Exp Brain Res (1986) 61: 614–624

Experimental Brain Research

Enhancement of cerebellar Purkinje cell complex discharge activity by microiontophoretic serotonin

J.C. Strahlendorf, H.K. Strahlendorf, and M. Lee

Departments of Physiology and Medical and Surgical Neurology, Texas Tech University Health Sciences Center, School of Medicine, Lubbock, TX 79430, USA

Summary. The major finding of the present study is that iontophoretically applied serotonin increased markedly (average 94.0%) the number of complex discharges within the majority (74%) of cerebellar Purkinje cells tested.Twenty-five percent of the cells showed an average 23% decrease in complex discharges, whereas 1% of the cells failed to respond. The effects of serotonin on complex activity were not related to any single effect of this amine on simple spike activity. It was apparent that the actions of serotonin on complex discharge activity were correlated with the initial simple-spike firing rate of the Purkinje cell and the predrug number of complex discharges. The other component of the complex discharge pattern analyzed in this study was the mean post-complex-discharge interval (MPCI). Purkinje cells evincing lower MPCI values were those in which serotonin increased the MPCI value preferentially, whereas cells in which serotonin depressed MPCI values exhibited higher predrug MPCI values. The serotonin antagonists methysergide and metergoline antagonized serotonin-induced enhancements in the numbers of complex discharges, whereas ketanserin failed to alter the response, suggesting a degree of receptor specificity. Comparisons between the present study and our previous work identifying a rate-dependent component to the actions of serotonin on simple spike activity are described.

Key words: Serotonin – Purkinje cell – Complex discharge – Cerebellum – Microiontophoresis

Introduction

Numerous anatomical studies have identified within the cerebellar cortex apparent synaptic and nonsynaptic serotonergic terminals originating from various raphé nuclei (Chan-Palay 1976; Shinnar et al. 1975) and the nuclei reticularis pontis oralis, paragigantocellularis, and gigantocellularis (Bishop and Ho 1985). Purkinje cells are among the recipients of these serotonergic fibers (Chan-Palay 1976; Bishop and Ho 1985).

Initial electrophysiological studies reported that approximately 50% of the recorded Purkinje cells were excited by microiontophoretically administered serotonin (5-hydroxytryptamine, 5-HT), whereas the remaining 50% were inhibited (Bloom et al. 1972; Hoffer et al. 1969). In our initial studies, we found that stimulation of the central superior and central inferior raphé nuclei elicited both excitation and inhibition of cerebellar Purkinje cells (Strahlendorf et al. 1979, 1981). We have reported that 5-HT administered iontophoretically to cerebellar Purkinje cells in urethane-anesthetized rats elicited one of three effects: inhibition (62% of the cells), biphasic response (27%), and excitation (11%) (Strahlendorf et al. 1983). These actions of 5-HT on the spontaneous discharge rates of Purkinje cells appeared to be correlated with the initial firing rates of the cells (Strahlendorf et al. 1984). Specifically, Purkinje cells that responded to serotonin with increases in firing rate had significantly slower predrug firing frequencies than those cells that were suppressed by 5-HT. We surmised that serotonin was modulating Purkinje cell activity, biasing the rate to that of a preferred frequency, possibly via transmitter-activated, voltage-dependent ionic channels or activation of excitatory and inhibitory receptors. Recently, we have shown that low currents of microiontophoretically administered serotonin appear to attenuate glutamate-evoked excitations preferentially rather than to alter significantly the spontaneous firing rate of the cell, a response that often is blocked by the serotonin antagonist methysergide (Lee et al. 1985). These

Offprints requests to: J.C. Strahlendorf (address see above)

studies have prompted us to suggest a neuromodulatory role for serotonin in the cerebellum.

Our previous studies have addressed the actions of serotonin on spontaneous and glutamate-driven simple spike (SS) activity. Numerous investigators have suggested that climbing fiber (CF) afferents originating from the contralateral inferior olive govern the responsiveness of the Purkinje cells to mossy fiber afferents (Campbell et al. 1983; Colin et al. 1980; Rawson and Tiloskulchai 1982; Ebner and Bloedel 1981, 1984; Savio and Tempia 1985; Ito et al. 1979, 1982); however, the ability of serotonin to alter the responsiveness of Purkinje cells to CF afferent activity as measured by the number of spontaneously occurring complex discharges (CDs) has not been examined. The present study was designed to monitor the effects of iontophoretic serotonin on the absolute number of complex discharges and the mean post-complex-discharge interval (MPCI) and to correlate these effects with predrug spontaneous activity in urethane-anesthetized rats. Our findings indicate that serotonin produces a marked enhancement of spontaneously occurring CDs. This study provides further evidence for the hypothesis that serotonergic afferents to the cerebellum may modulate impulse traffic in the cerebellar neuronal network.

Material and methods

Animal preparation

We performed our experiments on male Sprague-Dawley rats (200–350 g) anesthetized by an intraperitoneal injection of urethane, 1.2 g/kg. A proportionally controlled DC heating pad maintained body temperature between 36.5–37.5° C. A craniotomy exposing the cerebellum at the level of the fissura prima (which separates lobules V and VI) facilitated the exploration of the anterior and posterior cerebellar vermis out to the lateral edge of pars intermedia of the anterior lobe. To reduce movement artifacts and prevent the cerebellar surface from drying, we placed 3% agar in a saline solution on the exposed cerebellar cortex as necessary.

