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NORADRENALINE MODULATION OF THE RESPONSES OF THE CEREBELLAR PURKINJE CELL TO AFFERENT SYNAPTIC ACTIVITY

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Noradrenaline, applied by microiontophoresis to rat cerebellar Purkinje neurones, selectively depressed spontaneous neuronal discharge. Simple spike and complex spike excitations, evoked by stimulation of the mossy and climbing fibres, were relatively preserved during the inhibition of spontaneous activity, and the number of full-sized action potentials in the complex spike increased. Inhibition mediated by the basket and stellate cells was augmented. Thus, relative to the change in spontaneous activity, noradrenaline increased the responsiveness of the Purkinje cell to afferent input.

Introduction Demonstration of catecholamine-containing fibres in the cerebellar cortex suggested the presence of previously unknown cerebellar afferent fibres (Hokfelt & Fuxe, 1969). Subsequent work has identified these fibres as a noradrenaline (NA)-containing pathway, originating in the nucleus locus coeruleus and synapsing on the Purkinje neurone (Olson & Fuxe, 1971; Bloom, Hoffer & Siggins, 1971). Physiological characterization of this pathway by stimulation of the locus coeruleus or application of NA by microiontophoresis has thus far shown that it depresses Purkinje cell spontaneous discharge, while hyperpolarizing the membrane and increasing its resistance (Hoffer, Siggins, Oliver & Bloom, 1973). However, many current formulations of cerebellar function emphasize integration of input from the mossy and climbing fibres as the most important role of the Purkinje cell. In the work presented here, therefore, we have examined the influence of NA on the activity evoked by stimulation of the other known afferents to the cerebellum. Specifically, complex spike excitation evoked by the climbing fibre, simple spike excitation evoked by mossy fibre activation of the granule cells and their parallel fibres, and inhibition evoked by the basket and stellate cells were each interacted with NA.

Methods Twenty Sprague-Dawley rats were anaesthetized with urethane (1.5 g/kg, i.p.) or halothane (1% in O_2). Five-barrelled micro-pipettes were used to record extracellularly from cerebellar Purkinje cells and to apply NA by microiontophoresis. The centre recording barrel was filled with 5 M NaCl; three side barrels were filled with NA (0.5 M, (–)-noradrenaline hydrochloride, pH 4.5) or γ-amino-butyric acid (GABA, 1.0 M, pH 4.0). Artifacts of iontophoretic current, pH, local anaesthesia, or failure to release drug were controlled, as previously described (Hoffer, Siggins & Bloom, 1971). Climbing and mossy fibres were activated by triple shocks (500 Hz) to the limbs, snout, or cerebral cortex (Allen, Azzena & Ohuo, 1974). 'Off beam' surface stimulation (single shock) was used to activate inhibitory basket and stellate cell inputs (Eccles, Ito & Szentagothai, 1967). Action potentials of single Purkinje cells, converted to constant voltage pulses by a gating circuit, were used by a digital computer to generate a post stimulus time histogram (PSTH). A control PSTH was computed for each cell to quantify the evoked and spontaneous activity. As shown in the examples of Figure 1, epochs of evoked and spontaneous activity were selected in the histogram, and the discharge rates in each were calculated by dividing the number of counts by the time and the number of sweeps. Subsequently, NA was applied by microiontophoresis. When a new steady state level of spontaneous discharge was reached, another PSTH was computed. Identical epochs in the control and NA histograms were then compared to quantify the percentage change in evoked and spontaneous activity (Freedman, Hoffer & Woodward, 1975). After cessation of NA administration, histograms were serially computed for demonstration of recovery of discharge rates to control levels.

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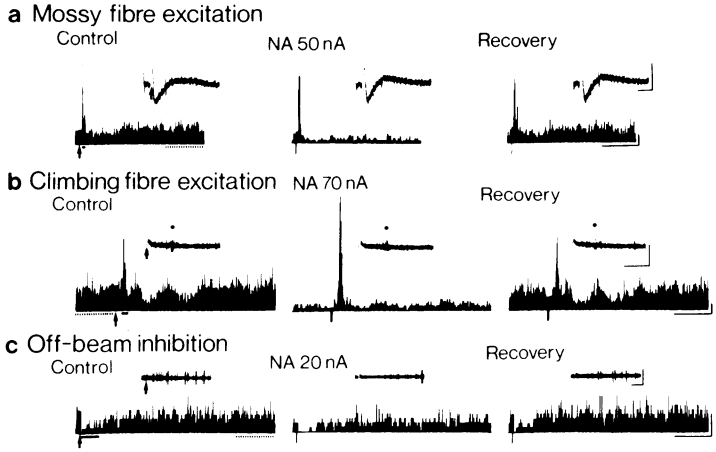

