nearly normal spectrum of fundamental periodicity of 178 Å, but with a somewhat reduced 300 reflection. Mammalian peripheral nerves are reported to have a fundamental periodicity near to 184 Å (*4*). The neutron diffraction pattern involved a remarkable suppression of the 300, 400, and 500 reflections of the human myelin x-ray pattern. The suppression of the third, fourth, and fifth orders of the x-ray diffraction pattern was confirmed by examination of deuterated rabbit sciatic nerves (Fig. 4).

The most direct interpretation of the observed neutron diffraction pattern is that the neutrons see a sinusoidal distribution of scattering material. This could arise if neutrons diffracted either from the protein portion only, or the lipid portion only, of the membrane. Alternatively, the third, fourth, and fifth reflections may be suppressed because they contain a large contribution of neutron scattering from hydrogen (which is 180° out of phase with the carbon scattering). However, it seems remarkable that the antiphase contribution would exactly equal the in-phase contribution. A detailed quantitative interpretation of the neutron and x-ray data is being prepared.

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Cyclic Adenosine Monophosphate: Possible Mediator for Norepinephrine Effects on Cerebellar Purkinje Cells

Abstract. *Microelectrophoretic application of norepinephrine or cyclic adenosine monophosphate reduces the discharge frequency of Purkinje cells in the rat cerebellum. In contrast, other nucleotides accelerate the discharge rate of most units. Parenterally administered theophylline, which inhibits the hydrolysis of cyclic adenosine monophosphate enhances the effects of norepinephrine and cyclic adenosine monophosphate. Therefore, norepinephrine may be able to regulate Purkinje cells functionally by metabolic stimulation of cyclic adenosine monophosphate synthesis.*

Despite the wealth of information about the neuronal connections to cerebellar cortex (*1*), the synaptic transmitters operating within this structure have not yet been defined. Histochemical (*2*) and biochemical (*3*) studies, confirming the presence of norepinephrine-containing nerve endings and high turnover rates of norepinephrine (NE) in the cerebellar cortex, prompted us to test the responsiveness of Purkinje cells to this drug when administered electrophoretically from 5-barrel micropipettes. We now report that almost all Purkinje cells exhibit reproducible reductions in spontaneous discharge rate in response to NE (*4*) and suggest that this response may be mediated by 3′,5′-adenosine monophosphate (cyclic-AMP).

Adult albino rats were decerebrated or anesthetized with chloral hydrate (350 mg/kg). Routine techniques of single-unit recording and microelectrophoretic drug administration were used (*5*); standard electrical methods prevented both polarization of the electrode tip during drug ejection and the undesirable diffusion of drugs from the pipette.

Spontaneously active nerve cells were identified as Purkinje cells on the basis of the so-called "inactivation potentials" or "climbing fiber responses" (*6*), namely, high-frequency (300 to 500/sec) bursts of two to five spikes, superimposed on a slow wave, seen in capacitance-coupled recordings. In our study, neurons exhibiting such bursts also showed a rapid irregular rate of single spike spontaneous discharge (usually 50 to 100 per second).

Nearly all (98 percent) of the 143 Purkinje cells studied responded to NE, by a reduction in their spontaneous discharge rate (*7*). Although the mean discharge rate decreased markedly during the administration of NE (Fig. 1A), Purkinje cell discharge tended to occur at the same preferred interspike intervals observed during the control period. This is reflected in the interspike interval histograms shown in Fig. 1, B and C. Furthermore, "climbing fiber" responses were rarely affected by microelectrophoresis of NE (Fig. 3). A characteristic feature of the response to NE was its slow onset and a persistence for many seconds after termination of the ejection current.

