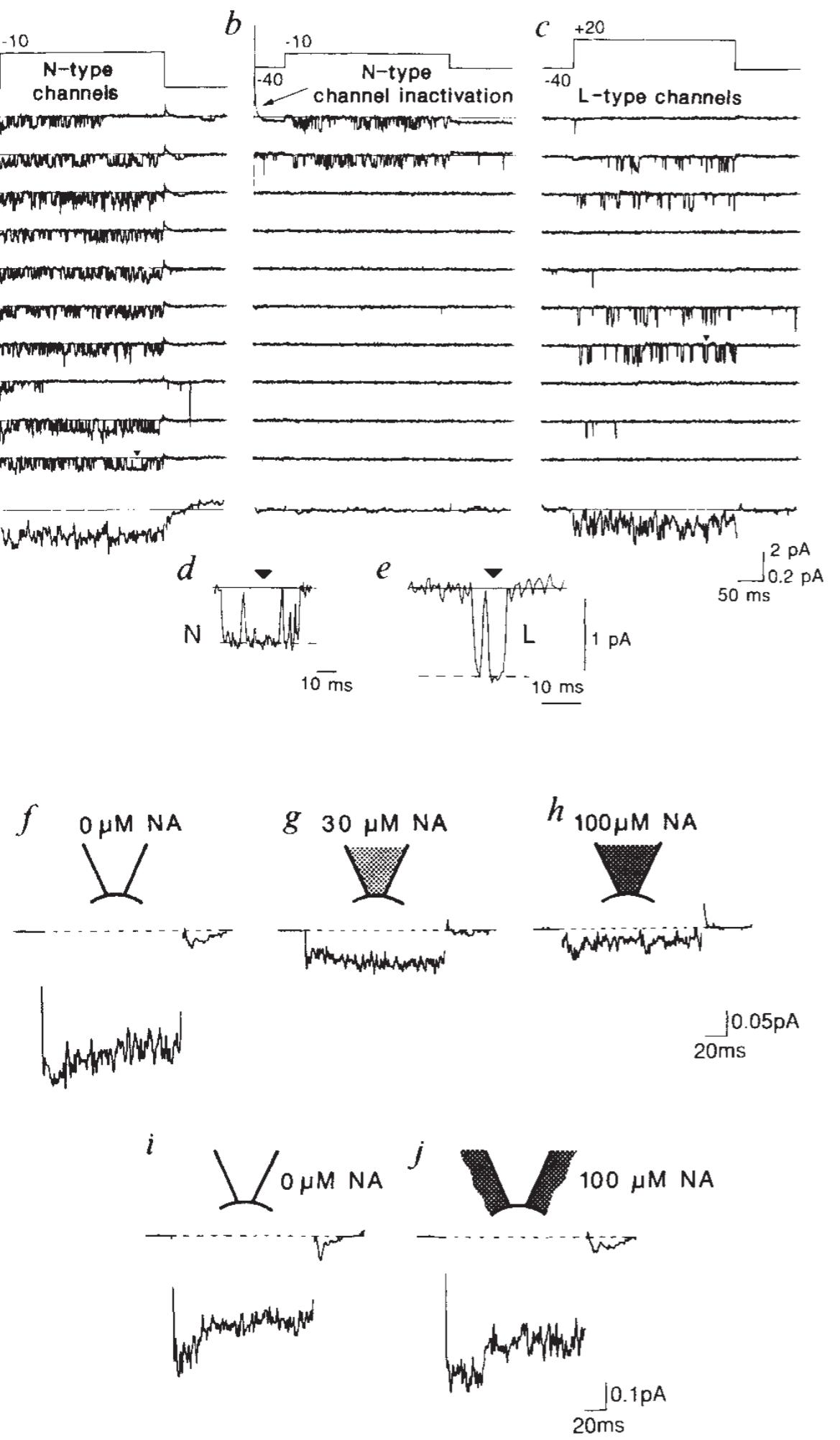
## Noradrenaline decreases single N-type, but not on L-type Ca channel currents

holding potential<sup>24,27</sup>. To assess the effect of NA, a series of cell-attached patch recordings were obtained with or without NA (10–100 μM) in the recording pipette. The average N-type Ca<sup>2+</sup> channel currents were reduced from  $0.24 \pm 0.09$  pA ( $n = 22$ ) to  $0.09 \pm 0.05$  pA ( $n = 10$ ) in 30 μM NA, and to  $0.03 \pm 0.02$  pA ( $n = 5$ ) in 100 μM NA (Fig. 3f–h). The L-type Ca<sup>2+</sup> current was not significantly changed ( $P > 0.05$ ), although there was some hint of inhibition at 100 μM NA. Noradrenaline did not significantly change unitary current amplitude at any concentration tested (legend to Fig. 4). By contrast, there was a marked change in rapid gating kinetics. The mean open time decreased from  $0.87 \pm 0.14$  ms (control;  $n = 23$ ) to  $0.38 \pm 0.07$  ms in 30 μM NA ( $n = 7$ ), or  $0.40 \pm 0.09$  ms in 100 μM NA ( $n = 5$ ). This more than two-fold abbreviation of N-type channel openings contributes substantially to the large decrease in average current seen overall (Fig. 3f–h). Changes in gating kinetics on a time scale slower than the pulse duration are suggested by an increase in the percentage of blank sweeps from 8% in the control to 24% in 30–100 μM NA.

