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Modulation of Parallel Fiber Excitability by Postsynaptically Mediated Changes in Extracellular Potassium

Abstract. *Field potentials and extracellular potassium concentration ($[K^+]_o$) were simultaneously monitored in the molecular layer of the rat cerebellar cortex during stimulation of the parallel fibers. The synaptic field potential elicited by stimulation was reduced by several methods. Reduction of synaptic field potentials was accompanied by a marked increase in the excitability of the parallel fibers. This change in excitability was related to the degree of extracellular K^+ accumulation associated with parallel fiber stimulation. These findings support the proposal that increases in $[K^+]_o$ associated with activity in postsynaptic elements can modulate the excitability of presynaptic afferent fibers.*

Neuronal communication at chemical synapses in the mammalian central nervous system is often assumed to be unidirectional, proceeding from pre- to postsynaptic elements. However, several studies indicate that ionic changes associated with postsynaptic activity may modulate the excitability of presynaptic elements (*1*, *2*). In contrast to invertebrate systems, where simultaneous pre- and postsynaptic intracellular recording is possible (*1*), pre- and postsynaptic responses in the mammalian brain are difficult to study simultaneously. The cerebellum, with its laminar organization and well-defined pre- and postsynaptic electrophysiological responses, allows for such an analysis. The excitability (*3*) of the parallel fibers (PF's) of the cerebellar cortex is enhanced by the reduction of activity in postsynaptic elements (*4*). This implies that an event secondary to postsynaptic activity can influence presynaptic fiber excitability. In these experiments, the excitability of the PF's was studied before and after reduction of surround synaptic activity and was shown to be related to evoked changes in extracellular potassium concentration ($[K^+]_o$). The findings support the hypothesis that increases in $[K^+]_o$ secondary to activity in postsynaptic elements can modulate the excitability of afferent PF's and perhaps affect information processing of the cerebellum.

Adult male Wistar rats were anesthetized with urethane (150 mg per 100 g of body weight). The cerebellar vermis was exposed (*4*) and superfused with warmed (38°C), oxygenated Ringer solution (*5*). Potassium-sensitive microelectrodes (*6*) were used to record $[K^+]_o$ and field potentials from the same location. Glass-coated tungsten stimulating microelectrodes (tip exposures, 5 to 20 μm) were located 0.3 to 1.5 mm from the recording electrode. Recordings were obtained within 70 μm of the cerebellar surface (*7*).

The field potential elicited by a stimulus applied to the folial surface (*8–10*) consists of an initial triphasic potential (P1, N1, P2 in Fig. 1A) representing the synchronous volley of PF action potentials, followed by a negative potential (N2) corresponding to the synaptic current generated by monosynaptic activation of dendrites in the molecular layer. In response to a 50-Hz, ten-pulse stimulation train (Fig. 1B), latency is reduced in the second and next few field responses, but subsequent responses show increased latency with corresponding amplitude (P1 peak to N1 peak) reductions. During the stimulation train, $[K^+]_o$ increases from a baseline of 3.0 m*M* to a

