Cyclic Adenosine Monophosphate and Norepinephrine: Effects on Transmembrane Properties of Cerebellar Purkinje Cells

Abstract. *Electrical properties of Purkinje cells were recorded by intracellular microelectrode during extracellular electrophoretic application of gamma aminobutyrate, norepinephrine, cyclic adenosine monophosphate, and dibutyryl cyclic adenosine monophosphate. All these substances hyperpolarized Purkinje cells. Transmembrane resistance decreased during gamma aminobutyrate hyperpolarization. In contrast, norepinephrine and the cyclic nucleotides generally elevated resistance. These results show that cyclic nucleotides mimic the unique effects of norepinephrine on the bioelectric properties of neuronal membranes.*

Adenosine 3′,5′-monophosphate (cyclic AMP) is considered an intracellular mediator of many humoral and neurohumoral responses of the peripheral nervous system (*1*). Among central nervous structures, the rat cerebellum is unique in its high activity of adenyl cyclase (*2*), the enzyme that synthesizes cyclic AMP from adenosine triphosphate. Moreover, increased levels of cyclic AMP are found in slices of rat cerebellum exposed to norepinephrine (*3*). Recent studies indicate that norepinephrine-containing afferents project to Purkinje cells (*4*) and may be functionally inhibitory (*5*). Furthermore, with extracellular recordings of spontaneous activity, electrophoretically applied cyclic AMP and its dibutyryl derivative mimic the inhibitory action of norepinephrine (*6*). These and other studies have led us to propose that cyclic AMP mediates the effect of norepinephrine (NE) on Purkinje cells (*5, 6*).

If the NE inhibition were mediated through activation of adenyl cyclase, the mechanisms of this response might differ from the mechanisms of inhibitory transmission as classically conceived (*7*) and as exhibited by the putative inhibitory transmitters gamma aminobutyrate (*8*) and glycine (*9*). These two substances hyperpolarize excitable membranes by increasing the membrane conductance to ions whose equilibrium potentials lie at a more negative level than the resting potential of the membrane. Thus, the inhibitory effects of these agents are associated with decreased membrane resistance (*8, 9*).

We now report that the hyperpolarizing responses of Purkinje cells to NE (*10*) appear to be associated with an increase in membrane resistance (that is, decreased membrane conductance) and that these transmembrane effects are mimicked by cyclic AMP and its dibutyryl derivative.

Albino rats (130 to 200 g) anesthetized with 1 percent halothane were mounted in a stereotaxic unit and their cerebellums exposed (*5*). Multibarrel micropipettes were constructed so that drugs could be applied outside the neuronal membrane, while a recording barrel was positioned intracellularly (Fig. 1A) (*11, 12*). Conventional electronic techniques (*13*) were used for recording and amplifying potentials of Purkinje cells, identified by the spontaneous climbing fiber responses (Fig. 1B) unique to these cells (*14*). A standard Wheatstone bridge circuit was used to balance current (0.5 to 5 na) passed through the recording micropipette, so that membrane "input" resistance could be determined and membrane potential adjusted artificially (*15*).

Upon impalement of a Purkinje cell and recording of a stable resting membrane potential with action potentials or excitatory postsynaptic potentials (EPSP's), the drug under investigation was applied extracellularly (*16*). We measured the following three parameters before, during, and after drug application: (i) membrane potentials, (ii) input membrane resistance, and (iii) amplitude of spontaneous climbing fiber EPSP's. The longest (5 to 35 minutes) recordings with high membrane potentials (−40 to −63 mv) were recorded by electrodes with only two extracellular barrels. Factors such as the bulkiness of the micropipette assembly and the small size (about 20 μm in perikaryal diameter) and glial envelopment of rat Purkinje cells probably account for the shorter recordings with low resting potentials sometimes encountered (*16*).