Recording and microiontophoresis

For iontophoretic experiments, we used 5-barrel micropipettes with 5- to 6-μm tips to record extracellularly the spontaneous discharges of Purkinje cells. Purkinje cells were identified by their characteristic electrophysiology, including their complex discharges (Eccles et al. 1967). The outer drug barrels of the multibarrel pipette contained 5-hydroxytryptamine creatinine sulfate (Sigma Chemical Co., 0.05 M, pH 4.5), methysergide bimaleate (UML) (Sandoz, 0.01 M, pH 4.0), metergoline (Farmitalia, 0.01 M in 1% ascorbic acid, pH 4.0), and ketanserin tartrate (Janssen, 0.01 M, pH 4.0), all dissolved in distilled water with the exception of metergoline to which ascorbic acid was added to enhance its stability. We applied a retaining current of 10 nA with appropriate polarity to all drug barrels between periods of ejection. Current effects that could influence cell discharges directly were minimized by an automatic balancing circuit that passed a current of equal magnitude and opposite polarity to the sum of all currents through one side barrel of the electrode filled with 4 M NaCl. To control for artifacts caused by local anesthetic effects, we led action potentials into an analog delay, the output of which was displayed on another oscilloscope. Cells that displayed nonspecific responses to drug applications (e.g., change in action potential shape) were eliminated from the study. The center barrel was filled with 4 M NaCl (3-5 MΩ impedance) for recording action potentials (simple spikes and complex discharges), which we amplified by conventional means, monitored on an oscilloscope, and converted to a uniform voltage pulse by passage through a window discriminator. The pulses were summed by a rate meter over 1-s epochs (cumulative records) and displayed on a strip-chart recorder. The amplified signals were stored on a multichannel magnetic tape recorder (bandpass: 25 Hz to 24 KHz). The window discriminator output also was led on line to a computer (Apple IIe, Apple Computer, Inc.), which quantitated firing characteristics by interspike-interval histogram software developed in this laboratory. Interspike-interval histograms (ISHs) were compiled over 120-s epochs containing typically between 2400 and 9600 intervals. Our ISH software identified CF-evoked bursts on the basis of two or more successive spikes separated typically by an interval of 3 ms or less and followed by an interval of greater than 4 ms. Quantitatively, the program computed the total number of events counted, the mean and standard deviation of all intervals, the median and modal intervals, the number of CDs, the average number of spikes per CD, and the MPCI period. The number of spikes per burst included the spike preceding the minimum interval (i.e., the initial spike of the burst). ISHs were stored on magnetic disks for future reference.

Extreme care was taken to insure proper window settings for complex discharge detection. Spike amplitudes ranged from 0.6 to 1.5 mV with noise levels typically around 0.08 mV. In addition, when the computer detected the occurrence of a complex discharge on the basis of interval analysis, it triggered a storage oscilloscope, which displayed the complex discharge after passage through an analog delay. By these means, it was possible to inspect each discharge and verify the correctness of the window threshold and minimum interval for detection. Figure 1 depicts typical

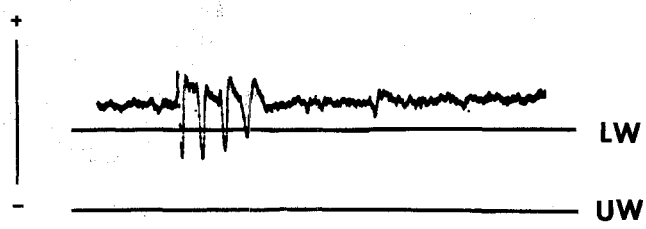

Fig. 1. Oscilloscopic recording of a typical extracellularly recorded spontaneous CF-evoked response. This CD representation consists of 4 individual spikes. Square waves generated by action potentials that fell within the window were led to the computer for ISH analysis. Solid lines represent the upper window (UW) and the lower window (LW) settings from the amplitude analyzer (gated negative). When the computer detected the CD, a trigger pulse was generated for a second oscilloscope that displayed each CD after passage of the signal through an analog delay. This allowed for visual verification of the window setting. Calibration bars: horizontal, 10 ms; vertical 0.1 mV