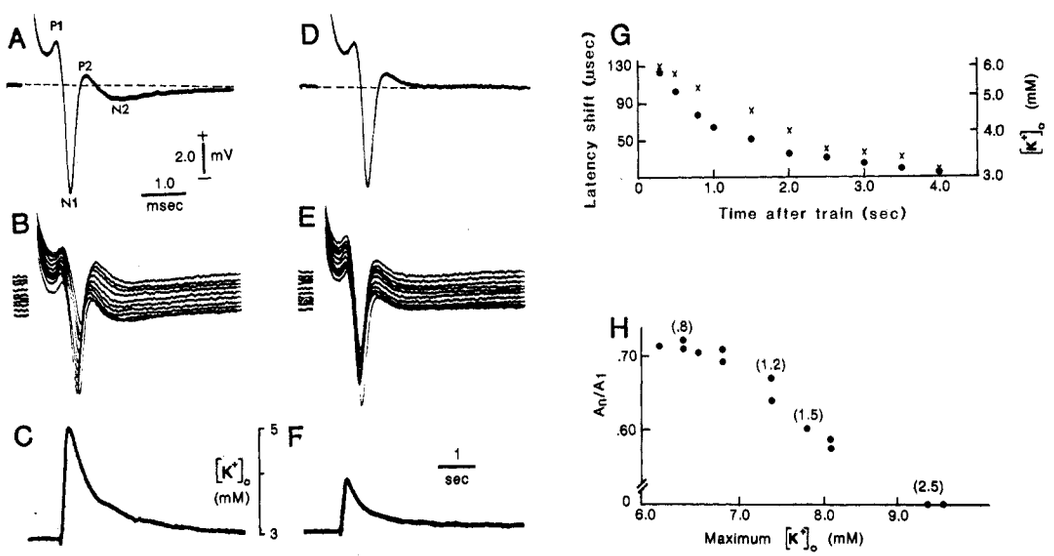

Fig. 1. Field potentials and $[K^+]_o$ changes elicited by PF stimulation in normal Ringer solution (A to C) and 10 m*M* Mg^{2+}-Ringer solution (D to F). (A) On-beam field potential. (B) Consecutive field responses elicited by a 50-Hz, ten-pulse stimulation train. (C) The simultaneous increase in $[K^+]_o$ for response in (B) recorded with a K^+-sensitive microelectrode. (D) Field potential recorded from the same location as in (A to C), but in the presence of 10 m*M* Mg^{2+}. The N2 synaptic potential is obliterated. (E) Field responses elicited by the 50-Hz, ten-pulse stimulation train in the presence of 10 m*M* Mg^{2+} exhibit enhanced frequency-following. (F) The $[K^+]_o$ signal simultaneous to the responses in (E). (G) Latency shift of the field potential (×) and $[K^+]_o$ (●) as a function of the interval after completion of a 50-Hz, 25-pulse stimulation train. $[K^+]_o$ is plotted on a logarithmic scale. (H) A_n/A_1 ratio elicited by 50-Hz, 25-pulse stimulation train plotted as a function of the maximum $[K^+]_o$ elicited by the stimulation train. Points on the graph were obtained by intermittent cerebellar superfusion with a 2 m*M* Mn^{2+}-Ringer solution. Amplitude (in millivolts) of the N2 potential is indicated by numbers in parentheses. $[K^+]_o$ is plotted on a logarithmic scale. Calibrations in (A) pertain also to (B), (D), and (E).

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peak of 5.2 m*M* (*11*) and returns to its baseline in 5.3 seconds (Fig. 1C).

Calcium is required at the presynaptic terminal for transmitter release (*12*). Altering the availability of Ca^{2+}, by adding calcium antagonists (Mg^{2+} or Mn^{2+}) or by lowering Ca^{2+}, selectively reduces or obliterates the N2 synaptic component of the field potential without altering the PF component (Fig. 1D). Field potentials elicited in the presence of 10 m*M* Mg^{2+} (Fig. 1E) show less latency increase and amplitude reduction during repetitive stimulation than the corresponding changes obtained in normal Ringer (Fig. 1, A to C). In Mg^{2+}-Ringer, the ratio of the amplitude of the last PF volley of the train (A_n) to that of the first volley (A_1) is 0.85; the ratio is 0.60 in normal Ringer where the synaptic field potential is present. The peak $[K^+]_o$ rise during the stimulation train in the presence of synaptic potentials was 5.2 m*M* (Fig. 1C) and 3.9 m*M* in their absence (Fig. 1F).

Excitability of the PF's, as measured by changes in field latency and amplitude (*13*), is related to increases in $[K^+]_o$. When single stimuli are presented at various intervals after a stimulation train, the PF volley returns to its control latency and amplitude with a time course similar to the dissipation of the $[K^+]_o$ elicited by the stimulation train (Fig. 1G). When the efficacy of synaptic transmission was variably reduced by intermittent superfusion with 2 m*M* Mn^{2+}-Ringer solution, there were concomitant variations in the extent of amplitude reductions (shown as the A_n/A_1 ratio) and the $[K^+]_o$ increases elicited by stimulation trains (Fig. 1H) ($r = -.97, P < .01$). Finally, the relationship between PF volley amplitude and latency, and $[K^+]_o$ was quantified by varying the $[K^+]_o$ in the superfusate (Fig. 2, A and B). The latency and amplitude of the PF volley vary with $[K^+]_o$, and steady state increases in $[K^+]_o$ alter the PF volley in a qualitatively similar manner to the dynamic increases in $[K^+]_o$ observed during high-frequency stimulation.

