# Reversal Potential for Noradrenaline-Induced Hyperpolarization of Spinal Motoneurons

Abstract. *By using two separate electrodes with tips inside a single feline motoneuron, current-voltage characteristics were studied during extracellular iontophoresis of noradrenaline. The usually observed hyperpolarization was accompanied by an increase in membrane resistance and became larger with polarizing and smaller with depolarizing currents. During large depolarizing current injections, the noradrenaline-induced potential reversed its direction, usually at a membrane potential of about −20 millivolts. These data are compatible with the concept that noradrenaline hyperpolarizes nerve cells by decreasing resting membrane conductances to sodium and potassium ions. The observation could also be explained by a nonspecific decrease in ion permeability that is associated with a hyperpolarization due to sodium pump activation.*

Noradrenaline (NA) appears to be an important chemical transmitter in the mammalian central nervous system, and noradrenergic neurons of the locus coerulus have been associated with diverse behavioral functions such as sleep, stress, and several activities controlled by the autonomic nervous system (*1*). In spite of intensive study, however, the mechanisms of the effects of NA on membrane excitability have not yet been clarified. Contributions toward an understanding of these mechanisms have come particularly from studies in the spinal cord and the cerebellar cortex, where iontophoretically applied NA depresses neuronal activity.

Intracellular recordings from these sites show that both spinal motoneurons (*2*) and Purkinje cells (*3*) respond to iontophoretic applications of NA with a hyperpolarization having a relatively slow time course. Investigations in which membrane input resistance has been measured during NA application have shown that resistance increases can be recorded during the hyperpolarizations. Such changes have been observed in spinal motoneurons (*4*), Purkinje cells (*3*), pyramidal cells of the hippocampus (*5*), and cultured brain cells of unknown regional origin (*6*).

Decreases in membrane conductance (that is, resistance increases) have been proposed as a mechanism of transmitter action (*7*) and could be responsible for the hyperpolarization observed during NA application. If this is the case, and if the potential change is generated by a conductance decrease to one or a combination of the monovalent ions usually associated with synaptic effects ($Na^+$, $K^+$, and $Cl^-$), then a reversal potential should exist and should be within the extremes of the equilibrium potentials for the individual ions (*8*).

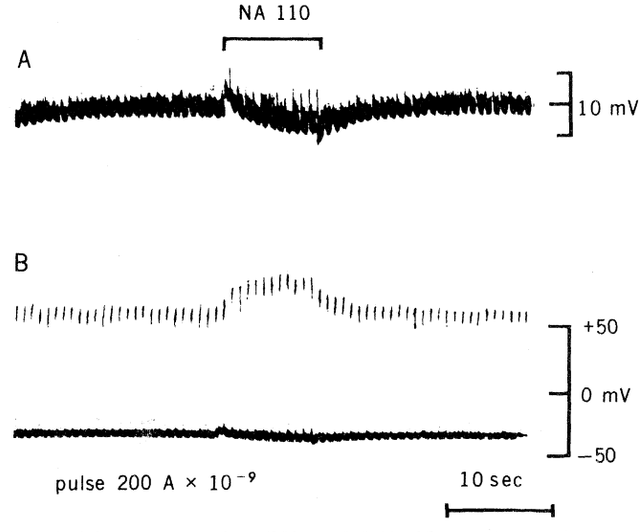


Fig. 1. (A) High-gain intracellular recording of membrane potential demonstrating the hyperpolarizing response to the iontophoretic application of NA ($110 \times 10^{-9}$ A) during the time indicated by the bar. At the onset of the NA ejection current there is a positive displacement of the recording trace due to coupling between the iontophoretic and the recording electrode. This voltage shift is reversed when the current is shut off. (B) The lower trace is identical to that shown in (A) but at lower gain. Depolarizing current pulses ($200 \times 10^{-9}$ A) 100 msec in duration were injected through the second electrode at a frequency of one per second to illustrate the resistance change that accompanied the hyperpolarization. Although the rising and falling phases of the pulses are too fast to be registered by the recorder, the plateau phases are observed as the discontinuous upper trace. The change in amplitude of the pulses during the NA application indicates a resistance increase of about 25 percent. The envelope formed by the pulses shows the shape of the reversed NA-induced potential change if the membrane potential were displaced to about +60 mV. Comparison of this trace with that of (A) shows the coincident time courses of the hyperpolarization (A) and the resistance change.