## No readily diffusible second messenger involved in coupling alpha-adrenoreceptor and N-type Ca channel inhibition

The coupling between the α-adrenoceptor and N-type channel inhibition does not involve a readily diffusible second messenger, as application of 100 μM NA to the bath had no effect on N-type channels under the patch pipette (Fig. 3i,j; see also ref. 17). In particular, the messenger is unlikely to be cAMP, because dibutyryl cAMP had no effect on N-type channels, whereas it did increase the activity of L-type channels. Protein kinase C is also unlikely to be involved, because phorbol esters increase N-type and L-type Ca<sup>2+</sup>-channel activity in these cells<sup>29</sup>.

Our results demonstrate that NA acts through α-adrenoceptors to selectively inhibit the activity of N-type Ca<sup>2+</sup> channels, and link this inhibition to attenuation of sympathetic transmitter release. Noradrenaline reduces the probability of channel opening by accelerating the rate of channel closing, and possibly slowing the kinetics of opening, without changing unitary channel flux. Because the mechanism of inhibitory modulation of unitary HVA neuronal Ca<sup>2+</sup>-channel currents has not previously been reported, it will be interesting to see if this pattern of modulation of rapid gating kinetics holds true for other neurotransmitters and other cells<sup>24,26</sup>. Changes in the kinetics of opening and closing might be consistent with allowed voltage-dependence of gating reported by Bean<sup>19</sup>.



**FIG. 3 a–e.** Separation of unitary N-type and L-type  $\text{Ca}^{2+}$ -channel currents in the same cell-attached patch. **a**, Sequential recordings of N-type  $\text{Ca}^{2+}$ -channel currents evoked every 10 s by 320 ms pulses from  $-80\text{ mV}$  to  $-10\text{ mV}$ . **b**, Sequential current recordings showing N-type  $\text{Ca}^{2+}$  channels inactivating within 10–20 seconds following a displacement of the holding potential from  $-80$  to  $-40\text{ mV}$  (arrow marks corresponding capacitive transient). **c**, Sequential recordings of L-type  $\text{Ca}^{2+}$ -channel currents evoked by pulses from  $-40\text{ mV}$  to  $+20\text{ mV}$ . Average currents, each calculated from  $\sim 30$  sweeps, are shown below individual current records. **d**, **e** Openings of N- and L-type  $\text{Ca}^{2+}$  channels (arrow heads in **a**, **c**) enlarged to show clearly resolved and different unitary amplitudes. As the unitary L-type currents at  $+20\text{ mV}$  (**e**) are larger in amplitude than unitary N-type currents at  $-10\text{ mV}$  (**d**), despite the smaller driving force for  $\text{Ba}^{2+}$  entry, they must represent different  $\text{Ba}^{2+}$  conductances (N-type, 15–16 pS; L-type, 26–28 pS; refs 24, 27). **f–h**, Mean currents from separate groups of cell-attached patches, showing inhibition of N-type  $\text{Ca}^{2+}$ -channel current evoked by 130 ms pulses from  $-80\text{ mV}$  to  $-10\text{ mV}$ . Channel activity was identified as N-type by unitary conductance and sensitivity to holding potential as in **a**, **b**. Averages weighted individual patches equally. **f**, Control ( $n=22$  patches); **g**, **h**,  $30\text{ }\mu\text{M}$  NA ( $n=10$ ) or  $100\text{ }\mu\text{M}$  NA ( $n=5$ ) in the patch pipette. Noradrenaline ( $10\text{ }\mu\text{M}$ ) reduced N-type channel currents only slightly; a difference in NA-sensitivity between recordings with  $110\text{ mM}$  external  $\text{Ba}^{2+}$  (**f–h**) and  $2\text{ mM}$   $\text{Ba}^{2+}$  (Fig. 2) might be expected from alterations in surface potential and local catecholamine concentration. **i**, **j**, Evidence against involvement of a readily diffusible messenger. In five cell-attached patches (pipettes  $\sim 1\text{ }\mu\text{m}$  in diameter), the mean current through N-type channels remained unchanged following application of  $100\text{ }\mu\text{M}$  NA to the rest of the cell. In none of the experiments was there a detectable decrease in activity after the drug addition.

**METHODS.** Unitary currents recorded in a series of cell-attached patches with and without NA present in the patch pipette. Attempts at perfusing the pipette with drug while continuously monitoring channel activity were hampered by instability of the recordings, and outside-out patches gave inconsistent results. Recording pipettes had resistances of  $5$ – $10\text{ M}\Omega$  and contained (mM):  $\text{BaCl}_2$  ( $110$ ), HEPES ( $10$ ), (pH adjusted to  $7.4$  with TEA-OH) and TTX ( $1\text{ }\mu\text{M}$ ). The external bathing solution, used to set the membrane potential to zero, contained (mM): K-aspartate ( $140$ ), Glucose ( $10$ ), HEPES ( $5$ ), EGTA ( $10$ ) (pH adjusted to  $7.4$  with KOH). A computer (PDP 11/23) controlled the command voltage; and digitized and stored the filtered currents ( $-3\text{ dB}$  at  $1\text{ kHz}$ ). Individual current recordings were leak-subtracted before determination of average currents.