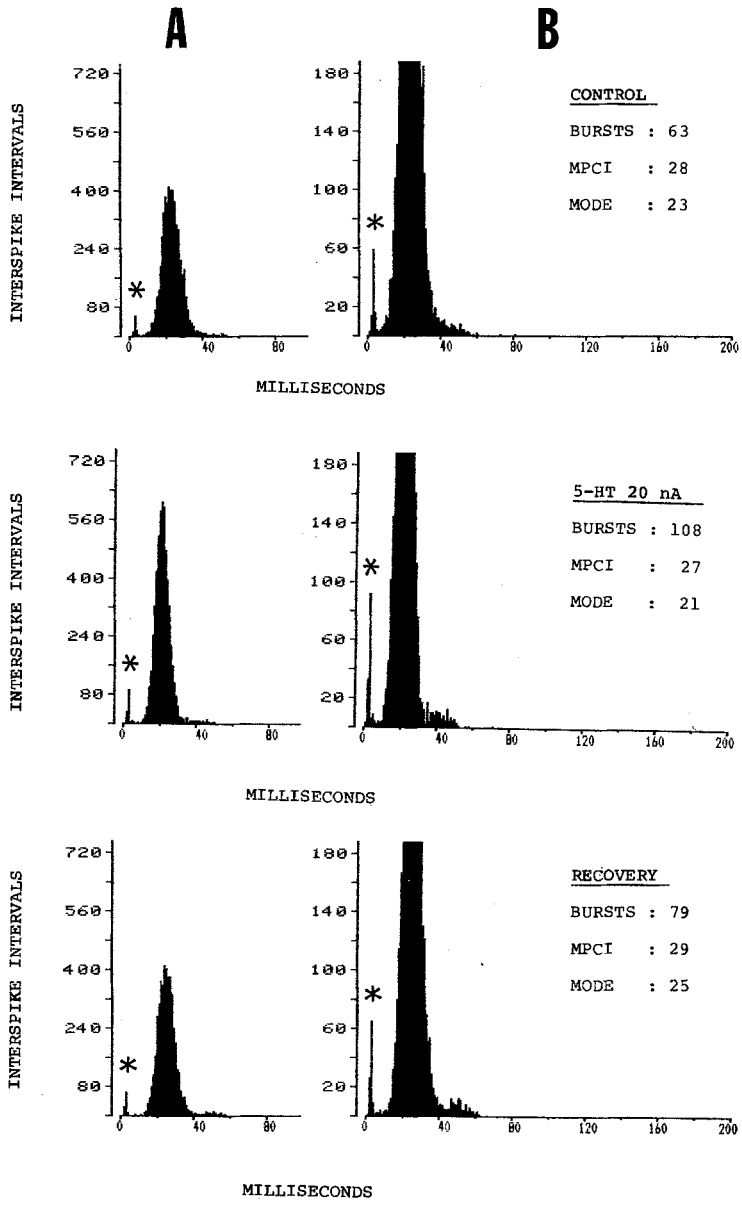

Fig. 2A and B. Computer-generated interspike interval histograms from a single Purkinje cell demonstrating the effect of continuous iontophoretic administration of serotonin (20 nA) on the number of spontaneously occurring complex spikes. Column **B** is identical to column **A** except that the ordinate has been expanded by a factor of 4 for better resolution of the leftmost bins containing complex spike intervals. This scaling factor affects the graphic display only; the actual numbers of intervals per bin is unchanged. In this instance, 5-HT caused a marked increase in the number of complex spikes (BURSTS) as indicated by the leftmost bins of the histogram. The asterisks correspond to CD interval range. The modal SS interval and MPCI value changed minimally. An increase in the regularity of firing induced by 5-HT can be seen also (narrowing of the histogram). The numerical values given for the burst parameter represent the number of CDs occurring within 120 s. The mode parameter represents the modal interval (measured in ms), i.e., the interval that occurred most frequently. The reciprocal of the mode served as an index of the frequency of the SS activity. Bin width is 1 ms. Recovery ISH was made within 10 min after terminating the ejection current for 5-HT

window settings for the detection of complex unit activity. Furthermore, when we began the studies and at frequent times thereafter, two or more members of the laboratory manually counted the number of CDs to insure that the discriminator and computer parameters were properly set to detect the complex activity. Because all recordings were made extracellularly, only CF responses that elicited complex discharges with two or more spikes could be counted by the computer. CF responses that may have elicited only an initial single spike succeeded by wavelets superimposed on a long-lasting depolarization and that were below the window threshold could not be detected.

Data analysis

We assessed drug effects qualitatively by tabulating the total number of cells tested versus the number of cells evincing a prescribed response. We quantitated drug effects on SS frequency and CF-evoked responses by comparing parameters of cell firing obtained during the iontophoresis of the drug with those recorded before administration of the drug and expressing the value as a percent change. For statistical analysis, a Student's t-test was used to compare singulary two means. A correlation coefficient was calculated as an index of the strength of a particular relationship.

Results

In the present experiments, Purkinje cells displayed spontaneous firing patterns of CDs interposed between SS activity. Complex discharges occurred at frequencies of 0.02–3 CDs/s (corresponding to a range of 2.4–360 CDs/120 s, average ± S.E.M., 0.47 ± 0.032 CDs/s), whereas SS ranged in firing rate

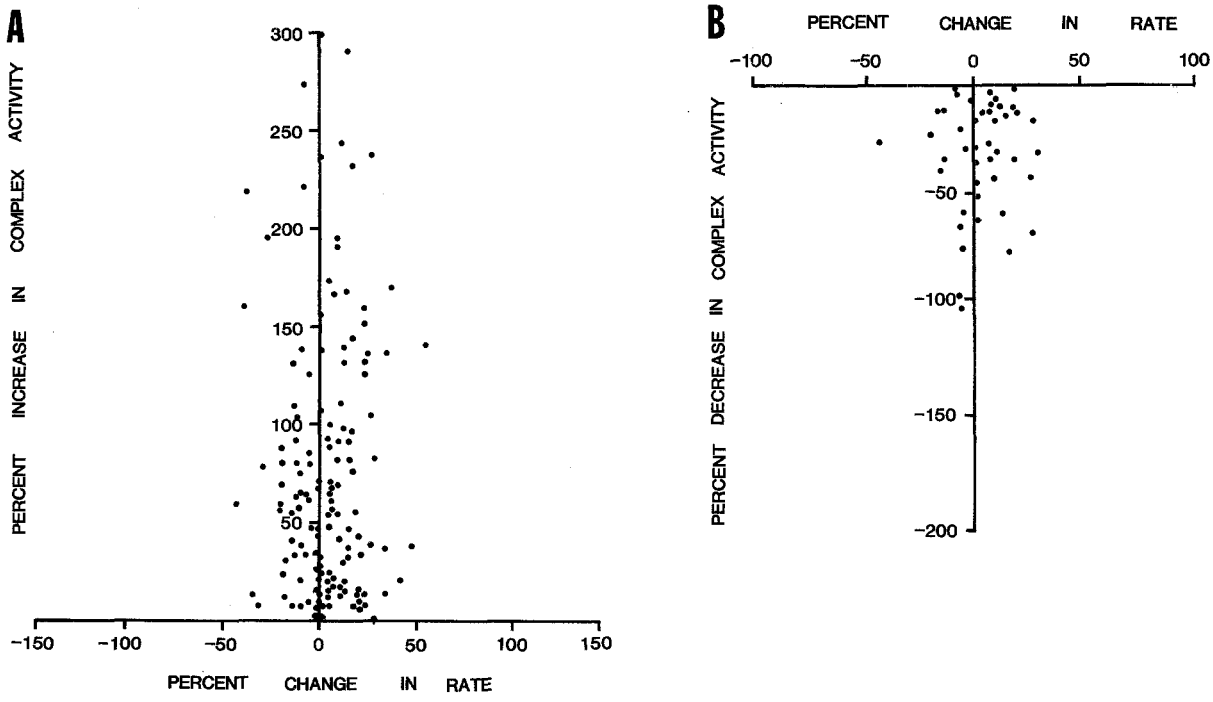

Fig. 3. **A** Scattergram comparing changes in spontaneous SS firing rate to increases in number of spontaneously occurring CDs in response to serotonin (20–30 nA). Serotonin produced a more marked enhancement of CD activity than SS activity. **B** Scattergram comparing changes in spontaneous SS firing rate to decreases in the numbers of spontaneously occurring CDs in response to serotonin (20–30 nA). Again, serotonin produced a more marked effect on CD activity than SS activity. Each point represents the response of a single Purkinje cell

