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Neuron–Glia Signaling via α_1 Adrenoceptor-Mediated Ca^{2+} Release in Bergmann Glial Cells *In Situ*

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Adrenoceptors were among the first neurotransmitter receptors identified in glial cells, but it is not known whether these receptors meditate glial responses during neuronal activity. We show that repetitive nerve activity evoked a rise of intracellular calcium in Bergmann glia and neighboring Purkinje neurons of cerebellar slices of mice. The glial but not the neuronal calcium transient persisted during block of ionotropic and metabotropic glutamate receptors. In contrast, the glial calcium response was abolished by cyclopiazonic acid and prazosin; however, prazosin affected neither the inward current nor the resulting depolarization that accompanied the stimulus-induced glial calcium transients. The glial depolarization was attenuated by 38% by the mixture of glutamate receptor blockers, which abolished the evoked neuronal depolarization and afterhyperpolarization. Ba^{2+} reduced the glial currents by 66% without affecting the concomitant calcium transients. In the presence of Ba^{2+}, the mixture of glutamate receptor blockers exerted no effect on the glial inward current or calcium rise. Furthermore, Ba^{2+} greatly potentiated both the activity-related Purkinje cell inward current and the accompanying neuronal calcium rises. The results indicate that release of noradrenaline from afferent fibers activates a glial α_1 adrenoceptor that promotes calcium release from intracellular stores. Glial calcium rises are known to stimulate a diversity of processes such as transmitter release, energy metabolism, or proliferation. Thus the adrenoceptor-mediated mechanism described here is well suited for feedback modulation of neuronal function that is independent of glutamate.

Key words: adrenoreceptor; epinephrine; locus coeruleus; neuroglia; neurotransmitter receptors; noradrenaline

There is increasing evidence that glial cells are pivotal for signaling within the brain. Neuronal activity induces a diversity of glial responses, such as membrane depolarization, release of neurotransmitters, proliferation, or stimulation of aerobic metabolism, that modulate neuronal excitability in reverse (Murphy et al., 1993; Ballanyi, 1995; Tsacopoulos and Magistretti, 1996; Pfrieger and Barres, 1997; Newman and Zahs, 1998). Glial cells possess numerous types of receptors that are thought to constitute the major pathway of information transfer from neurons to glia during activity-related interstitial accumulation of neuroactive substances (Murphy and Pearce, 1987; Porter and McCarthy, 1997). Elevation of the concentration of free intracellular Ca^{2+} (Ca_i), which follows activation of most of these receptors, plays a crucial role in the induction of glial activity (Verkhratsky and Kettenmann, 1996; Verkhratsky et al., 1998). However, analysis of the origin of glial Ca_i rises, which occur during (electrically) induced neuronal activity *in situ*, is hampered by the fact that several neuroactive substances accumulate in the interstitial space because of synaptic or nonsynaptic release or reversed transmitter uptake (Kriegler and Chiu, 1993; Ballanyi, 1995; Robitaille, 1995; Kang et al., 1998). In those few cases that provide experimental evidence for one particular mechanism of activity-related Ca^{2+} signaling in glia of functionally intact central nervous tissue, metabotropic glutamate receptors appear to be involved (Dani et al., 1992; Porter and McCarthy, 1996; Pasti et al., 1997).

Besides glutamate receptors, adrenoceptors constitute one of the most abundant classes of neurotransmitter receptors in glia of different phyla (Murphy and Pearce, 1987; Porter and McCarthy, 1997). In particular for astrocytes, it was demonstrated that agonist-evoked activation of α_1 adrenoceptors induces a glial Ca_i transient (Duffy and MacVicar, 1995; Porter and McCarthy, 1997). Adrenoceptors were among the first neurotransmitter receptors identified on glial membranes several decades ago (Clark and Perkins, 1971; Gilman and Nirenberg, 1971). Nevertheless, evidence of their involvement in neuron–glia signaling is still lacking. In the present study, we have thus tested whether adrenoceptors mediate a rise of glial Ca_i during nerve activity *in situ*. For this purpose, Ca_i was microfluorometrically measured in Bergmann glial cells of cerebellar slices from juvenile mice. These glial cells are well suited for such analysis because they respond to bath-applied noradrenaline (NE) with a noticeable Ca_i rise (Kirischuk et al., 1996a), and also activation of afferent nerve fibers induces a robust rise of Ca_i (Grosche et al., 1999). Furthermore, Bergmann glia are closely associated (Grosche et al., 1999) with Purkinje neurons, in which the modulating effects of stimulation of NE-containing afferent nerve fibers from locus coeruleus on neuronal excitability were thoroughly studied (Bickford-Wimer et al., 1991; Woodward et al., 1991).