Hyperpolarizations of excitable cells that may be generated by transmitter-induced ionic conductance decreases have been reported in frog sympathetic ganglia (*9*), *Aplysia* buccal neurons (*10*), mammalian cortical neurons (*11*), and crustacean heart cells (*12*) and reversal potentials have been demonstrated in three cases. For the 5-hydroxytryptamine (5-HT)–induced hyperpolarization in *Aplysia* (*10*), the reversal level was found to be usually −20 to −30 mV, while close arterial injections of 5-HT in cat cerebral cortex gave a hyperpolarization of Betz cells having a reversal potential of about −30 mV (*11*). For the nerve-induced hyperpolarizing potential in crustacean heart, it was about −50 mV (*12*).

Reversal potentials for the other examples of hyperpolarization with conductance decrease have not been observed, but this may be because of the several technical difficulties in making such measurements. When the reversal potential lies in the depolarizing direction, large currents are usually required to displace the membrane potential sufficiently, because of the large conductance increases (delayed rectification) that result from the depolarizations. A related difficulty is that the relatively small electrodes usable for intracellular recordings from most vertebrate cells may not be capable of passing such large currents. Even when this is possible, the electrical noise generated often obscures the desired membrane potential changes, if they are being recorded from the same electrode as is used to inject the current.

To achieve reliable potential measurements during the injection of large currents, we impaled single spinal motoneurons with two independent electrodes (*13*), a technique that has permitted us to study current-voltage relations during the application of NA and to identify a reversal potential (*14*). One of the electrodes was attached to and extended about 50 $\mu$m beyond the tip of a multibarreled glass electrode assembly that was used for the iontophoretic application of NA (*15*). Hind-leg nerves of cats were dissected, and a lumbosacral laminectomy was performed under ether anesthesia. Before discontinuation of the ether, an anemic decerebration (*16*) and spinal transection at the thoracic level were carried out. The animal was subsequently paralyzed by using gallamine triethiodide and artificially respired.


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Spinal motoneurons were first impaled by the assembly-bearing microelectrode, identified by antidromic activation from a dissected ventral root nerve, and tested for responsiveness to NA. Cells showing a response to NA and having a resting membrane potential of at least −50 mV were then impaled with the second electrode. Membrane potential measured by one electrode and changes induced by drug application or by current injection through the second electrode were continuously monitored on a Mingograph ink recorder (*17*).

On some occasions, we used steady currents of constant value to displace membrane potential while potential changes resulting from NA applications were recorded. These were then compared with the NA responses during injection of larger or smaller currents. In general, however, measurements were easier and neuron viability was better when 0.1-second current pulses were used, at a repetition rate of about one per second. Figure 1 shows a record from such a test, using large depolarizing pulses. A particular advantage of this method is the clear demonstration of the correspondence of the time courses of the potential change and the increase in membrane resistance demonstrated by the changing pulse amplitudes. In fact, the envelope of the pulse potentials indicates the shape of the reversed NA-induced potential.

For each cell, we used current pulses of several different amplitudes so that current-voltage plots could be made for the identification of the reversal potential. Figure 2 shows two plots obtained in this manner, indicating reversal potentials of about −5 and −20 mV. Reversal potentials obtained for 11 neurons ranged from −5 to −30 mV, the usual value being close to −20 mV. Resting membrane potentials between −35 and −55 mV were observed in these neurons at the time of identification of reversal potential levels. The NA hyperpolarizations at the resting membrane potential were usually 5 to 10 mV in amplitude and were most often accompanied by 10 to 20 percent increases in membrane resistance.

In some cases, we were unable to demonstrate a reversal of the NA hyperpolarization. At least part of this difficulty is attributable to the delayed rectification observed during passage of depolarizing currents. In some cases, the large currents necessary could not be passed by the electrode. Another important contributing factor may be that the shunting effect of the delayed rectification, plus the reduced size of the NA-induced potential in the region of the equilibrium potential, renders the detection of the reversal particularly difficult.

In most cells, monosynaptic excitatory postsynaptic potentials (EPSP's) were evoked by stimulation of leg nerves. In no case did the initial phase of the EPSP reverse at the reversal potential of the NA hyperpolarization. This initial phase has, however, been found to reverse at membrane potentials of 0 to +10 mV (*18*). Our attempts to define ionic mechanisms for the NA effects have been restricted to changes in chloride equilibrium potentials. Intracellular injections of $Cl^-$ that reversed inhibitory, postsynaptic potentials (IPSP's) evoked by nerve stimulation caused little or no change in the amplitude or time course of the NA hyperpolarization. Similar observations have been made in studies of NA hyperpolarizations of Purkinje cells (*3*).