from approximately 20–80 spikes/s (average ± S.E.M., 35.9 ± 1.99 spikes/s). The low frequency of CD occurrences necessitated a protracted analysis to ensure an accurate count of CDs. For this reason, we examined 120-s epochs before and during iontophoretic administration of serotonin. Serotonin (20–30 nA) increased the responsiveness of Purkinje cells to CD afferent activity preferentially without eliciting equivalent changes in SS activity. The effects of serotonin on CD activity lasted generally 10–15 min after the current was terminated, although on occasions the cell required 45 to 60 min for recovery to control values. In the majority of cases (132 of 177 cells), microiontophoretically administered serotonin enhanced the number of CDs as seen in Fig. 2 and 3A. Figure 2 displays interval histograms of a representative response from a Purkinje cell to iontophoretically administered serotonin. As the initial peak in the histogram (asterisk) shows, serotonin caused a 71.4% increase (from 63 CDs/120 s to 108 CDs/120 s) in the number of CDs while decreasing the SS modal interval only minimally from 23 ms to 21 ms, representing a 8.7% change. The scattergram in Fig. 3A depicts the percent increase in the number of complex spikes versus percent changes in SS activity for 132 cells. The average (± S.E.M.) percent increase in the number of complex spikes in the presence of serotonin was 94.0 ± 8.6 ($P < 0.0001$, $n = 132$, paired Student's t-test). The modal SS interval was decreased insignificantly by 3.7 ± 1.5% ($n = 132$). Of the remaining 47 cells, 45 responded to serotonin with a decrease in the number of CDs, and 2 failed to respond. Figure 3B reveals that decreases in the number of complex spikes were of lesser magnitude than were increases. The average percent decrease in the number of complex spikes in the presence of serotonin was 23.0 ± 4.0 (± S.E.M.). SS activity increased by an average of 2.6 ± 0.8% (± S.E.M.). Thus, from a qualitative and quantitative viewpoint, serotonin may exert a permissive action on the spontaneous CF-evoked activity of Purkinje cells.

Although in the majority of cells, 5-HT produced a net increase in the number of CDs, it is apparent from Fig. 4 that the qualitative effect is related in part to the initial firing rate of the Purkinje cell. A larger percentage of the faster-firing cells responded to serotonin with an increase in numbers of complex spikes, whereas serotonin increased spontaneous complex activity in a smaller percentage of cells that fired in the lower frequency ranges. Specifically, 67% of the Purkinje cells firing in the 10–20 spikes/s range

displayed increases in CS activity in response to iontophoretic serotonin, but such increases occurred in 85% of the cells firing in the 61–100 Hz range. The product moment correlation coefficient from this graph is $r = 0.85$ ($P < 0.05$).

Quantitatively, it was not possible to establish a strong linear correlation between SS interval and the corresponding number of complex spikes; therefore we divided those Purkinje cells in which 5-HT enhanced CDs arbitrarily into two approximately equal-sized groups on the basis of modal SS intervals (1–24 and 25–75 ms, corresponding to 42–1000 and 13–41 spikes/s, respectively). The numbers of CDs corresponding to each of these groups were tabulated before serotonin was administered. The average number (± S.E.M.) of complex discharges detected in 120 s for the 1- to 24-ms interval group (average modal interval of 18.9 ± 0.37) was 48.25 ± 6.11, whereas the 25- to 75-ms group (average modal interval of 35.9 ± 1.1) averaged 69.1 ± 8.2 CDs. The numbers of CDs in the two groups were significantly different from each other ($P < 0.05$). Generally, Purkinje cells with larger modal intervals (slower simple spike rates) tended initially to have higher numbers of CDs. One additional correlation was made to relate directly the magnitude of the changes in CD activity in response to serotonin to the predrug number of complex spikes. A relationship between the predrug number of CDs and the magnitude of change elicited in this number by serotonin was evident; these data are displayed in Table 1. There is an apparent inverse relationship between these two parameters in that a greater percentage increase in the number of CDs was observed in those cells that displayed intially fewer CDs (product moment correlation coefficient, $r = 0.799$, $P < 0.05$). A similar correlation for serotonin-induced decreases in CDs was not evident. Thus, serotonin enhanced the number of CDs in the majority of Purkinje cells tested, and the magnitude of the observed enhance-

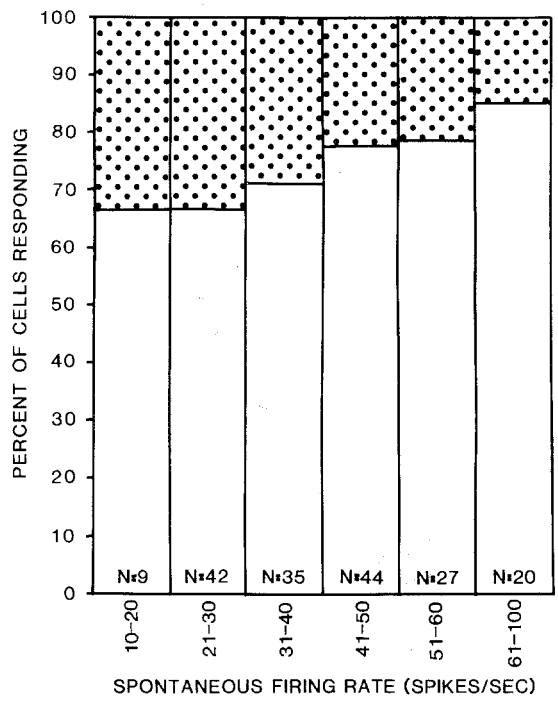

Fig. 4. Histogram demonstrating a correlation between the average predrug SS frequency of Purkinje cells and the action of serotonin (20–30 nA) on the number of spontaneously occurring CDs. The clear bars represent the percentage of Purkinje cells responding to serotonin with an increase in CDs, whereas the dotted bars represent the percentage of cells in each of the frequency ranges that responded to serotonin with a decrease in CDs. The product moment correlation coefficient is $r = 0.85$ ($P < 0.05$)