The pharmacological properties of NA inhibition of whole-cell N-type channel current and transmitter release are very similar. This points to a functional relationship between these phenomena and reinforces earlier evidence that dihydropyridine-insensitive N-type  $\text{Ca}^{2+}$  channels are the main  $\text{Ca}^{2+}$  entry mechanism controlling transmitter release from sympathetic neurons<sup>6</sup>. Interestingly, the triggering of transmitter release may be dominated by L-type channels in certain other neuronal systems<sup>30-32</sup>. Stimulation of  $\alpha$ -adrenergic receptors is coupled to inhibition of N-type  $\text{Ca}^{2+}$ -channels and reduction of transmitter release by means of a G protein but not by a readily diffusible second messenger such as cAMP (as in current hypotheses<sup>2-4</sup>), nor by protein kinase C (as in sensory neurons<sup>33</sup>). A relatively direct coupling mechanism would be appropriate for rapid feedback control of transmitter release.

The feedback may also be localized, because (1) the concentration of NA falls off steeply with increasing distance from the release sites, (2) the  $\alpha$ -adrenergic modulation of N-type  $\text{Ca}^{2+}$  channels works only at short range, and (3) the attenuation of  $\text{Ca}^{2+}$  entry may strongly affect only nearby release sites. Our results do not exclude additional effects of NA on potassium channels<sup>34,35</sup>, possibly mediated by clonidine-sensitive  $\alpha_2$ -receptors<sup>34</sup> and lowered cAMP<sup>35</sup>, that would result in less localized decreases in transmitter release through reduction of action-potential duration and global  $\text{Ca}^{2+}$  entry. □