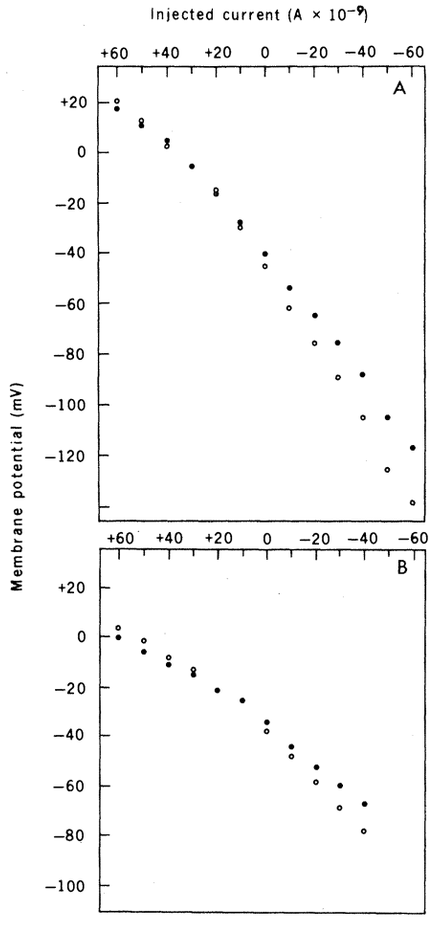


Fig. 2. Transmembrane voltage-current plots of two different motor neurons. (●) Potential of the cell at resting level (no current) or during intracellular injection of current. (○) Comparable potentials during iontophoretic application of NA to the cell. Values have been corrected for any electrical coupling between the current-passing and the recording electrode. (A) Reversal potential appears in the range of 0 to −10 mV. (B) Reversal potential is about −20 to −25 mV.

The characteristics of the NA hyperpolarization are similar to those of the 5-HT hyperpolarization in *Aplysia* (*10*) and the hyperpolarizing potential in crustacean heart (*12*). In both of these examples, a decrease in $Na^+$ and $K^+$ conductance was the mechanism proposed, on the basis of experiments in which the extracellular ionic environment was altered. Although reversal potentials were not observed in studies of NA hyperpolarizations of Purkinje cells, the pattern of increased amplitude of the hyperpolarization during increased polarization of the membrane by injected currents was seen (*3*). Similarly, the hyperpolarizing response to acetylcholine and the slow IPSP of frog sympathetic ganglion cells decrease with depolarizing and increase with hyperpolarizing currents (*9*), although a reversal has not been demonstrated, perhaps for the technical reasons cited earlier. The mechanism suggested for these latter potentials is a decreased conductance to $Na^+$, but no clear reason for the elimination of $K^+$ involvement is apparent. We feel, because of the value of the observed reversal potentials, that the NA hyperpolarization is more likely to be related to a $Na^+$ and $K^+$ conductance decrease. A more positive value would have suggested a selective effect on $Na^+$ conductance.

The relationship of the hyperpolarizations by iontophoretically applied NA to the effects of synaptically released NA is not clear from our studies; in fact, it is disturbing that similar responses are observed during iontophoretic application to spinal motoneurons of many amines (*19*) and also of calcium ion (*20*) or hydrogen ion (*21*). However, it has been observed that stimulation of the locus coeruleus, the source of a noradrenergic pathway, causes depression of cells in the hippocampus (*22*) and Purkinje cells (*23*). In the latter case, a close parallelism of the effects of locus coerulus stimulation and NA application was observed, including hyperpolarization and increased membrane resistance, indicating similar actions of iontophoretically and synaptically released NA. The interesting possibility that the NA-induced hyperpolarization and conductance change are mediated by adenosine 3',5'-monophosphate (*24*) is neither supported nor negated by our experiments.

The simplest explanation of our observations is that NA depresses neuronal activity by a hyperpolarization that is

generated by a decrease in resting $Na^+$ and $K^+$ conductance.