Table 1. The relationship between the number of spontaneously occurring complex discharges in a 120-s control epoch and the enhancement of this activity by serotonin (20–30 nA). Serotonin-induced increases in the number of complex discharges are inversely related to the complex discharge activity in the control situation. A paired Student's t-test was employed for statistical analysis

Range of complex discharges in 120 s	Predrug average number of complex discharges ± SEM in 120 s	Average number of complex discharges ± SEM during serotonin (120 s)	Percent increase in average number of complex discharges with serotonin	Number of neurons
1–10	6.15 ± 0.66	16.08 ± 2.57[a]	161.5	19
11–30	20.81 ± 1.00	50.92 ± 4.44[a]	144.7	48
31–50	39.08 ± 0.98	75.62 ± 6.71[a]	93.5	32
51–70	58.20 ± 1.42	87.87 ± 5.92[a]	51.0	27
71–90	80.67 ± 1.43	127.11 ± 15.79[a]	57.6	16
91–110	100.71 ± 2.63	123.71 ± 6.90[a]	22.8	12
111–130	118.38 ± 2.04	154.75 ± 10.06[a]	30.7	11
131–400	195.44 ± 28.54	261.89 ± 62.71[a]	34.0	12

[a] Indicates a significant difference from the predrug value ($P < 0.05$)

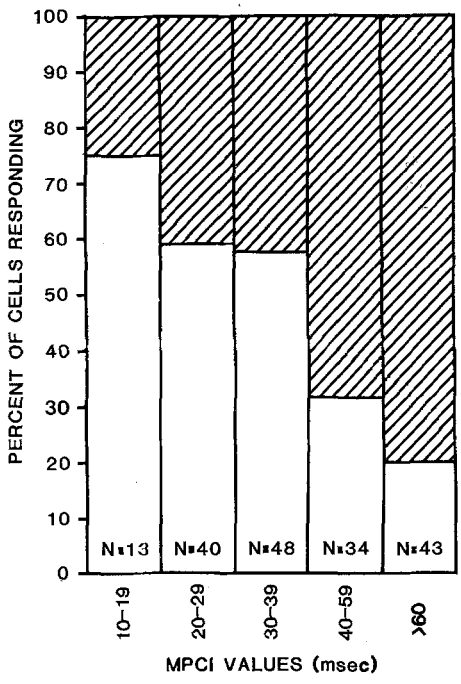

Fig. 5. Histogram depicting a correlation between the average predrug MPCI values of Purkinje cells and the action of serotonin on MPCI values. The clear bars represent the percentage of Purkinje cells responding to serotonin with an increase in MPCI value, and the hatched bars represent the percentage of cells in each of the MPCI categories responding to serotonin with a decrease in MPCI. Qualitatively, the effectiveness of 5-HT in altering the MPCI value is dependent on the initial MPCI value of the Purkinje cell. The product moment correlation coefficient is $r = 0.939$ ($P < 0.05$)

ment was related inversely to the predrug number of CDs.

Another component of the CD pattern analyzed in this study was the MPCI phase. We performed qualitative analyses of factors that could serve to predict the effects of serotonin on the MPCI value of a cell and found that the predrug MPCI values could serve as an index of the probability of an increase or decrease in this pause as shown in Fig. 5. The total population of cells was divided into 5 groups on the basis of the predrug MPCI duration (ms): 10–19, 20–29, 30–39, 40–59, and 60 or longer. The proportions of Purkinje cells in which serotonin increased or decreased the MPCI were calculated separately for each category. It is evident that with increasing MPCI values, the proportion of cells evincing a shortening in the MPCI became greater and vice versa (product moment correlation: $r = 0.939$, $p < 0.05$).

We sought further to determine whether in addition to this qualitative relationship, a similar correlation could be shown by quantitative analysis of the effects of 5-HT. Inspection of Table 2 reveals a direct relationship between the average predrug MPCI value and the average predrug SS-interval mode. It is evident from this comparison that cells that display longer modal intervals (slower-firing cells) exhibit higher MPCI values, whereas cells that display shorter modal intervals (faster-firing cells) have characteristically lower MPCI values ($r = 0.987$, $P < 0.05$). Serotonin exhibited a bimodal effect on the computed MPCI values by enhancing significantly the average MPCI values of cells displaying low MPCI values (20–29 ms range) and decreasing significantly the average MPCI values of cells displaying high values (50 ms or longer). The cells exhibiting MPCI values in the middle range (30–49 ms) were not affected by iontophoretically administered serotonin. Thus, cells that had relatively high SS frequencies responded to serotonin with an increase in MPCIs and vice versa.