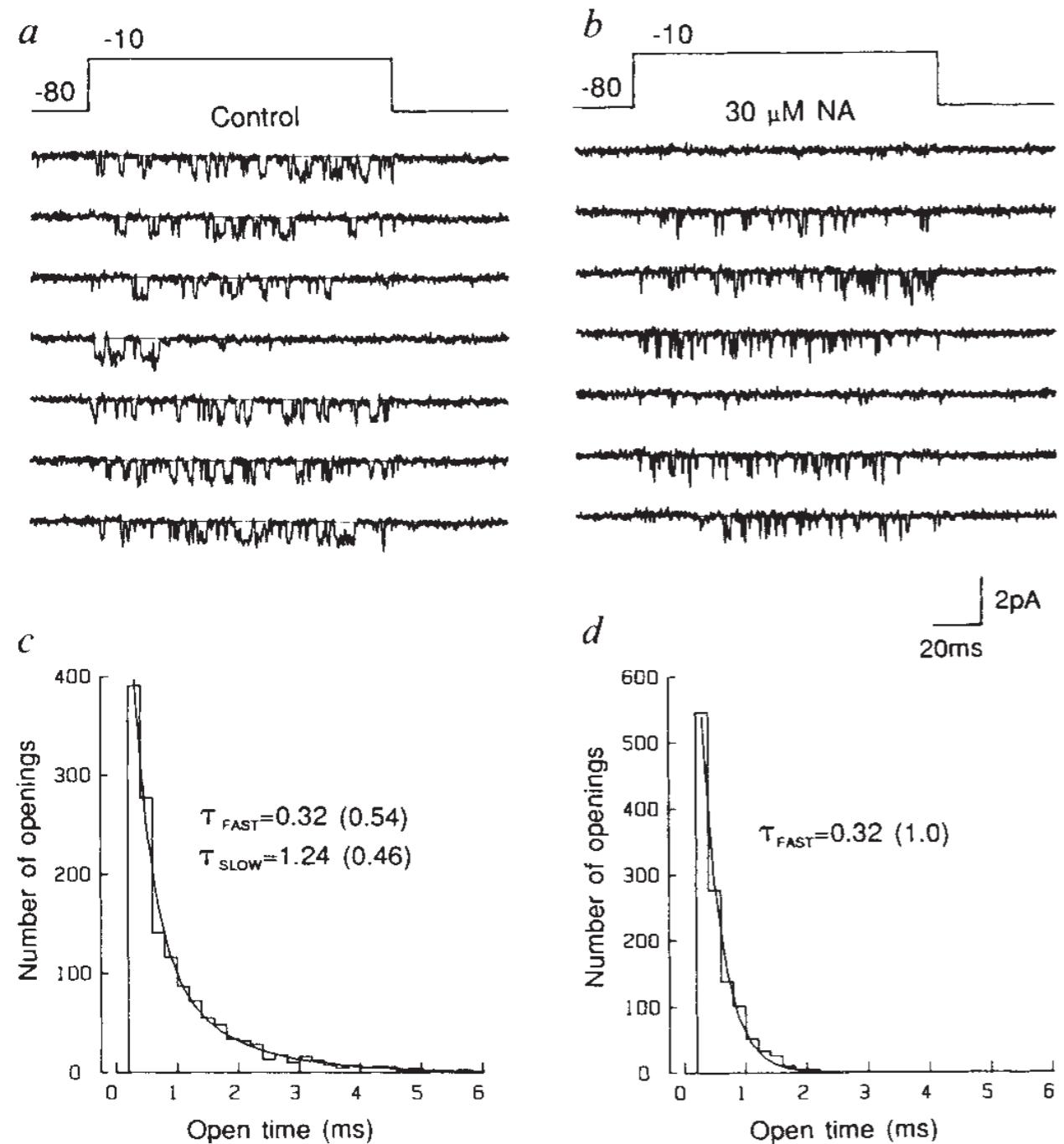


FIG. 4 Effect of NA on the gating of unitary N-type  $\text{Ca}^{2+}$  channels. **a, b**, Sequential sweeps of N-type  $\text{Ca}^{2+}$  channel currents recorded in two cell-attached patches, in the absence of NA (**a**) and with 30  $\mu\text{M}$  NA present in the pipette solution. (**b**). Noradrenaline altered gating kinetics, but did not change unitary current size: control,  $0.92 \pm 0.02 \text{ pA}$  ( $n=22$ ); 30  $\mu\text{M}$  NA,  $0.95 \pm 0.05 \text{ pA}$  ( $n=8$ ); 100  $\mu\text{M}$  NA,  $1.0 \pm 0.02 \text{ pA}$  ( $n=5$ ). **c, d**, Histograms of open time durations measured from the patches illustrated in **a, b**. Opening and closing events were detected as crossings of a threshold set halfway between open and closed levels. The distribution of open times in the absence of NA (**c**) was fitted by least-squares with the sum of two exponential components of the form  $B \exp(-t/\tau)$  with time constants and relative area [ $B \cdot \tau / \Sigma(B \cdot \tau)$ ] as indicated. In the presence of 30  $\mu\text{M}$  NA, the slow component was essentially absent (**d**), consistent with the diminished appearance of long openings in the current records (**b**). In other experiments, single channel analysis was performed on records with little or no overlap of unitary currents, like those illustrated in **a, b**. The slow component was too small to measure in 6 out of 12 patches with 30–100  $\mu\text{M}$  NA. In the other 6 patches, a slow component remained detectable, but it decayed more rapidly and was smaller in amplitude and relative area.

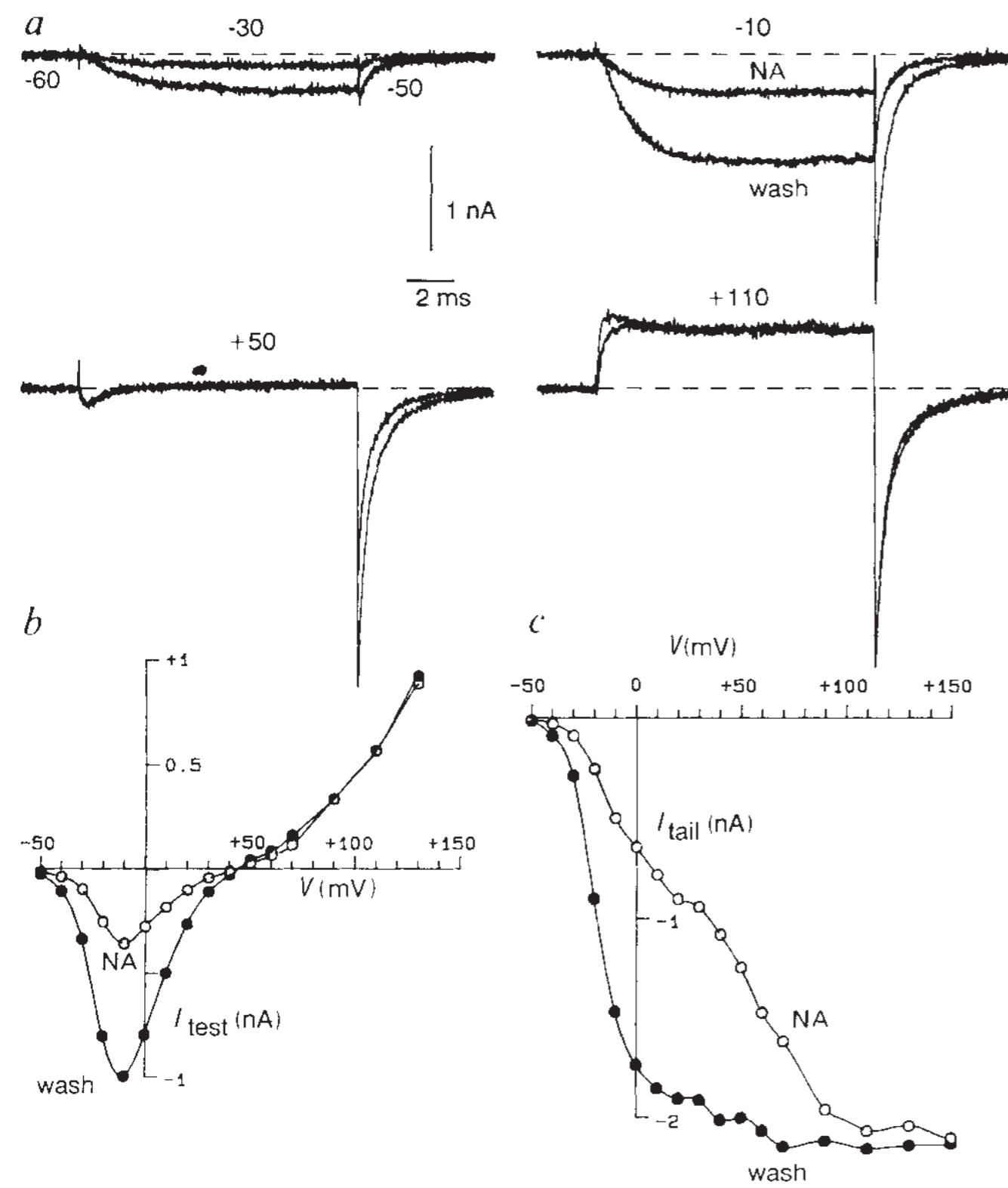
# **Neurotransmitter inhibition of neuronal calcium currents by changes in channel voltage dependence**