The results show for the first time that nerve activity produces a rise of intracellular Ca^{2+} in glia that is mediated via α_1 adrenoceptors. Because these adrenoceptor-mediated glial Ca_i transients are not affected by block of both ionotropic and metabotropic glutamate receptors, the noradrenergic system con-

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stitutes a pathway of signal transfer from neurons to glia that does not depend on glutamatergic neuronal activity.

MATERIALS AND METHODS

Slice preparation and solutions. The experiments were performed on cerebellar slices obtained from 18- to 29-d-old mice of either sex. The animals were anesthetized with ether and decapitated. The cerebellum was isolated and kept for 5 min in ice-cold artificial CSF (standard solution). Sagittal slices (150 μm) were cut and stored (<7 hr) at 30°C in standard solution. In the recording chamber (volume 3 ml), slices were superfused at 30°C with oxygenated standard solution (flow rate 5 ml/min) of the following composition (in mM): 118 NaCl, 3 KCl, 1 $MgCl_2$, 1.5 $CaCl_2$, 25 $NaHCO_3$, 1.2 NaH_2PO_4 and 10 D-glucose. The pH was adjusted to 7.4 by gassing with 95% O_2 and 5% CO_2. In the Ca^{2+}-free solution, which also contained 1 mM EGTA as a Ca^{2+} buffer, the Mg^{2+} concentration was elevated to 5 mM. Drugs were purchased from Sigma (München, Germany), Biomol (Köln, Germany), or Tocris Cookson (Bristol, UK). For Ca_i measurements in intact Bergmann glia, slices were exposed for 30 min at 37°C to 10 μM fura-2 AM. Under these conditions, exposure to fura-2 AM resulted in a selective staining of Bergmann glia in the Purkinje cell layer and of granule cells in the granule cell layer (Kirischuk et al., 1995, 1996a). The smaller size of the glial cell somata and the characteristic shape and orientation of their processes differed considerably from those of Purkinje neurons.

Intracellular recording. Patch pipettes were produced from borosilicate glass capillaries (GC 150TF, Clark Electromedical Instruments, Pangbourne, UK) using a horizontal electrode puller (Zeitz, München, Germany). The standard patch pipette solution (osmolarity 270–285 mOsm) contained (in mM): 140 K-gluconate, 1 Na_2-ATP, 1 $MgCl_2$, 0.5 $CaCl_2$, 1 K_2-BAPTA, 10 HEPES, pH 7.3–7.4. The DC resistance of the electrodes ranged from 4 to 6 MΩ. For measurements of Ca_i, 100–200 μM fura-2 (Molecular Probes, Eugene, OR) was added to the BAPTA- and Ca^{2+}-free patch pipette solution before the experiment. Whole-cell recordings were performed on superficial Bergmann glial cells and Purkinje neurons under visual control (Axioscope Zeiss, Oberkochen, Germany; equipped with a 63× water immersion objective Achroplan, NA 0.9). The EPC-9 patch-clamp amplifier (HEKA, Lambrecht, Germany) was driven by Pulse/Pulsefit software (HEKA) on a PowerPC (Apple Computer, Cupertino, CA). Seal resistance ranged from 1 to 3 GΩ, and series resistance was between 10 and 25 MΩ. Holding potential in voltage-clamp was −80 mV (Bergmann glia) or −60 mV (Purkinje neurons), unless otherwise indicated.

Stimulation. A glass microelectrode (outer diameter 10–15 μm) that was filled with superfusate (DC resistance 1 MΩ) was used for electrical stimulation (Digitimer stimulator 3072, Master-8 A.M.P.I., Jerusalem, Israel; single-pulse duration 200 μsec). In an initial series of experiments, the stimulation electrode was positioned in the molecular layer. Repetitive stimulation with 50 Hz revealed Ca_i rises of between 30 and 100 nM in ~50% of ester-loaded Bergmann glial cells (n = 17). Basically, similar glial Ca_i rises were detected when the stimulation electrode was positioned in the granule cell layer at a distance >100 μm from the recorded Bergmann glial cell. Because the latter type of stimulation evoked with high probability (>80%), on average, larger Ca_i transients and these responses were also more stable on consecutive application of tetanic stimuli, pharmacological analysis was performed in slices in which the stimulation electrode was positioned in the granule cell layer.