Microelectrophoretic applications of gamma aminobutyrate (GABA), NE, cyclic AMP, or dibutyryl cyclic AMP all produced hyperpolarization of Purkinje cells (Figs. 1C and 2). Although GABA appears to be much more

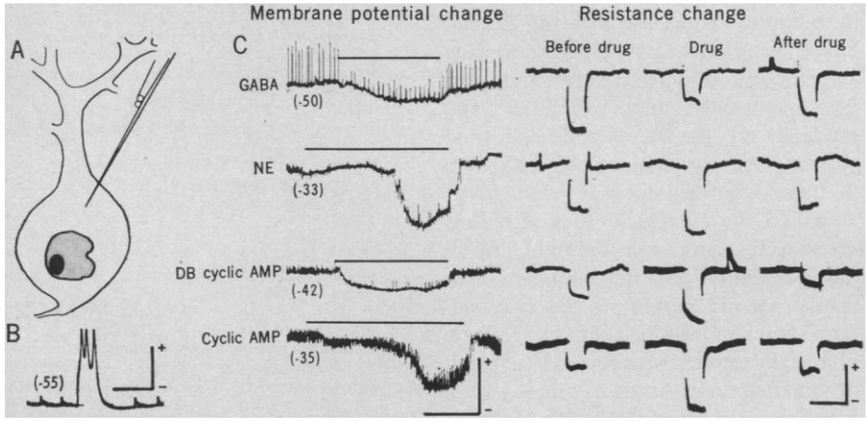

Fig. 1. Intracellular recordings from rat cerebellar Purkinje cells. (A) Schematic representation of a three-barrel micropipette with a Purkinje cell. The intracellular electrode protrudes beyond the orifices of the two extracellular microelectrophoresis barrels. (B) Multispiked spontaneous climbing fiber discharge obtained during intracellular recording from a Purkinje cell. Number in parentheses is resting potential in millivolts (mv); calibration bars are 20 msec and 25 mv. (C) Changes in membrane potential and membrane resistance of four different Purkinje cells in response to gamma aminobutyrate (*GABA*), norepinephrine (*NE*), dibutyryl cyclic AMP (*DB cyclic AMP*), and cyclic AMP. All specimens in each horizontal row of records are from the same cell. Solid bar above each record indicates the extracellular electrophoresis of the indicated drug (100 to 150 na). Number in parentheses below each recording is resting potential in millivolts; calibration bar under membrane potential records is 10 seconds and 20 mv for NE, DB cyclic AMP, and cyclic AMP, and is 5 seconds and 10 mv for GABA. The effective input resistance was judged by the size of pulses resulting from the passage across the membrane of a brief constant current (1 na) pulse before, during, and after electrophoresis of the respective drugs (1 mv = 1 megohm). Discontinuities in the fast transients of the pulses result from the loss of high frequencies (> 10 khz) and from the chopped nature of the frequency-modulated magnetic tape recording used. All "pulse" records were graphically normalized to the same baseline level. Calibration bar on right indicates 80 msec and 15 mv for all pulse records.

potent than NE or the cyclic nucleotides in reducing both spontaneous single spikes and climbing fiber bursts (*5*), the hyperpolarization produced by NE (34 cells; mean hyperpolarization, 9 mv), cyclic AMP (8 cells; mean hyperpolarization, 7 mv), and dibutyryl cyclic AMP (27 cells; mean hyperpolarization, 9 mv) was generally greater than that produced by GABA (17 cells; mean hyperpolarization, 6 mv) when applied with comparable ejection currents. This was apparent even though mean resting membrane potentials of the cells treated with NE, cyclic AMP, dibutyryl cyclic AMP, and GABA were nearly identical (29, 33, 35, and 27 mv, respectively).

The discrepancy between the discharge-reducing and hyperpolarizing effects of NE and the nucleotides on the one hand and of GABA on the other may be explained by the disparate actions of these substances on Purkinje input resistance and climbing fiber EPSP size. Gamma aminobutyrate reduced input resistance in all cells (mean input resistance reduced by 5 megohms from mean control value of 12 megohms), but NE, cyclic AMP, and dibutyryl cyclic AMP generally increased resistance (Fig. 1C) (by +4, +4, and +2 megohms from mean control values of 11, 7, and 12 megohms, respectively). In 88 percent of the cells NE increased resistance, and it had no effect on the resistance of 12 percent. The cyclic nucleotides elevated resistance in 61 percent of the cells, had no effect on the resistance of 37 percent of the cells, and lowered resistance in only 2 percent.