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THE voltage-dependent calcium current of many neurons is depressed by transmitters such as noradrenaline<sup>1–3</sup>, GABA<sup>4–6</sup>, and kappa-opiate agonists<sup>7–12</sup>. This modulation probably constitutes a major mechanism of presynaptic inhibition<sup>1,8,12</sup>. Although recent work has implicated GTP-binding proteins in the mechanism of current inhibition<sup>13–19</sup>, it is still unknown how the activation of those proteins alters the operation of the channels. In their initial description of the phenomenon<sup>1</sup>, Dunlap and Fischbach proposed that noradrenaline acts by somehow reducing the number of functional calcium channels in the cell. By contrast with this hypothesis, I have found that inhibition of  $\text{Ca}^{2+}$  current is primarily due to a transmitter-induced change in the voltage-dependence with which channels are opened. Transmitters profoundly alter the voltage-dependence of channel activation, but there is little or no change in the number of functional channels activated by very large depolarizations. There is also little effect on the voltage-dependence of inactivation.



**FIG. 1** Noradrenaline (NA) inhibition of  $\text{Ca}^{2+}$ -channel currents in a bullfrog neuron. **a**,  $\text{Ca}^{2+}$ -channel current at various potentials in a bullfrog DRG neuron in the presence and absence of  $30 \mu\text{M}$  NA. After completing the series the cell was transferred to a solution containing  $100 \mu\text{M}$   $\text{La}^{3+}$ ; the currents remaining in  $\text{La}^{3+}$  (which were small) were subtracted to correct for non- $\text{Ca}^{2+}$ -channel currents. Currents in NA were recorded 40–80 s after application and those labelled ‘wash’ were recorded 80–120 s after washing out the NA. The current at  $-10 \text{ mV}$  declined from a control value of  $1,165 \text{ pA}$  to  $359 \text{ pA}$  in NA and was restored to  $998 \text{ pA}$  after washing; currents in NA are compared to those after wash-out to restrict the comparison to the reversible effects of NA. **b**, Current during test pulse, averaged over the last 1 ms, as a function of voltage. **c**, Tail current, averaged over a  $160\text{-}\mu\text{s}$  interval beginning  $160 \text{ }\mu\text{s}$  after the end of the test pulse, as a function of the test voltage. Cell E72C; compensation for 1.2 of  $1.6\text{-MOhm}$  series resistance.

**METHODS.** Dorsal root ganglia from adult bullfrogs were incubated at  $35^\circ\text{C}$  for 45 min in  $\text{Ca}^{2+}$ -free Ringer’s solution (in mM:  $100 \text{ NaCl}$ ;  $2.5 \text{ KCl}$ ;  $5 \text{ MgCl}_2$ ;  $10 \text{ glucose}$ ;  $10 \text{ HEPES}$ , pH adjusted to 7.4 with NaOH) containing  $1 \text{ mg/ml}^{-1}$  collagenase (Sigma Type 1) and  $5 \text{ mg/ml}^{-1}$  Dispase (Boehringer-Mannheim), then at  $22^\circ\text{C}$  for 45 min with Dispase alone. Single cells were released by trituration, stored at  $5^\circ\text{C}$  in Tyrode’s solution (in mM:  $150 \text{ NaCl}$ ;  $4 \text{ KCl}$ ;  $2 \text{ CaCl}_2$ ;  $2 \text{ MgCl}_2$ ;  $10 \text{ glucose}$ ;  $10 \text{ HEPES}$ , pH adjusted to 7.4 with NaOH), and used within 24 h. Cells were voltage-clamped at  $22^\circ\text{C}$  using patch pipettes<sup>32</sup> with resistances of  $0.3\text{--}2 \text{ M}\Omega$ . Internal solution (in mM):  $126 \text{ Cs}^+$  glutamate;  $9 \text{ EGTA}$ ;  $4.5 \text{ MgCl}_2$ ;  $9 \text{ HEPES}$ ;  $3.6 \text{ MgATP}$ ;  $14 \text{ creatine phosphate}$  (Tris salt);  $1 \text{ GTP}$  ( $\text{Na}^+$  salt);  $50 \text{ U ml}^{-1}$  creatine phosphokinase; pH adjusted to 7.4 with CsOH. External solution (in mM):  $3 \text{ BaCl}_2$ ;  $154 \text{ TEA chloride}$ ;  $10 \text{ HEPES}$ ; pH adjusted to 7.4 with TEA hydroxide.