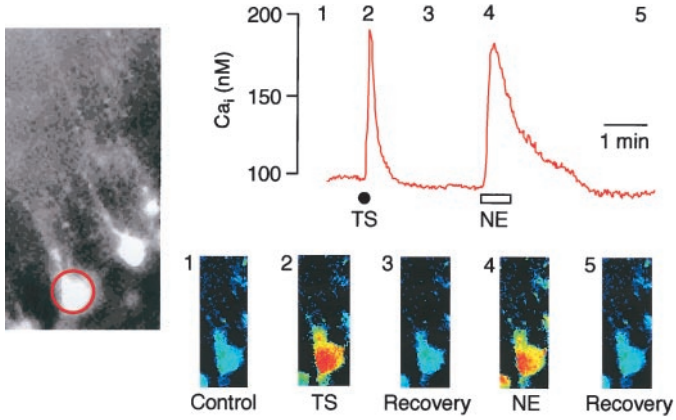

Figure 1. Stimulus-evoked and norepinephrine (*NE*)-induced rises of intracellular Ca^{2+} (Ca_i) in a Bergmann glial cell. Ca_i was ratiometrically measured with digital imaging techniques in the soma (region of interest marked in *red*) of a Bergmann glial cell in a cerebellar slice that was ester-loaded with the Ca^{2+} indicator fura-2 AM. Tetanic stimulation (*TS*; 75 V, 50 Hz, 2 sec) in the granule cell layer evoked a robust Ca_i transient, similar to that elicited by bath application of 10 μM NE. The *bottom row* shows a series of Ca_i images measured at the time indicated by the corresponding *numbers* in the continuous Ca_i recording.

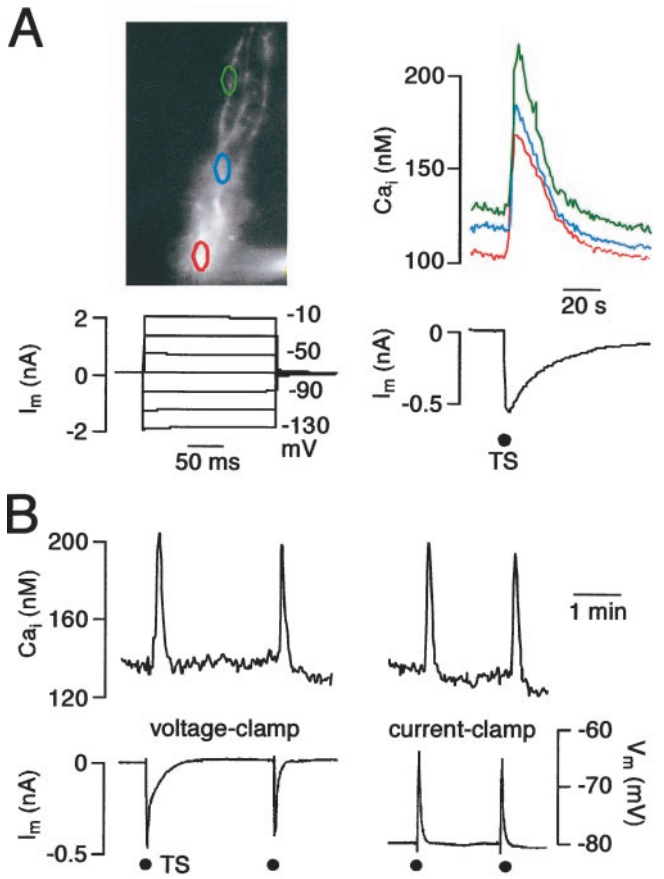

Figure 2. Activity-induced Ca_i rises and membrane response in whole-cell recorded Bergmann glial cells. *A*, Dialysis of a cell with fura-2 via the patch electrode revealed the typical morphology of Bergmann glia (patch electrode was positioned at the right side of the soma). In voltage-clamp, tetanic stimulation (*TS*, 75 V, 50 Hz, 2 sec) caused an inward current (holding potential −70 mV), accompanied by Ca_i rises in the soma as well as in the cell processes (color of traces correspond to those of regions of interest). The linear current–voltage relation caused by voltage pulses between −130 and −10 mV is typical for a glial cell. *B*, In a different Bergmann glial cell, the stimulus-induced Ca_i rise did not change after switching from voltage-clamp (holding potential −80 mV) to current-clamp. I_m, Membrane current; V_m, membrane potential.