Alternative explanations are possible that are consistent with the demonstration of a reversal potential for the hyperpolarization. The NA effect could be to reduce the ongoing release of depolarizing transmitter substance (*25*). We have not, however, observed decreases in synaptically evoked potentials during NA application, except those explicable by the NA-induced hyperpolarization, so that such an effect seems unlikely. A third possibility is the generation of two effects by the NA, a hyperpolarization (for instance, by activation of an ion transport system) and a general decrease in membrane conductance. Such a combination would exhibit an apparent reversal potential during the intracellular injection of current. The value of this reversal level would occur when the product of the injected current and the change in cell input resistance is equal to the amplitude of the hyperpolarization.

The third possibility would be compatible with recent proposals that catecholamines may hyperpolarize frog sympathetic ganglion neurons (*26*), mammalian central neurons (*27*), or striated muscle (*28*) by stimulation of a $Na^+$-$K^+$ pump. In addition, association of pump activation with decreases in membrane conductance has been observed in striated muscle (*29*).

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### References and Notes

1. D. G. Amaral and H. M. Sinnamon, *Prog. Neurobiol.* **9**, 147 (1977).
2. J. W. Phillis, A. K. Tebecis, D. H. York, *Eur. J. Pharmacol.* **4**, 471 (1968).
3. G. R. Siggins, A. P. Oliver, B. J. Hoffer, F. E. Bloom, *Science* **171**, 192 (1971).
4. I. Engberg and K. C. Marshall, *Acta Physiol. Scand.* **83**, 142 (1971).
5. A. P. Oliver and M. Segal, *Soc. Neurosci. 4th Annu. Meet. Abstr.* (1974), p. 361.
6. L. Bonkowski and W. F. Dryden, *Neuropharmacology* **16**, 89 (1977).
7. F. F. Weight, in *The Neurosciences, Third Study Program*, F. O. Schmitt and F. G. Worden, Eds. (MIT Press, Cambridge, Mass., 1974), p. 929.
8. J. E. Brown, K. J. Mullen, G. Murray, *Science* **174**, 318 (1971).
9. F. F. Weight and A. Padjen, *Brain Res.* **55**, 219 (1973); *ibid.*, p. 225.
10. H. M. Gerschenfeld and D. Paupardin-Tritsch, *J. Physiol. (London)* **243**, 427 (1974); *ibid.*, p. 457.
11. C. C. Huang and A. S. Marrazzi, *Soc. Neurosci. 3rd Annu. Meet. Abstr.* (1973), p. 292.
12. J. C. Delaleu, *J. Exp. Biol.* **65**, 117 (1976).
13. I. Engberg, Y. Källström, K. C. Marshall, *Acta Physiol. Scand.* **84**, 4A (1972). The electrode attached to the multibarrelled assembly was first driven into the gray matter of the cord. After a stable penetration, the prealigned single electrode was lowered into the same region. With cells that were less than 1.5 mm below the (lateral) cord surface, penetration of the same cell was usually achieved by the second electrode.
14. A preliminary report of these findings has been given in I. Engberg and K. C. Marshall, *J. Gen. Physiol.* **61**, 261 (1973).
15. The NA was passed by using a positive current, from a 0.5*M* solution, *p*H 5. Current controls were either in the form of balancing opposite currents from another electrode of the assembly or of comparing NA effects with responses to equivalent or higher sodium currents passed from another electrode. Intracellular electrodes were usually filled with KCl, sometimes with citrate.
16. P. E. Voorhoeve, *Acta Physiol. Pharmacol. Neerl.* **9**, 1 (1960).
17. Electrical coupling between the screened intracellular current-passing and recording electrodes was often so small that it could be neglected. In other cases, the coupling was measurable by oscilloscopic examination of the rising and falling phases of short current pulses. The amplitude of the coupling was a linear function of the current amplitude, and was easily corrected by subtracting the appropriate value from the apparent potential displacement resulting from a given current pulse.
18. K. C. Marshall and I. Engberg, *Can. Physiol.* **4**, 188 (1973); I. Engberg and K. C. Marshall, *Neuroscience*, in press.
19. I. Engberg, J. A. Flatman, K. Kadzielawa, *Acta Physiol. Scand.* **96**, 137 (1976).
20. ———, *ibid.* **91**, 3A (1974).
21. K. C. Marshall and I. Engberg, *Proc. Can. Fed. Biol. Soc.* **16**, 33 (1973).
22. M. Segal and F. E. Bloom, *Brain Res.* **72**, 99 (1974).
23. G. R. Siggins, B. J. Hoffer, A. P. Oliver, F. E. Bloom, *Nature (London)* **233**, 482 (1971); B. J. Hoffer, G. R. Siggins, A. P. Oliver, F. E. Bloom, *J. Pharmacol. Exp. Ther.* **184**, 553 (1973).
24. B. J. Hoffer, G. R. Siggins, A. P. Oliver, F. E. Bloom, *Ann. N.Y. Acad. Sci.* **185**, 531 (1971).
25. H. M. Gerschenfeld, in *International Cell Biology, 1976-1977*, B. R. Brinkley and K. R. Porter, Eds. (Rockefeller Univ. Press, New York, 1977), p. 93.
26. K. Koketsu and M. Nakamura, *Jpn. J. Physiol.* **26**, 63 (1976).
27. J. W. Phillis, *Life Sci.* **15**, 213 (1974); G. G. Yarbrough, *Neuropharmacology* **15**, 335 (1976); U. Heinemann, H. D. Lux, K. J. Zander, in *Iontophoresis and Transmitter Mechanisms in the Mammalian Central Nervous System*, R. W. Ryall and J. S. Kelly, Eds. (Elsevier/North-Holland, Amsterdam, 1978), p. 419.
28. E. T. Hays, T. M. Dwyer, P. Horowicz, J. G. Swift, *Am. J. Physiol.* **227**, 1340 (1974); B. H. Bressler, J. W. Phillis, W. Kozachuk, *Eur. J. Pharmacol.* **33**, 201 (1975).
29. T. Clausen and J. A. Flatman, *J. Physiol. (London)* **270**, 383 (1977); D. Geduldig, *ibid.* **194**, 521 (1968).
30. We thank Hegström for technical assistance and G. W. Mainwood for critically reviewing the manuscript. The experiments described here were conducted at the Physiology Institute, University of Göteborg, Göteborg, Sweden.