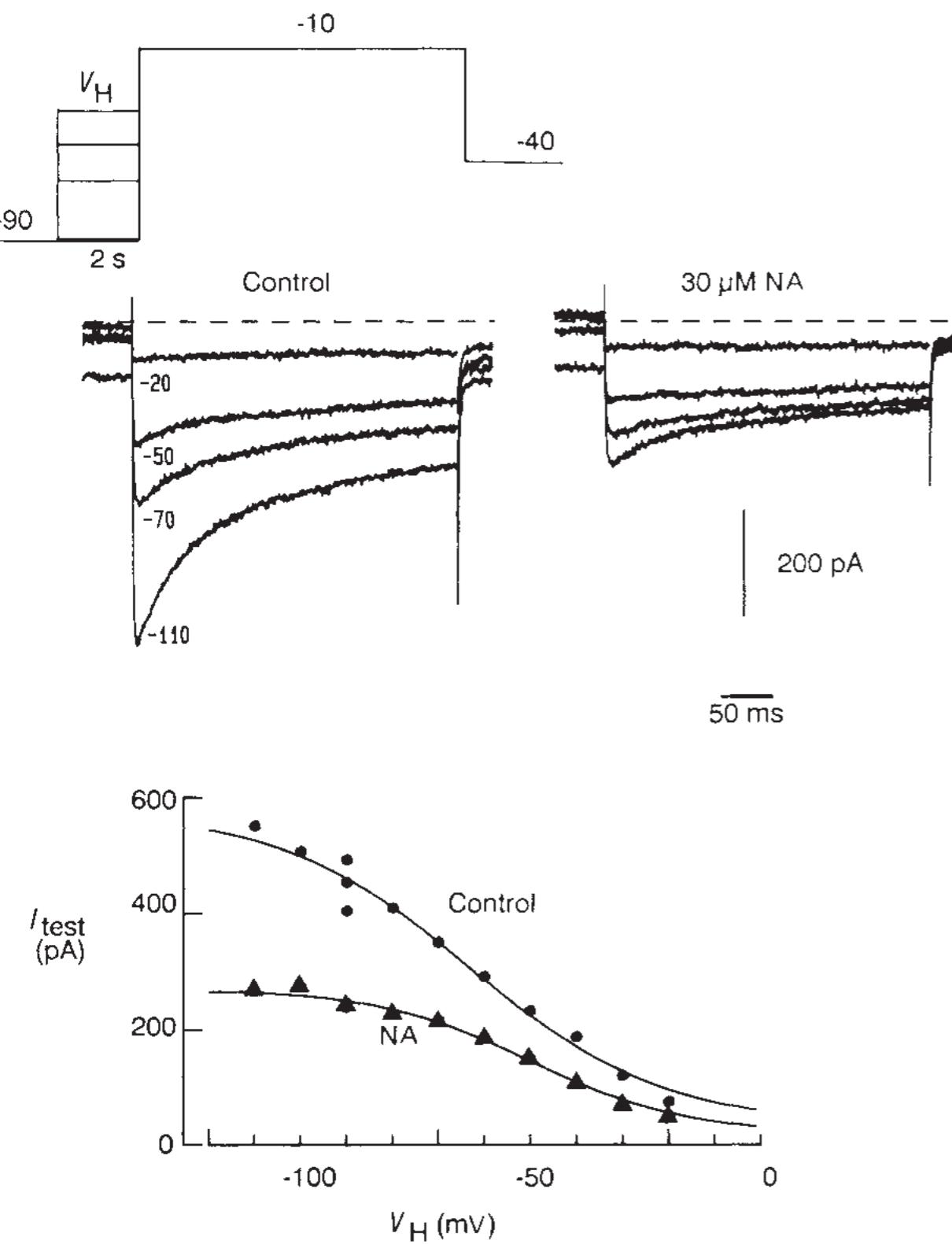


FIG. 2 Effect of NA on  $\text{Ca}^{2+}$ -channel inactivation in a bullfrog DRG neuron. A test pulse to  $-10$  mV was given after different holding potentials were maintained for 2 s; the currents shown have been corrected for leak and capacitative currents obtained from a step from  $-90$  to  $-98$  mV. Top right: currents after  $30 \mu\text{M}$  NA was applied for 0.5–2.5 min. Top left: currents recorded 1–3 min after NA was washed out. Bottom: peak test-pulse current versus holding potential. Solid curves are drawn according to  $544/(1 + \exp((V_H - (-63))/21)) + 33$  for control points (circles) and  $253/(1 + \exp((V_H - (-50))/17)) + 18$  for NA points (triangles). Conditions as in Fig. 1; cell E95C. Inactivation determined with this voltage protocol and these experimental conditions (internal EGTA,  $\text{Ba}^{2+}$  as charge carrier) is probably limited to voltage-dependent processes; possible effects of NA on current-dependent inactivation<sup>22</sup> might not be evident.