Fluorescence measurements. Fluorescence measurements were performed with either a photomultiplier (Luigs & Neumann, Ratingen, Germany) or an imaging system using a 12-bit CCD camera (T.I.L.L. Photonics, Planegg, Germany) fixed to an upright microscope (Axioskop or Standard 16, Zeiss, Oberkochen, Germany). The microscope was equipped with epifluorescence optics and a monochromator (Polychrome II, T.I.L.L. Photonics) to allow alternating fluorescent excitation at 360 and 380 nm. Emission light was measured at 510 nm. While the photomultiplier system was being used, a pinhole diaphragm limited the region from which light was collected to a circular spot of 20 μm diameter to avoid disturbances from background illumination. Fluorescence ratios were converted into Ca_i by using Equation 1 ($Ca_i = K\,(R - R_{min})/(R_{max} - R)$, in which R is the fluorescence ratio (360 nm/380 nm) and K is the effective dissociation constant of fura-2. *In vivo* calibration to determine

R_{min}, R_{max}, and K was performed. Briefly, measurements were performed with three different pipette solutions that contained (in mM): (1) 130 KCl, 1 $MgCl_2$, 10 BAPTA, 10 HEPES, 1 Na_2-ATP (low Ca^{2+}; R_{min}); (2) 130 KCl, 1 $MgCl_2$, 3 $CaCl_2$, 4 BAPTA, 10 HEPES, 1 Na_2-ATP (intermediate Ca^{2+}; 300 nM, according to a K_D of 107 nM for BAPTA; (3) 130 KCl, 1 $MgCl_2$, 10 $CaCl_2$, 10 HEPES, 1 Na_2-ATP (high Ca^{2+}; R_{max}); 100 μM fura-2 was added to each solution. The resulting intracellular fluorescence ratios were calculated according to Equation 1. K was calculated as $K = 300\ nM\ (R_{max} - R)/(R - R_{min})$. For further details and references, see Ballanyi and Kulik (1998).

Data analysis. Fluorescence and electrophysiological signals were sampled at 3 Hz and 1 kHz, respectively, by the PowerPC (Apple) via the ITC-16 interface of the EPC-9 amplifier using the X-Chart extension of the Pulse/Pulsefit software (HEKA). Analysis of the data was performed with IGOR software (Wavemetrics, Lake Oswego, OR). Images were sampled on an IBM-compatible computer using T.I.L.L. vision software. Further image processing was performed using Adobe Photoshop software (Adobe Systems, Mountain View, CA) and CANVAS (Deneba software, Miami, FL). Values are mean ± SEM.

RESULTS

Stimulus-evoked Ca_i rises in fura-2 AM-loaded Bergmann glia

As demonstrated recently (Grosche et al., 1999), stimulation of afferent fibers with parameters that induce routinely synaptic responses in Purkinje neurons (Bickford-Wimer et al., 1991; Llano et al., 1991; Batchelor et al., 1996; Takechi et al., 1998) elicits local Ca_i signals in the soma and processes of Bergmann glial cells. In the present study, tetanic stimulation with 50 Hz was performed within the granule cell layer. In a total of 12 preparations, such stimulation increased Ca_i by 40–200 nM in fura-2 AM ester-loaded Bergmann glia cells within an area of ±50 μm along the perpendicular axis in the Purkinje cell layer with regard to the stimulation electrode. As measured in eight cells, NE (10 μM) led to a similar (Fig. 1) Ca_i transient (98.0 ± 6.2 nM vs 101.5 ± 8.8 nM during stimulation). In contrast, administration of NE did not change Ca_i either in four whole-cell-recorded Purkinje neurons or in 12 fura-2 AM ester-loaded granule cells (data not illustrated).