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## Dual Mechanism Mediating Opiate Effects?

Jacquet has proposed (*1*) that morphine acts on two different receptors in the brain: a naloxone-sensitive endorphin receptor that mediates the analgesic and catatonic effects of morphine, and a naloxone-insensitive adrenocorticotropic hormone (ACTH) receptor that mediates the opiate abstinence syndrome and the excitatory effect of the drug. We see value in some aspects of this formulation but take exception to others.

Jacquet reports that the opioid peptides β-endorphin and Met-enkephalin (*2*) fail to mimic the behavioral excitation produced by morphine or ACTH. These peptides, according to Jacquet, should not produce opiate-like dependence. Earlier findings indicate that repeated injections of endorphins into the periaqueductal gray (PAG) result in physical dependence as manifested by the occurrence of withdrawal symptoms following naloxone injections or cessation of endorphin administration (*3*). Jacquet has attributed these findings to PAG damage caused by the large cannula (*4*) used to deliver endorphin. Indeed, we (*5*) observed explosive motor behavior (EMB) immediately after lesions to the PAG. In Wei and Loh's study (*3*), however, infusion of water to the PAG through identical large cannulas did not trigger EMB. Thus, the withdrawal signs observed by Wei and Loh (*3*) may be attributed to endorphins and not to procedural artifacts. Furthermore, the opioids levorphanol and etonitazene produce physical dependence in animals and in humans (*6*), but fail to precipitate EMB when injected intracerebrally in naloxone-treated or -naïve animals (*7*). These findings demonstrate that the ability of opioids to produce physical dependence is not conditional upon their ability to elicit behavioral excitation (that is, EMB).

We question the use of the label "ACTH receptor" to describe the site at which ACTH and opiates are producing EMB. Wei *et al.* (*8*) reported that intracerebral injections of thyrotrophin-releasing hormone in rats induce opiate withdrawal-like, wet-dog shakes. We observed (*9*) EMB after intraventricular injections of either lithium or various calcium chelators, the latter at molar doses below those effective in morphine EMB. Given that the class of substances capable of producing EMB may be fairly large, the designation ACTH receptor may be misleading. In fact, there is evidence that ACTH may act at the endorphin receptor. Some aspects of ACTH-induced behavioral excitation are modified by naloxone (*10*). ACTH has affinity for opiate receptors in vitro and it antagonizes morphine analgesia in animals (*11*). Thus, the excitatory effects of ACTH may not be independent of its action at the stereospecific endorphin receptor. While we agree that the mechanism subserving EMB may play a role in


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