We administered by iontophoresis the putative serotonin antagonists methysergide ($n = 11$), metergoline ($n = 3$), and ketanserin ($n = 5$) in a limited number of experiments to determine their various abilities to block the effects of serotonin. The compounds were applied at low currents (< 10 nA) for relatively long periods of time (minutes). None of the compounds had any direct effect on SS or CD activities when administered in this manner, although higher currents could lead to alteration of firing patterns. Specifically, higher currents of all three antagonists appeared to exert weak agonistic actions. Methysergide reduced serotonin-mediated increases in complex bursts from 138% to 24%, a difference of 114% ($P < 0.05$, $n = 11$, paired Student's t-test), whereas metergoline antagonized the actions of serotonin effectively by a difference of 88%, Table 3. Ketanserin was ineffective as an antagonist in all cases (data not shown, 5 cells). These results suggest a degree of receptor specificity for serotonin effects. Figure 6 displays interval histograms of a representative response from a Purkinje cell to iontophoretically administered serotonin before and during iontophoretic administration of methysergide. Serotonin increased the number of CDs from 51 CDs/120 s to 117 CDs/120 s, representing a 129.4% increase. Methysergide attenuated the 5-HT induced suppression of the MPCI value. A question arises as to whether the effects of iontophoretic serotonin may be caused in part by actions of the creatinine sulfate salt complex. We have documented previously that creatinine sulfate alone is ineffective in altering SS activity of Purkinje cells (Lee et al. 1985) and have found recently that this agent fails to alter CDs (unpublished observations).

Table 2. The relationship between the predrug MPCI ranges and the effect of serotonin (20–30 nA) on the average modal simple spike interval and MPCI. A Student's t-test was employed for statistical analysis

Predrug average modal simple spike interval ± SEM (ms)	Average modal simple spike interval with serotonin ± SEM (ms)	% Change	Range of predrug MPCI values (ms)	Predrug average MPCI values (ms) ± SEM (ms)	Average MPCI values (ms) with serotonin ± SEM (ms)	% Change	Number of neurons
16.85 ± 1.36	17.08 ± 1.40	+ 1.4	10–19	16.38 ± 0.70	19.46 ± 1.87	+18.1	13
20.06 ± 0.84	20.06 ± 1.17	0.0	20–29	24.33 ± 0.50	27.28 ± 1.57[a]	+12.1	40
24.04 ± 0.79	23.6 ± 0.77	− 1.8	30–39	35.04 ± 0.42	35.15 ± 1.85	+ 0.3	48
29.17 ± 1.90	27.94 ± 1.74	− 4.2	40–49	43.78 ± 0.77	43.00 ± 3.08	− 1.9	20
31.58 ± 1.67	28.25 ± 2.00	−21.1	50–59	53.83 ± 1.04	37.92 ± 2.45[a]	−29.6	14
34.25 ± 1.92	29.75 ± 1.84	−13.1	60–69	63.82 ± 0.76	42.87 ± 2.56[a]	−32.8	16
45.62 ± 3.89	37.77 ± 3.32	−17.1	>70	109.42 ± 8.19	67.37 ± 5.2[a]	−38.0	27

[a] indicates a significant difference from the predrug value ($P < 0.05$)

Table 3. Effects of two putative serotonin antagonists on serotonin-elicited increases in the average number of complex discharges in 120 s. A Student's t-test was employed for statistical analysis

	Methysergide			Metergoline		
	Predrug CDs (SEM)	CDs during 5-HT (SEM)	CDs during 5-HT and methysergide (SEM)	Predrug CDs (SEM)	CDs during 5-HT (SEM)	CDs during 5-HT and metergoline (SEM)
	27.9 (7.2)	66.4 (13.8)[a]	34.6 (10.5)[b]	49.3 (13.5)	115.3 (11.7)[a]	72.0 (13.8)[b]
% Change	–	138	24	–	134	46
# of neurons	11	11	11	3	3	3

[a] indicates a significant difference from the predrug value ($P < 0.05$)
whereas [b] indicates a significant difference from the response obtained with serotonin alone

Discussion

Recent electrophysiological and neurochemical studies have revealed multiple receptors for serotonin in the central nervous system (CNS). Microiontophoretic administration of serotonin to single CNS neurons has shown that this amine inhibits cell firing predominantly in some structures such as the lateral geniculate nucleus and amygdala (Rogawski and Aghajanian 1980; Wang and Aghajanian 1977); in the neocortex, excitatory and biphasic responses are seen also (Bradshaw et al. 1983; Reader et al. 1979; Lakoski and Aghajanian 1985). Moreover, in brainstem motor nuclei and spinal cord ventral horn, serotonin facilitates excitatory neurotransmission, but produces no direct effect on cell firing itself (McCall and Aghajanian 1979; White and Neuman 1980). On the basis of the diverse nature of neuronal responses to serotonin, the term modulator has been employed frequently to describe this amine (Aghajanian 1981).

Previous reports from our laboratory have detailed a complex action of iontophoretically administered serotonin and raphe stimulation on SS firing characteristics of cerebellar Purkinje cells (Strahlendorf et al. 1979, 1984). The present study extends these findings to include a specific action of serotonin on spontaneously occurring CD activity. Within the majority of Purkinje cells tested, iontophoretically administered 5-HT induced a marked enhancement in the number of CDs. In the present study, we surmised that the SS frequency is an important factor in determining the effect of serotonin on CD activity after we observed a progressive increase in the probability of serotonin enhancing the number of CDs in Purkinje cells with increasing SS firing rates. Our earlier study (Strahlendorf et al. 1984) revealed a rate-dependency component for the actions of serotonin on SS activity in that with increasing firing rates, the proportion of cells depressed by serotonin increases. From the present and previous studies, it is apparent that the cell popula-