The question arises as to how NE reduces firing rate. In many peripheral sympathetically innervated tissues, the influence of NE may be mediated metabolically by cyclic-AMP. Norepinephrine is thought to increase the synthesis of this nucleotide by stimulating the activity of adenyl cyclase (*8*). This enzyme, which catalyzes the conversion of adenosine triphosphate (ATP) to cyclic-AMP, has a high specific activity within the cerebellum (*9*) and is still further activated by NE in this structure in vitro (*10*). Therefore, we tested the responsiveness of Purkinje cells to cyclic-AMP administered by microelectrophoresis. Cyclic-AMP (Fig. 1) also reduced mean discharge rates of Purkinje cells with the same minimum effects upon the most probable interspike interval and climbing fiber bursts as seen with NE. Of 59 Purkinje cells studied, 44 (75 percent) responded to cyclic-AMP application. Within this responsive group of cells, 73 percent (32 cells) exhibited a reduction in discharge rate and 18 percent (8 cells), an elevation. Nine percent of the responsive cells showed a biphasic or reversible type of response, consisting of a sequential acceleration and reduction of rate during or shortly after drug application, or of a complete reversal of the direction of response upon subsequent testing. In contrast to the delayed onset and termination of the response to NE, more rapid effects were observed with microelectrophoresis of cyclic-AMP. The unresponsiveness of 25 percent of the cells tested with cyclic-AMP may be accounted for by the presence of phosphodiesterase, a soluble enzyme that hydrolyzes cyclic-AMP, in the cerebellar cortex (*9*). Although the dibutyryl derivative of cyclic-AMP is generally more potent in peripheral tissues (*11*), we found no ob-

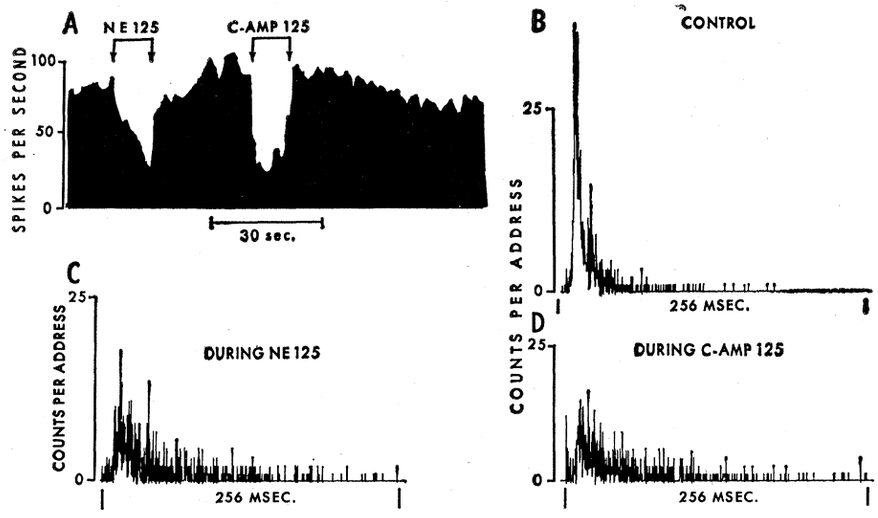


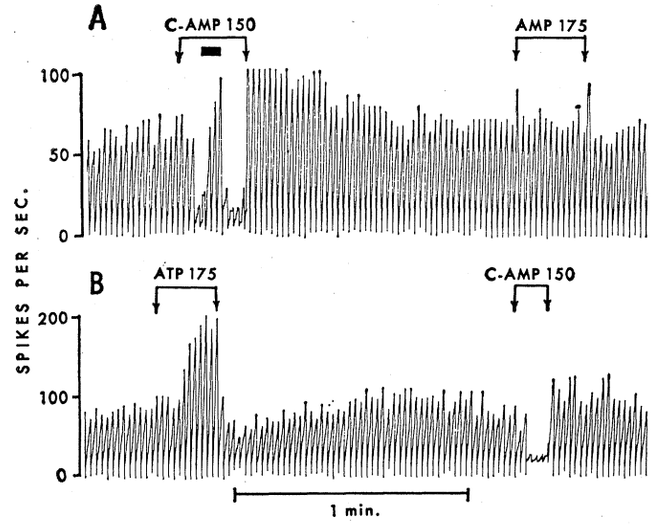
Fig. 1. Effects of microelectrophoretic application of NE and cyclic-AMP on spontaneous Purkinje cell discharge. (A) Effects of drug application on mean discharge frequency. Duration of drug application is indicated by arrows. Numbers after each drug indicate ejection current in nanoamperes. (B, C, and D) Interspike interval histograms of the same cell during the control period, and during application of NE and cyclic-AMP, respectively. Each histogram was computed from 2000 action potentials deposited in address intervals of 0.25 msec. The peak of each histogram indicates the most probable interspike interval of single spike discharge. Despite the large decrease in mean frequency, there is little change in the most probable interspike interval (15.4 msec).