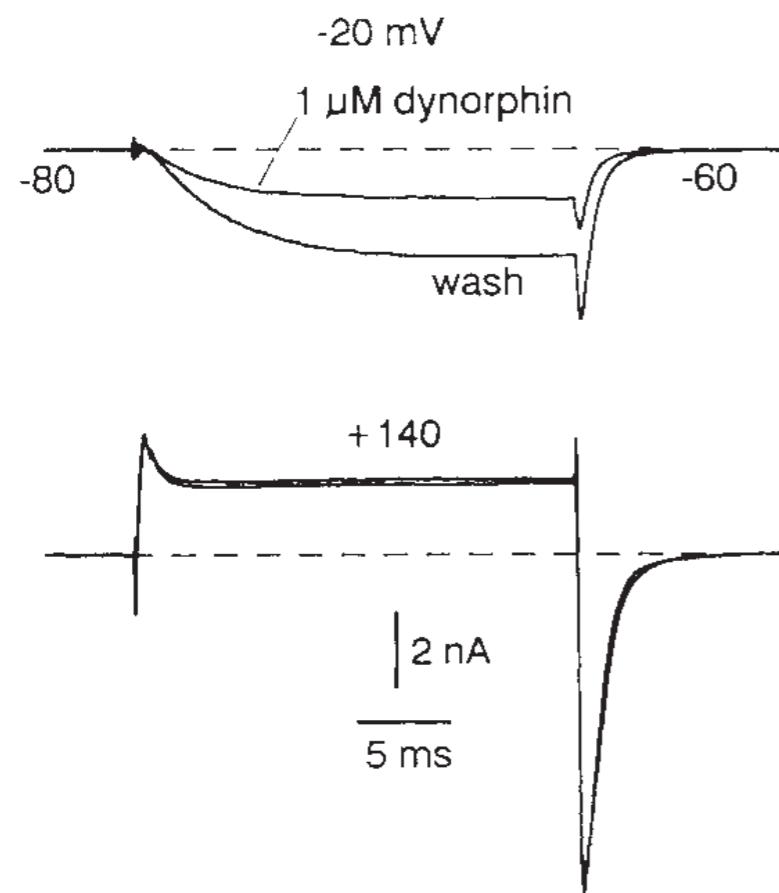
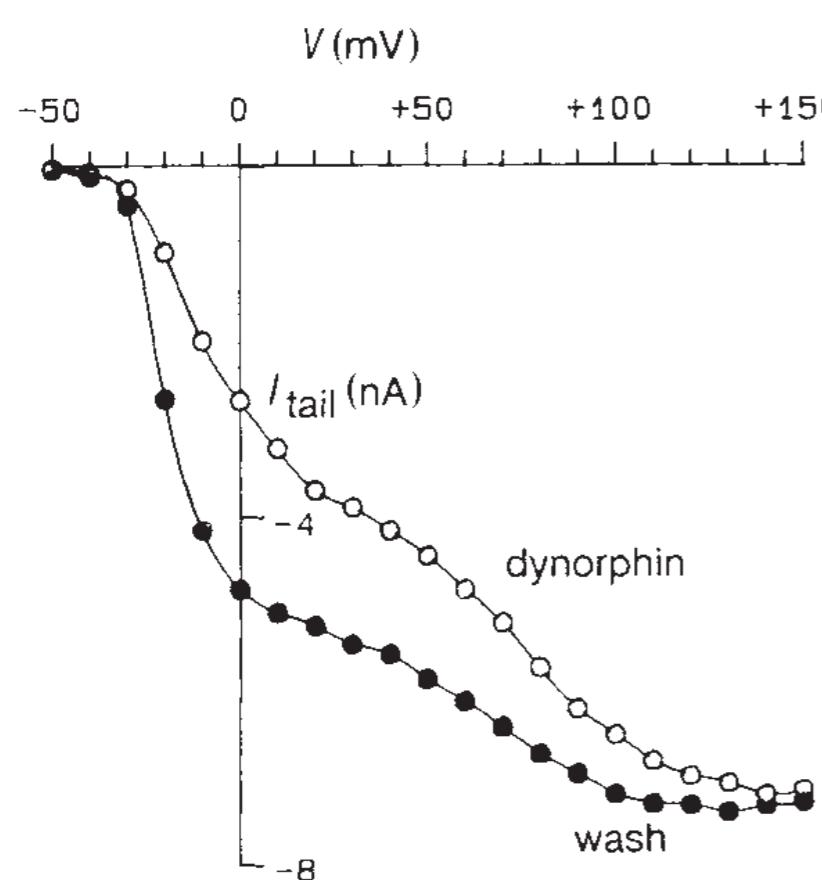
*a**b*

FIG. 3 Dynorphin A inhibition of  $\text{Ca}^{2+}$ -channel currents in a rat DRG neuron.  
*a*, As in Fig. 1, currents in dynorphin are compared with those after washout to focus on the reversible effect of dynorphin (there was a 20% decline in currents at all potentials between control and wash-out runs). *b*, Tail current, averaged over 400  $\mu\text{s}$  beginning 680  $\mu\text{s}$  after the end of the test pulse, versus test voltage. Cell W51D. Compensation for 2.9 of 4.2-MOhm series resistance; 22 °C.

METHODS. The isolation technique was as for frog DRG neurons (Fig. 1 legend) except that the enzyme solution contained 1.5 mg/ml  $^{-1}$  collagenase and 3 mg/ml  $^{-1}$  Dispase in  $\text{Ca}^{2+}$  free Tyrode's solution. Internal solution (in mM): 115 CsCl; 5 MgATP; 5 creatine phosphate (Tris salt); 0.3 GTP (Tris salt); 10 BAPTA; and 10 HEPES; pH adjusted to 7.4 with CsOH. External solution (in mM): 3  $\text{BaCl}_2$ ; 160 TEA chloride; 10 HEPES; 3  $\mu\text{M}$  tetrodotoxin; pH adjusted to 7.4 with TEA hydroxide.