A difference was also seen with respect to spontaneous climbing fiber EPSP's; GABA always reduced the size of EPSP's, whereas NE, cyclic AMP, and dibutyryl cyclic AMP generally increased EPSP size (Fig. 1C). The ratio of the mean amplitude of the climbing fiber EPSP during drug application to that before drug application was 0.70 for GABA tests, 1.7 for NE, and 1.3 for the cyclic nucleotides. Hence, it is likely that GABA can reduce spontaneous discharge by two mechanisms: (i) hyperpolarization of the cell to a membrane potential farther from spike threshold levels and (ii) reduction of EPSP size (Fig. 1C) by increasing membrane conductance. Both NE and dibutyryl cyclic AMP hyperpolarize Purkinje cells more than GABA does, but the concomitant increase in resistance and EPSP size

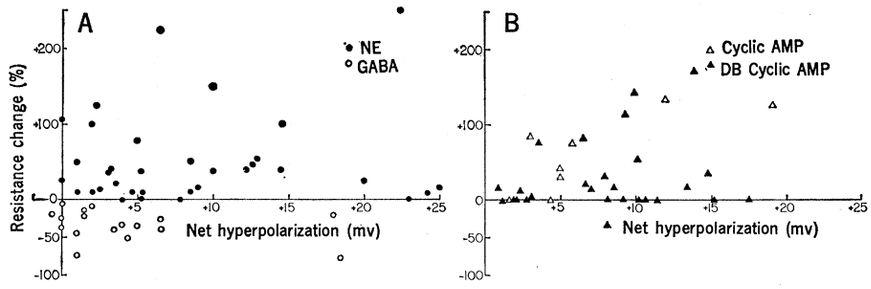

Fig. 2. Relationship of drug-induced hyperpolarizations in millivolts and the percentage of change in membrane resistance (change in effective input resistance × 100/original input resistance). (A) Effect of GABA and NE. Note that, although both drugs generally hyperpolarize Purkinje cells, only GABA responses are accompanied by a decrease in resistance. (B) Effect of cyclic AMP and dibutyryl cyclic AMP. Most hyperpolarizations are accompanied by either an increase or no change in input resistance.

results in a greater efficacy of synaptic currents, which may partially account for the apparent lesser potency of NE and the nucleotides in reducing discharge frequency (*5*).

Classical inhibitory neurotransmission produces hyperpolarization by elevating conductance to one or more ions, thus driving the membrane potential toward the more negative equilibrium potential for the ions involved (*7*). The result is that the magnitude of hyperpolarization produced by the transmitter is inversely dependent upon the membrane potential; when the membrane is artificially hyperpolarized beyond the equilibrium potential for the specific ion, the neurotransmitter produces a depolarizing response. In contrast, we observed no obvious relationship between initial resting potential and the hyperpolarization induced by NE or cyclic nucleotides in different Purkinje cells. Even when the membrane potential of the same cell was artificially adjusted to discrete levels on either side of the resting potential, no consistent changes in the magnitude of NE- or nucleotide-induced hyperpolarization were seen within the membrane potential range of −10 to −80 mv. Indeed, a few cells showed a greater response during artificial hyperpolarization than either before or after. Reversals of response were not seen.

These observations suggest that hyperpolarizations induced by NE and cyclic nucleotides differ strikingly from classical inhibitory postsynaptic potentials (IPSP's) in that no passive increase in ionic conductance occurs to drive the membrane potential toward a more negative equilibrium potential. The unique hyperpolarizations are unlikely to involve chloride because they are unaffected by intracellular injection of Cl^- sufficient to reverse the polarity of small spontaneous IPSP's to positive-going potentials (*17*).

It is difficult to determine if the increase in resistance usually seen with NE and the cyclic nucleotides in Purkinje cells is merely coupled indirectly to the hyperpolarization, as predicted by the Goldman equation (*18*). However, artificial hyperpolarization of Purkinje cells does not duplicate the NE- and nucleotide-induced increases in membrane resistance, since we observed linear current-voltage relationships over the range of membrane potentials from −20 to −100 mv (*18*). Although the drug-induced hyperpolarizations coupled to an increase in resistance are presently unexplained, they might be produced merely by a passive decrease in conductance to some ion or by activation of an electrogenic pump (*19*).