vious differences between the effects of either cyclic-AMP when Purkinje cells were tested with both substances.

Because 18 percent of the cells showed elevated mean rates with cyclic-AMP, its effects were compared to those of two other adenine nucleotides, ATP and 5′adenosine monophosphate. Each of these nucleotides increased (Fig. 2) the spontaneous discharge rate of the cells tested (ATP, 83 percent; 5′-AMP, 100 percent). These results suggest that the few elevations in discharge rate with cyclic-AMP may be nonspecific effects, since the other adenine nucleotides examined reproduced the direction and rapidity of such responses (*12*). The biphasic or reversible responses to cyclic-AMP seen in 9 percent of the cells probably represent a combination of the specific reduction and nonspecific elevation of discharge rate.

If the response of Purkinje cells to NE were mediated through adenyl cyclase, inhibition of phosphodiesterase would be expected to potentiate the response to NE. The diverse pharmacological effects of theophylline are thought to be produced by just such an action (*13*). We found only slight potentiation of the effect of either cyclic-AMP or NE with concomitant microelectrophoretic administration of theophylline from another barrel of the same micropipette. Intravenous administration of theophylline (60 mg/kg), on the other hand, dramatically increased the magnitude and duration of the response to NE and cyclic-AMP (Fig. 3). The extremely low water solubility of theophylline is probably responsible for its minimum effects when administered by microelectrophoresis. There is a greater enhancement of NE and cyclic-AMP effects with microelectrophoretically applied aminophylline, which is more soluble than theophylline.

Further support for the hypothesis that cyclic-AMP mediates responses to NE is derived from a study of the effects of prostaglandins. Microelectrophoretic administration of the prosta-



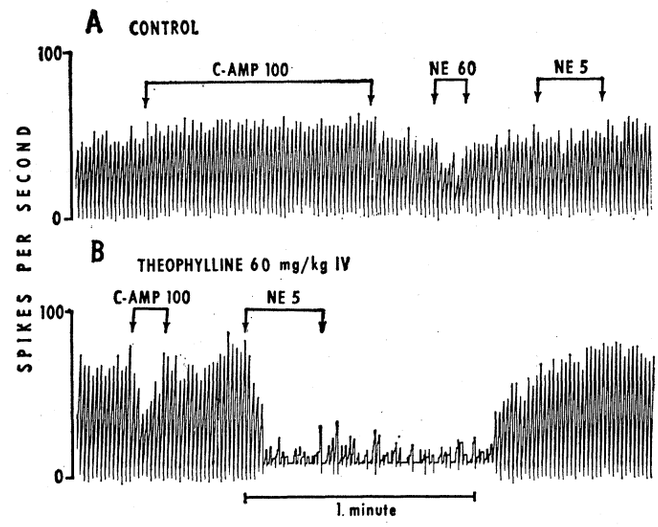


Fig. 2 (left). Effects of microelectrophoretic application of adenine nucleotides on spontaneous Purkinje cell discharge. A and B illustrate continuous records from the same cell. Duration of drug application is indicated by arrows, and numbers after each drug indicate ejection current in nanoamperes. The black bar in A represents application of a cathodal current through the micropipette. The restoration of spontaneous discharge by cathodal current indicates that the slowing seen with cyclic-AMP is not secondary to local anesthetic action or hyperdepolarization (cathodal block). Fig. 3 (right). Potentiation of microelectrophoretically administered NE and cyclic-AMP by parenteral theophylline. (A) Control. (B) Same cell after intravenous injection of theophylline (60 mg/kg). Duration of drug application indicated by arrows. Numbers after each drug indicate ejection current in nanoamperes. The residual discharge after 5 nanoamperes of NE in B consisted almost entirely of climbing fiber bursts.

glandins E_1 and E_2, thought to inhibit adenyl cyclase activity (*14*), reduces the slowing of spontaneous discharge obtained by application of NE from another barrel of the same micropipette.