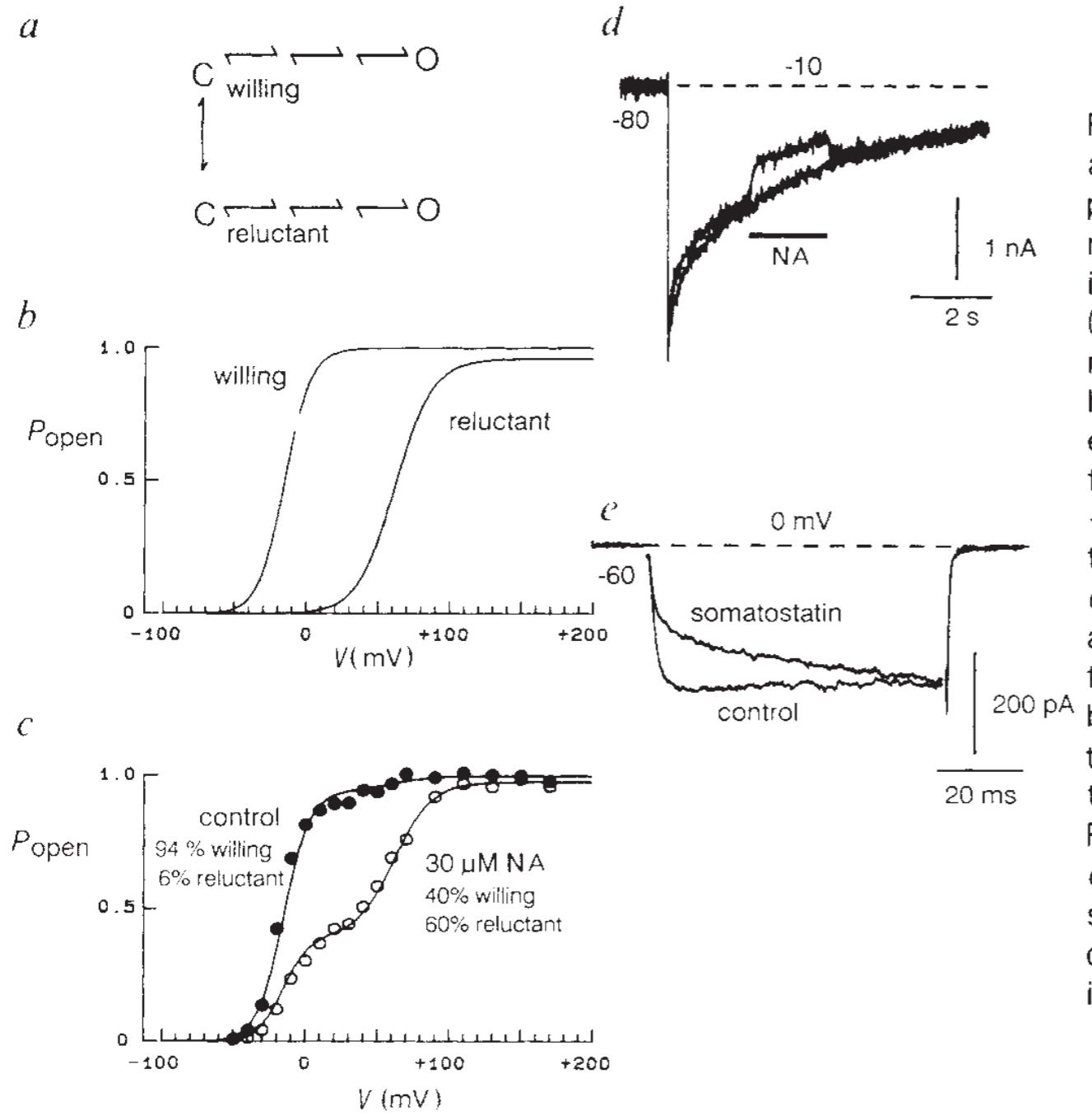


FIG. 4 A model for transmitter control of neuronal  $\text{Ca}^{2+}$  channels. **a**, It is assumed that channels exist in two modes, in equilibrium. **b**,  $P_{\text{open}}$  is the probability of a channel being open during a step to a given voltage. In one mode (willing), channels are opened readily by small depolarizations, whereas in the other (reluctant), channels require much larger depolarizations to open (and have a somewhat less steep voltage dependence and a slightly smaller maximal probability of being open). In each mode channels are assumed to have an activation curve given by a Boltzmann curve of the form  $P_{\text{max}}/(1 + \exp - (V - V_{0.5})/k)$ , where  $P_{\text{max}}$  is the maximal probability of being open (1 for willing channels, 0.94 for reluctant channels);  $V_{0.5}$ , the midpoint, is  $-15\text{ mV}$  for willing channels and  $+62\text{ mV}$  for reluctant channels; and  $k$  is the slope factor (9 mV for willing channels, 13 mV for reluctant channels). **c**, With these parameters, the scheme accurately predicts the change in activation curve produced by  $30\text{ }\mu\text{M}$  NA in a bullfrog sensory neuron (data from Fig. 1). **d**, Rapid kinetics of NA inhibition of  $\text{Ca}^{2+}$ -channel current in a bullfrog DRG neuron. Two successive currents elicited by long depolarizations to  $-10\text{ mV}$  are shown; during the second, the cell was transferred for 1.8 s to an external solution containing  $30\text{ }\mu\text{M}$  NA. Methods and solutions as in Fig. 1 except the external solution contained  $10\text{ mM}$  rather than  $3\text{ mM}$   $\text{Ba}^{2+}$ . **e**, Slow activation phase of  $\text{Ca}^{2+}$ -channel current after application of somatostatin ( $1\text{ }\mu\text{M}$ ) to a freshly dissociated spinal-cord neuron. Recording conditions as in Fig. 1 except that methanesulphonate replaced glutamate in the internal solution.