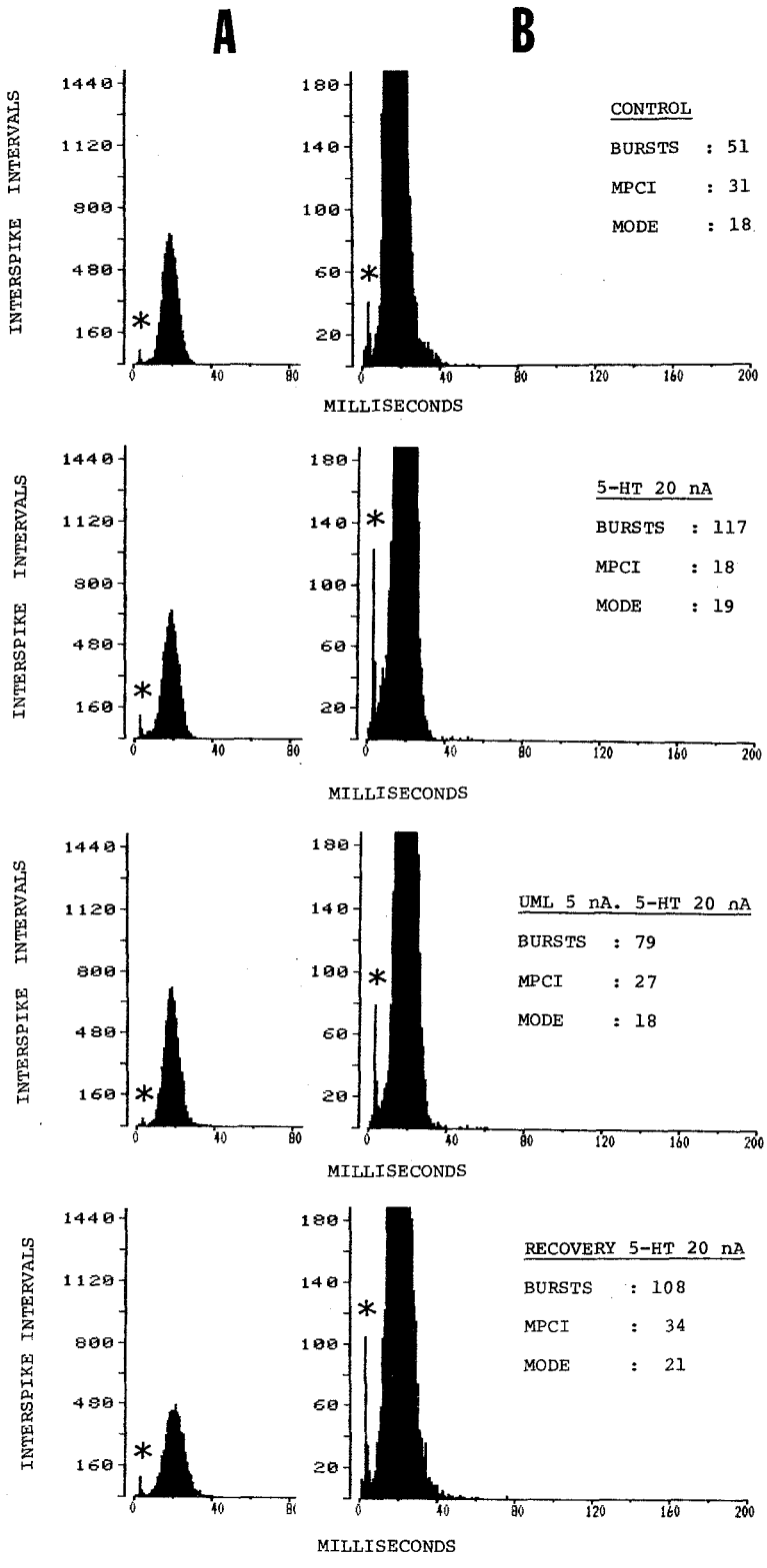

Fig. 6A and B. Computer-generated interspike interval histograms demonstrating the effectiveness of 5 nA methysergide (UML) as an antagonist of the facilitatory effects of 20 nA 5-HT on complex spike activity. Column **B** is identical to column **A** except that the ordinate has been expanded by a factor of 8 for better resolution of the leftmost bins containing complex spike intervals. This scaling affects the graphics display only, the actual number of intervals per bin is unchanged. The asterisks correspond to the CD interval range. The numerical values given for the burst parameter represent the number of CDs occurring within 120 s. The mode parameter represents the modal interval (measured in ms), i.e., the interval that occurred most frequently. Note the reversible nature of the antagonism by methysergide in the third and fourth panels. Methysergide did not affect the firing characteristics of the neuron. An antagonism of 5-HT effects on the MPCI is evident also. Bin width is 1 ms. Recovery ISH was made approximately 12 min after the ejection current for methysergide was terminated.

tion that responds most readily to serotonin with an increase in the number of CDs is the same population that would be expected to show a reduction in SS frequency after pulsatile administration of serotonin. The question arises as to whether the effects of serotonin on SS and CD activities are independent of one another or whether altering one of the parameters (e.g., increasing the number of CDs) would

result in a slowing of SS activity. In relation to this question, it is of interest that the slower-firing cells, in which serotonin would be predicted to accentuate SS activity, are as a whole less responsive to serotonin in terms of increased CDs. On the other hand, increases in CDs were accompanied frequently by small increases in SS frequency. Thus, the question remains unresolved.

On the basis of these qualitative findings, we made a series of quantitative comparisons in control situations and in the presence of 5-HT. Although no apparent linear correlation could be established between modal SS intervals and the number of CDs, it is still evident that as a class, faster-firing cells exhibit fewer numbers of CDs and that the reverse is true for the slower-firing cells. Such an inverse relationship between SS and CD firing rates has been reported by a number of other investigators (for review, see Ito 1984). It is apparent that this relationship is maintained in the presence of 5-HT. Although serotonin increased the numbers of CDs in both groups, it was less effective on a percentage (quantitative) basis in accentuating the number of complex spikes in the group with lower SS modal intervals and correspondingly higher numbers of CDs. This relationship is substantiated further by the fact that when the population of cells was sorted on the basis of the predrug numbers of CDs rather than SS modal intervals, a marked linear correlation was found between the initial number of CDs and the magnitude of the increases induced by serotonin. These correlations suggest a possible ceiling effect for the efficacy of the action of serotonin on CDs. The composite picture drawn from qualitative and quantitative analyses is that Purkinje cells with shorter SS intervals (faster firing) are more likely to be inhibited by pulsatile applications of serotonin: they display more frequently a lower number of CDs; they have a greater probability of increasing CDs during 5-HT application, and they respond with larger increases in CDs. On the other hand, neurons with slower simple spike frequencies (in which 5-HT would be predicted to increase SS activity) have a higher number of CDs initially, are less likely to increase their CD activity in response to serotonin, and exhibit increases of lesser magnitude.