Our studies of ultrastructure show nerve terminals in the cerebellar cortex containing large granular vesicles, characteristic of the nerve endings in the brain, which contain monoamine (*15*). Moreover, autoradiography of cerebellar cortex after subdural injection of H^3-NE reveals uptake and binding of this monoamine in nerve terminals adjacent to dendrites of Purkinje cells.

These data on ultrastructure, together with our findings on NE sensitivity of Purkinje cells and previous reports of NE-containing axons and high turnover rates of NE in the cerebellar cortex, suggest that this monoamine should be given serious consideration as a possible neurotransmitter in the cerebellum.

We propose that the action of NE on Purkinje cells may be specifically mediated by the formation of cyclic-AMP, although other possibilities cannot be eliminated. The lengthy time course of the effect of NE relative to that of cyclic-AMP, the similarity of response to these two substances, and the effects of theophylline and prostaglandins E_1 and E_2 all support such a stepwise mechanism. Synaptic inhibitions of long duration, reflecting underlying metabolic effects, have been described for vertebrate and molluscan ganglia (*16*). We suggest that, in the mammalian cerebellum, the NE released by the postulated adrenergic terminals affects Purkinje cells by transynaptic activation of a metabolic system.

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C-Fiber Responses in the Ventrolateral Column of the Cat Spinal Cord

Abstract. *Saphenous nerve C-fiber volleys generate, in the ventrolateral column of the cat spinal cord, a conducted postsynaptic response revealed by averaging the integrated temporally dispersed axonal discharges. This finding is compatible with the hypothesis that the feline ventrolateral column includes a major pathway related to nociception.*

Central reflex and sensory effects of unmyelinated C-fiber afferent volleys are well known, but summated central tract potentials evoked by C volleys have never been observed, even with closely spaced repetitive pulse stimulation. Their identification is hindered by temporal dispersion in nerve, polysynaptic relay, and central tracts where recordable amplitude from the fine fibers is small at best. Furthermore, the strength of the applied stimulus unavoidably engenders multifiring in myelinated A fibers and initiates a postsynaptic disturbance preceding C-evoked activity. Methods of differential block of myelinated fibers have been successfully applied to confine the input to C fibers, and spike activity responses have been described in the ventral thalamus, caudal medulla, and dorsolateral and propriospinal tracts of the cat spinal cord (*1*, *2*).

In a study of directly recorded ventrolateral column (VLC) discharges evoked by myelinated afferents, we failed to record responses that could be related to C volleys. However, depression of responses evoked by A afferents after conditioning volleys in C fibers alone suggested that this column does relay C input (*3*). Attempts to reduce temporal dispersion by stimulating C fibers in dorsal roots, or averaging the poststimulatory activity, or both, were also unsuccessful. The postsynaptic firing generated by C volleys is revealed by averaging the integrated evoked activity.

In nine cats, decerebrated under ether anesthesia, the saphenous nerve was dissected, cut distally, and placed on an array of stimulating, polarizing, and recording electrodes (Fig. 1D). After laminectomy and opening of the dura the animals were paralyzed with gallamine triethiodide and artificially ventilated; small doses of the drug were supplemented intravenously each 20 to 30 minutes. Contralateral VLC responses were recorded monopolarly through a stainless steel electrode. electrolytically sharpened (*4*) and insulated to within 30 to 70 μm of the tip (resistance 60 to 100 kilohm); a similar reference electrode was inserted into nearby muscle (Fig. 1, D and E). Trains of ten stimuli, 100 per second, at appropriate strength for A-delta and C fibers were delivered to the nerve