In conclusion, the data show marked similarities in the hyperpolarizing actions of NE, cyclic AMP, and dibutyryl cyclic AMP, in that increases in conductance are rarely encountered. This contrasts with elevated conductances seen during classical IPSP's (*7*) and during microiontophoresis of GABA (*8*). Nonsynaptic hyperpolarizations associated with an unchanged (*20*) or decreased (*21*) membrane conductance have been reported for excitable membranes. Similar properties have been described for the "slow IPSP" of vertebrate sympathetic ganglia (*22*), proposed to be adrenergic (*23*), and for several muscle preparations in response to catecholamines and cyclic nucleotides (*24*).

Furthermore, the results of the present investigation indicate that NE inhibition of Purkinje cells is brought about by hyperpolarization of the neuronal membrane (*10*), and these results further support a postsynaptic mediation of this inhibition by cyclic AMP

(*6*). The NE-containing afferents that terminate on rat Purkinje cells (*4*) and the enhanced formation of cyclic AMP in cerebellar slices treated with NE (*3*) may imply operation of a metabolically dependent postsynaptic response in the cerebellum. Final verification of such a physiological function awaits experimental activation of the proposed noradrenergic pathway.

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Cerebellar Hypoplasia in Neonatal Rats Caused by Lymphocytic Choriomeningitis Virus

Abstract. *Lymphocytic choriomeningitis virus, strain E-350, when inoculated intracerebrally in rats 1 to 7 days old, produces an acute destructive infection of the cerebellar cortex resulting in permanent cerebellar hypoplasia and ataxia. Several other arenoviruses may produce a similar lesion in neonatal rodents.*

The name arenovirus has recently been suggested for a group of agents which are enzootic in a variety of wild rodent species (*1*). Lymphocytic choriomeningitis (LCM) virus, the prototype of this group, is capable of producing several types of infections in its natural host, the mouse—acute and often lethal choriomeningitis, acute visceral infection, or persistent lifelong infection which may be followed by immune complex glomerulonephritis (*2*). Choriomeningitis also occurs in several other species of experimental animals after intracerebral inoculation of the virus (*3*). We now report the occurrence of an acute destructive process of the cerebellum after intracerebral inoculation of LCM virus into the neonatal rat.

Six groups of Wistar rats (*4*), of ages 1, 4, 7, 14, 21, and 90 days, respectively, were injected intracerebrally with the E-350 strain of LCM virus (*5*). Each animal received 0.03 ml of a clarified suspension of brain tissue from infected mice. This contained approximately 750 times the 50-percent lethal dose (LD_{50}) for intracerebrally inoculated adult mice. Animals were either held for observation or killed 3 weeks after injection for histological examination. Brains from infected and similarly aged, uninfected rats were embedded in paraffin, and parasagittal sections were stained with hemotoxylin and eosin.

Animals infected at 4 days of age showed maximum neurological signs of disease. About 2 weeks after infection they developed a permanent cerebellar ataxia consisting of hypotonia of hind limbs, inability to maintain balance, and slight resting tremor. Those injected at 1 or 7 days of age developed a transient ataxia, beginning about 2 weeks after injection and lasting for about 1 month. No deaths specifically attributed to the virus occurred in any of the animals inoculated at 1, 4, or 7 days; furthermore, no signs of illness were noted in rats which were injected at age 14 days or older.

Brains of sucklings inoculated at 4 days of age showed a marked hypoplasia of the cerebellum (Fig. 1), which was less severe in animals inoculated at ages 1 and 7 days. No gross abnormality was apparent in brains of rats inoculated at ages 14, 21, or 90 days. The major features of the typical lesion (Fig. 2) are: (i) Marked variation in attack on the cerebellar cortex, which totally destroys some folia, and partially or almost completely spares others (Fig. 2A). (ii) Choroid plexus, ependyma, and meninges show little, if any, inflammation (Fig. 2B). (iii) Deep cerebellar nuclei are spared. (iv) Demyelination is variable, and can be severe in folia in which cortical necrosis is minimum (Fig. 2C). Although all elements of the cerebellar cortex are affected, the evolving lesions (not illustrated) suggest that necrosis of granule cells precedes outfall of Purkinje cells.

The contrast between this cerebellar lesion and the classical choriomeningitis produced by LCM virus in mice, rats, guinea pigs, and monkeys (*1, 2*) raised the question as to whether our virus stock might contain a contaminating agent, such as rat parvovirus (*6*). Confirmation of the LCM virus etiology of the lesion was based on: (i) Production of cerebellar hypoplasia with E-350 virus from another