Figure 1 Effects of noradrenaline (NA) on cerebellar Purkinje neurones: changes in evoked and spontaneous activity induced by NA. Post stimulus time histograms and extracellular action potential recordings are shown for control (left), NA iontophoresis (centre) and recovery (right) periods. Changes in evoked or spontaneous activity were computed by selecting portions of the histogram to correspond with each type of activity and comparing these same portions during control and NA iontophoresis periods. (a) For mossy fibre stimulation, the sharp peak several ms after the stimulus (arrow) represents simple spike excitation (solid line). A typical extracellular recording of such a response is shown in the insert. A 100 ms period, 400 ms after the stimulus, was chosen to represent the spontaneous activity (dotted line) of the cell. During NA ejection (50 nA), this spontaneous activity was nearly abolished. Comparison of identical periods in the control and NA iontophoresis histogram shows an 87% decrease, from 54.3 spikes per s to 7.2 spikes per second. However, the peak of simple spike excitation remained almost intact during NA ejections; an average of 0.95 spikes per stimulus were elicited, compared to 1.18 spikes during the control period, a decrease of only 19%. Stimulus parameters were three 7 V shocks to the cerebral cortex at 500 Hz every 250 milliseconds. Each histogram contains 100 sweeps. (b) The complex spike response to climbing fibre stimulation often was potentiated during NA iontophoresis. In the example shown, the evoked excitation (solid line) increased during NA ejection from 1.02 spikes to 1.52 spikes per stimulus. This potentiation is a result of an increase in the number of full-sized action potentials in the complex spike. In the control recording, the complex spike (dot) contains one full-sized spike with smaller wavelets. During NA ejection, the complex spike (dot) contains 3 full-sized action potentials. Simultaneously, the spontaneous activity, selected here to be prior to the stimulus, fell from 30.8 to 5.0 spikes per s, an 81% inhibition. Stimulation parameters were three 12 V shocks to the cerebral cortex at 500 Hz every 2 seconds. Each histogram contains 200 sweeps. (c) The effect of NA on inhibitory pathways was assessed during 'off beam' responses, obtained by stimulating the cerebellar surface. The resulting inhibition was quantified by selecting a response period (solid line), in a manner similar to that used for the study of excitation. During NA ejection, in the example shown, this inhibition was augmented by 90%, while spontaneous activity (dotted line) was depressed by only 45%. Stimulus parameters were one 5 V shock every 2 seconds. Each histogram contains 100 sweeps; each extracellular spike recording contains 5 superimposed sweeps. Calibration bars are 0.5 mV, 20 ms for all recordings and 5 counts per address, 100 ms for histograms.

Results Mossy fibre activation is known to result in simple spike excitation of the Purkinje cell, with a latency of 5 to 10 ms (Allen *et al.*, 1974). An example is shown in Figure 1a. The initial peak in the histogram represents this excitation, which, after a brief period of inhibition, was followed by a return to a baseline spontaneous rate. During NA iontophoresis spontaneous discharge was inhibited profoundly, as previously observed (Hoffer *et al.*, 1971). However, simple spike excitation was much less affected. Spontaneous discharge was inhibited 87% in this case, but mossy fibre evoked spike activity was decreased only 19%. In all ten Purkinje cells similarly studied, spontaneous activity was depressed more than simple spike excitation.

Activation of the climbing fibre evokes a complex spike in the Purkinje cell, consisting of one or more full-sized action potentials, followed by smaller wavelets. In these experiments, PSTH's were computed with the gating circuit set to count only full-sized action potentials. As the inset spike record in Figure 1b (left) shows, generally there was only one such full-sized action potential in the complex spike. During NA iontophoresis, the spontaneous discharge rate again fell markedly, but evoked activity increased. Serial photographs of the complex spikes showed that

although the number of evoked complex spikes decreased somewhat, the number of full-sized action potentials in each complex spike increased during NA administration (Figure 1b). Of the 20 cells studied, all showed greater inhibition of spontaneous activity than of complex spike evoked activity; in 8 cells the total number of full-sized action potentials evoked by the climbing fibre actually increased. On the average, spontaneous activity fell 72% while evoked activity increased 5%.

Stimulation of the cerebellar surface activates basket and stellate cells, which inhibit Purkinje cell discharge (Figure 1c). The inhibition was quantified by examining a selected response period following the stimulus, analogous to the manner by which the excitations were studied. Iontophoresis of NA potentiated 'off beam' inhibition by an amount greater than would be expected from the inhibition of spontaneous activity. In the example shown, inhibition increased by 90%, while spontaneous activity was inhibited only 45%. For ten cells similarly studied, augmentation of inhibition was 85%, while inhibition of spontaneous activity was only 63%. In several cases, marked augmentation of basket and stellate cell inhibition was seen at doses of NA which caused only minimal changes in spontaneous activity or at times when spontaneous discharge returned to normal following NA administration.

Discussion The effects of NA on cerebellar neuronal circuitry include actions which cannot be predicted from the simple inhibition of spontaneous activity. Despite the depression of spontaneous activity, simple spike excitation was preserved. Complex spike excitation was similarly preserved, and the number of full-sized action potentials increased. 'Off beam' inhibition was augmented. Thus, NA had a selective effect, preserving or enhancing afferent-evoked excitation and inhibition, while depressing spontaneous activity.

This study raises questions about the mechanisms responsible for the differential inhibitory action of NA and its implication for behaviour. A hypothesis that spontaneous discharge and receptivity to afferent input are affected differently because they are independent functions can be supported by studies which show spontaneous activity in cerebellum isolated from afferent input by lesion (Eccles *et al.*, 1967), transplantation (Hoffer, Seiger, Ljungberg & Olsen, 1974), or culture (Geller & Woodward, 1974). Further studies are required to determine the respective roles of the hyperpolarization and increased membrane resistance produced by NA in altering receptivity. It should be noted, however, that GABA, which hyperpolarizes but decreases membrane resistance, also increases responses to afferent excitatory input (unpublished observations). Findings similar to those reported here were seen with NA and auditory cortical neurones in monkey (Foote, Freedman & Oliver, 1975). Determination of the functional relevance of these effects of NA awaits further evidence from studies of adrenergic input in behaving animals.

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