Although Mn^{2+} superfusion has no effect on the PF action potential elicited from a single stimulus (*9*), changing Ca^{2+} levels or introducing Ca^{2+} antagonists might directly influence axonal excitability (*14*). In order to reduce synaptic potentials without changing the superfusate, double stimulation trains were applied (Fig. 2, C and D). The conditioning train presumably diminishes temporarily the amount of releasable neurotransmitter, reducing the N2 synaptic component of the subsequent test field. The PF responses of the second train show an enhanced frequency-following capability with an A_n/A_1 ratio of 0.59 compared to 0.16 for the conditioning train. The evoked $[K^+]_o$ is markedly less for the test train than for the conditioning train (Fig. 2E).

Synaptic field potentials were also reduced by increasing K^+ concentration of the superfusate ($[K^+]_s$) from 3.0 m*M* to 25 m*M* for as long as 1 hour. After $[K^+]_s$ had been restored to 3.0 m*M* and $[K^+]_o$ had returned to baseline values, a field potential devoid of N2 synaptic component was elicited. Under these conditions the PF's exhibited markedly increased frequency-following capabilities concomitant with reduced K^+ signals (Fig. 2, H and I). Hence, when activity in postsynaptic elements was reduced or eliminated, the PF's always displayed improved frequency-following capabilities, and the $[K^+]_o$ increases associated with PF stimulation were always reduced.

Most of the postsynaptic K^+ efflux is probably associated with Purkinje cell activity since Purkinje dendrites constitute the majority of postsynaptic elements in the molecular layer receiving synaptic inputs from the PF's. The potassium conductance (G_K) associated with excitatory postsynaptic potentials from PF activation of the Purkinje cells, Ca^{2+}-activated G_K (*15*), and G_K associated with Purkinje action potentials could all contribute to an increase in $[K^+]_o$ after PF activation. The PF's are