In our present experiments, the most marked effect of iontophoretic 5-HT was to increase the number of CF bursts per unit time in the presence of slight increases or decreases in SS activity. This may appear at first to contradict the hypothesis that CDs serve to govern the SS rate (Colin et al. 1980; Montarolo et al. 1982; Ito 1984). It is probable that this apparent discrepancy derives from differences in experimental design. In the experiments of Ito et al. (1982), the inferior olive was activated at rates ranging from 10–100 Hz, presumably producing CF-evoked bursts of Purkinje cells at rates approximating these frequencies. Our experiments quantitated the number of spontaneously occurring CDs, which ranged in frequency from 0.02 to 3 Hz, frequencies much lower that those used by others. Thus, any effect of CDs on SS activity measured for 120 s was probably negligible.

At this point, it is tempting to speculate that the effects of 5-HT on CF-evoked activity provides a mechanism by which the serotonergic afferents to the cerebellum modulate impulse traffic in the neuronal network. We have reported recently that iontophoretic serotonin attenuates glutamate-induced excitations of Purkinje cells specifically and reversibly (Lee et al. 1985). Furthermore, Ito et al. (1982) have demonstrated conclusively that conjunctive stimulation of the inferior olive and the vestibular nerve causes depression of mossy-fiber responsiveness of Purkinje cells. Moreover, stimulation of the inferior olive and the subsequent CF responses of Purkinje cells depress the excitatory response of these target neurons to iontophoretic glutamate. Collectively, these data from our laboratory and Ito's might imply that serotonin-induced facilitation of CF-evoked bursts provides a modulatory tuning of the target Purkinje cell, thereby permitting CD activity to govern the responsiveness of Purkinje cells to subsequently occurring mossy-fiber signals. This could be extended to include coordinating effects in the inferior olive, which receives a rich innervation of serotonergic fibers (Wiklund et al. 1977), the function of which is believed to be involved in the electrotonic coupling of olivary cells (Sjölund et al. 1977).

Often after a CF-evoked burst, normal Purkinje cells exhibit depression of SS activity (pause) for 40 ms or more (Granit and Phillips 1956). As a component of the total CF response, these pauses probably contribute to the shaping of impulse traffic through the cerebellar cortex. Moreover, as potential modifiers of neuronal impulse flow, these inhibitory periods appear to be susceptible to modification by serotonin. It is significant that in our studies a direct relationship appears to exist between predrug values of MPCI and average modal SS intervals. In a similar relationship described previously, the duration of the CS pause (MPCI) was reported to be related inversely to the SS firing frequency in the period just preceding the CS (Latham and Paul 1971). Our observation that serotonin-induced decreases in MPCI were accompanied most commonly by an increase in SS activity supports and possibly extends the concept that SS frequency is related inversely to

the MPCI value. The possible significance of this, as suggested initially by others, is that the total CF response shapes neuronal traffic in the cerebellar cortex and that serotonin is able to modulate this tuning further. Our data demonstrated also a correlation between the initial predrug MPCI value and the effect of serotonin on the MPCI. Lower MPCI values tended to be increased by serotonin, whereas higher initial values were most often decreased. This bimodal effect of serotonin on the MPCI is very similar to effects of pulsatile serotonin on SS intervals reported previously by us (Strahlendorf et al. 1984). Collectively, the present data and those from our previous study demonstrate similar and apparently parallel effects of 5-HT on the SS interval and MPCI. In this respect, it is of interest that correlation histograms describing the effects of 5-HT on MPCI (Fig. 6) and spontaneous firing rates (see note added in proof, Strahlendorf et al. 1984) are almost identical in shape with matching product moment correlation coefficients.

We tested the effects of the serotonin antagonists ketanserin, metergoline, and methysergide on serotonin-induced enhancements of CDs because these enhancements were observed most frequently in our study. These antagonists have not been tested consistently in the few instances in which serotonin inhibited the CDs. As in our previous studies on SS activity, methysergide and metergoline were both effective in attenuating serotonin-induced enhancements, whereas ketanserin, the purported 5-HT_2 antagonist, has proven to be ineffective in antagonizing the enhancements of SS or complex discharges. These data suggest that a common 5-HT receptor may underlie 5-HT-induced excitations of SS and CD activities of Purkinje cells.

The results of these experiments demonstrate yet another action of serotonin on cerebellar Purkinje cells. As far as we are aware, these are the first reported studies of the action of serotonin on spontaneously occurring CF responses. These studies describe a facilitatory action of serotonin on electrophysiologic responses recorded from Purkinje cells, the target neurons of the climbing fibers. It is conceivable that the facilitatory action of serotonin on spontaneously occurring CF responses could arise from an enhanced efficacy of junctional events at the CF-Purkinje cell synapse. The sites at which these actions of serotonin are exerted may include both pre- and postjunctional elements because diffusion of serotonin from the micropipette cannot be limited to the area of the target neuron. Although delineation of the mechanisms underlying 5-HT actions awaits investigation by intracellular recording, it appears that 5-HT may, as a neuromodulator of many facets of Purkinje cell activity, function to shape and govern output to nuclear cells.

Acknowledgements. This work was supported by NIH grant R01NS19296 to J. C. and H. K. Strahlendorf. The authors extend their appreciation to Judith Keeling and Christina Bridges for assisting in the preparation of the manuscript, and to Randy Pierce for designing the computer software.

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Received June 4, 1985 / Accepted September 18, 1985