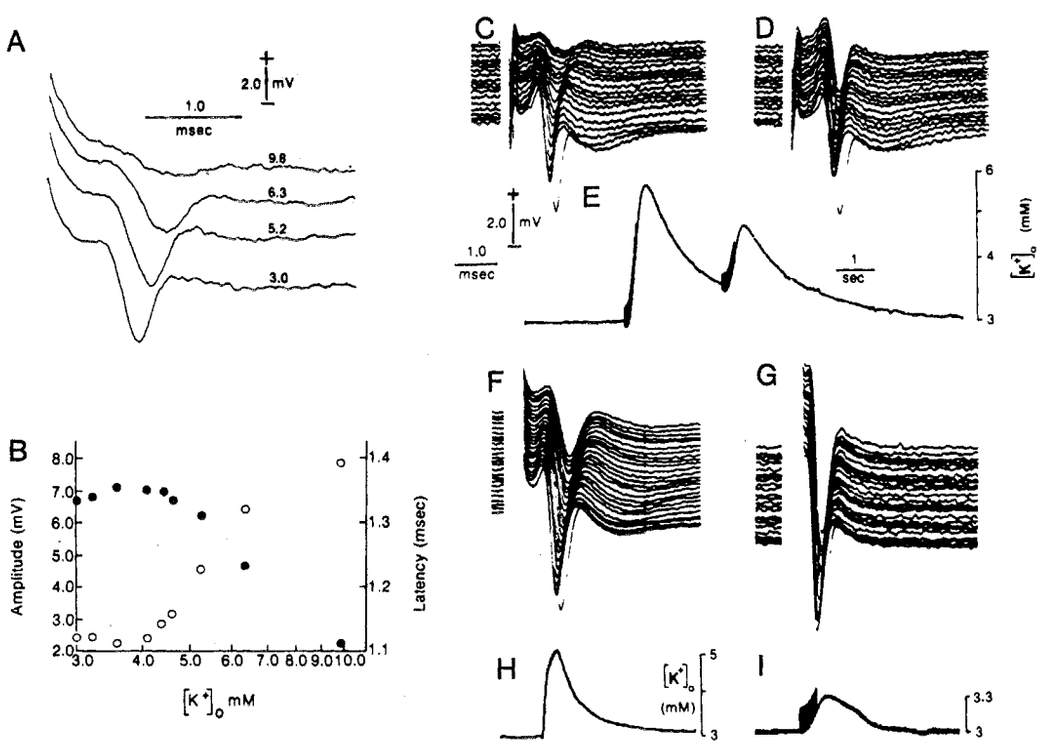

Fig. 2. (A) Field potentials at different levels of $[K^+]_o$. Numbers above traces indicate $[K^+]_o$ in millimoles measured at the site where the field was recorded. The $[K^+]_o$was altered by superfusing with an osmotically balanced 20 m*M* K^+-Ringer solution. (B) Amplitude (●) and latency (○) of PF volleys as a function of $[K^+]_o$, from the experiment shown in (A). (C) Consecutive field potential responses elicited by a 50-Hz, 20-pulse conditioning stimulation train, followed 2.8 seconds later by a test (D) 50-Hz, 20-pulse stimulation train. (E) The $[K^+]_o$ signal recorded simultaneously with the two stimulation trains. (F) Consecutive field responses to a 50-Hz, 25-pulse stimulation train in normal Ringer solution and the $[K^+]_o$ signal recorded simultaneously (H). (G) Field responses to a 50-Hz, 25-pulse stimulation train after superfusion with 25 m*M* K^+ for 30 minutes. Note the complete absence of an N2 potential. (I) The $[K^+]_o$ signal recorded simultaneously. The responses in (G) and (I) were obtained when $[K^+]_o$ had returned to baseline (3.0 m*M*). The calibrations in (C) pertain also to (D), (F), and (G). Time calibration in (E) pertains also to (H) and (I).

morphologically well suited for possible ionic interactions between molecular layer elements. They are densely packed, fine (0.1 to 0.3 μm) nonmyelinated axons passing through Purkinje dendrites, separated by a narrow extracellular space with few intervening glial elements (*16*). These characteristics might allow the PF's to be particularly sensitive to changes in $[K^+]_o$.

Changes in $[K^+]_o$ have been hypothesized to be functionally significant in several neuronal systems (*17*). Our results support the proposal that the excitability of the presynaptic afferent PF's is influenced by increases in $[K^+]_o$ resulting primarily from activity in postsynaptic elements. Accumulation of extracellular potassium could lead to membrane depolarization, which, if large enough, could inactivate the sodium channel and slow and possibly block conduction (*18*). Whether such changes occur during normal cerebellar activity is open to question. Granule cells discharge small bursts of action potentials at frequencies above 500 Hz (*19*). We demonstrated changes in PF frequency-following for short bursts at lower firing frequencies (ten impulses at 50 Hz). If changes in $[K^+]_o$ occur during normal activity in the cerebellum, then a mechanism might operate that could inhibit the afferent PF's in the microsurround of a Purkinje cell, by changing the extracellular ionic environment.

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Instrumental Control of Cardioacceleration Induced by Central Electrical Stimulation

Abstract. *Each of four monkeys* (Macaca mulatta) *was operantly conditioned to slow and to speed heart rate through a shock-avoidance procedure. During these sessions, electrical brain stimulation that produced tachycardia and pressor responses was delivered on alternate, 64-second segments to one of several brain regions. All animals were able to attenuate the increases in heart rate produced by brain stimulation during the slowing sessions when posterior hypothalamic and striatal regions were stimulated but not when anterior hypothalamic or subthalamic areas were stimulated. During speeding or control sessions during which heart rate was monitored, brain stimulation continued to increase heart rate.*

A number of experiments have examined the effect of electrical stimulation of the brain (ESB) on cardiovascular changes (*1*). With few exceptions [for example, (*2*)] nonhuman primates have been anesthetized and unable to interact with the environment (*3*). Therefore, there was no chance to observe the possible relationships among ESB, cardiovascular changes, and behavior. Our research was designed to investigate these relationships and to delineate the neural structures involved in cardiovascular control. We trained four monkeys (*Macaca mulatta*) to raise and to lower heart rate to avoid an electric shock to the tail (*4*); we then determined whether they could modulate cardiovascular function while receiving ESB, which produced tachycardia and pressor responses.

Sessions consisted of a 256-second baseline phase plus a 1024-second phase of either conditioning or heart rate monitoring (control) (*4*). The blood pressure signal was detected by a pressure transducer (P23 DB Statham) attached to a catheter permanently inserted in the abdominal aorta. The signal was controlled by computer (Raytheon 704), which also controlled experiments and recorded heart rate and systolic and diastolic blood pressure. [We report derived mean pressure—the sum of systolic pressure + 2 (diastolic pressure) divided by 3.] Animals were signaled to slow heart rate by a red cue light or to speed it by a green

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