Stimulus-evoked Ca_i rises in whole-cell recorded Bergmann glia

Because spatial resolution of the stimulus-induced Ca_i transients was limited in the ester-loaded slices, individual glial cells were dialyzed with 100 μM fura-2 via the patch electrode during whole-cell recording ($n = 54$). These cells (Fig. 2*A*) and also nine Bergmann glial cells that were dialyzed with 1 mg/ml lucifer yellow showed the typical morphology. Several parallel processes that extended through the molecular layer and terminated underneath the pia originated from a soma with a diameter of 6–10 μm [see also Tempia et al. (1996); Bergles et al. (1997); Grosche et al. (1999)]. The glial cells were electrophysiologically characterized by an input resistance of <25 MΩ, a linear current–voltage relation (Fig. 2*A*), and a resting potential (−81 ± 4.5 mV; $n = 6$) that was close to the expected K^+ equilibrium potential (Ballanyi et al., 1987; Ballanyi, 1995). As a further indication that recordings were performed on glia, electrical stimulation did not induce action potentials or postsynaptic potentials.

Fura-2 loading of individual Bergmann glial cells (Fig. 2*A*) revealed that the stimulus-induced Ca_i rises occurred both in the somatic region and in dendritic processes in 70% of cases ($n = 46$). In 18% of cells, stimulation evoked a Ca_i rise solely in the soma, and in 12% of observations Ca_i transients were seen exclusively in the processes. As described above for the fura-2 AM ester-loaded Bergmann glia (Fig. 1), NE led to a Ca_i transient of similar magnitude (86 ± 13.6 vs 83.9 ± 9.8 nM) in 10 cells that responded with a clear rise of intracellular Ca^{2+} to electrical stimulation. At a holding potential of −80 mV, the stimulus-evoked Ca_i transients were accompanied by an inward current with an amplitude of between 0.2 and 0.8 nA (Fig. 2*A*), whereas 10 μM NE induced an inward current of <50 pA (data not shown). As measured in six cells under current-clamp, the inward current on tetanic stimulation produced a depolarization by 21.2 ± 2.8 mV. In these glial cells, the magnitude of the stimulus-evoked Ca_i rise was not affected by changing from voltage- to current-clamp (Fig. 2*B*). Furthermore, a 50 mV depolarizing voltage step with a duration of 5 sec did not elevate Ca_i in six voltage-clamped cells (data not shown). These results are consistent with the view that Bergmann glia lack voltage-gated Ca^{2+} channels (Muller et al., 1992; Carmignoto et al., 1998; Grosche et al., 1999).

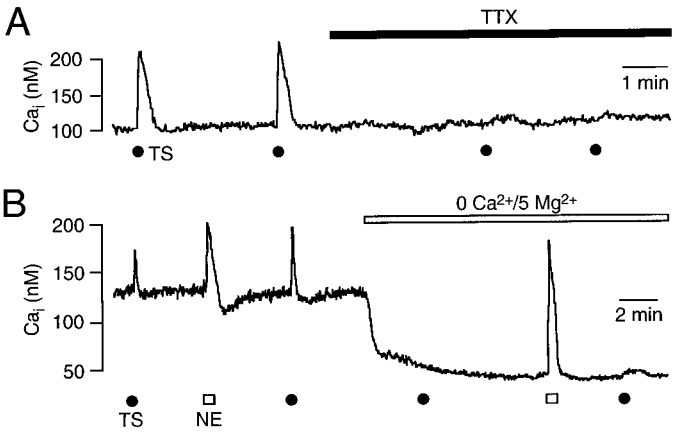

Figure 3. Glial Ca_i rises depend on synaptic transmission. *A*, Suppression of nerve conduction by bath application of 1 μM tetrodotoxin (*TTX*) abolished the stimulus-induced Ca_i transient of a Bergmann glia cell. *B*, Block of synaptic transmission with a Ca^{2+}-free superfusate suppressed the stimulus-evoked Ca_i rise in a different Bergmann cell, whereas the Ca_i response to 10 μM NE persisted.

Neuronal origin of the glial Ca_i rises

As shown in the examples of Figure 3, the stimulus-evoked responses were not caused by direct effects of the electrical stimulation on the glial cells. The Ca_i transients were abolished after block of action potential propagation with 1 μM tetrodotoxin ($n = 9$) (Fig. 3*A*) or on inhibition of synaptic transmission with Ca^{2+}-free solution ($n = 8$) (Fig. 3*B*). The solutions did not impair glial Ca_i signaling capabilities because NE ($n = 5$) (Fig. 3*B*) or 1 mM ATP ($n = 4$; data not shown) was still able to elevate Ca_i to those levels observed under control. These results strongly suggest that the evoked glial Ca_i transients are caused by synaptic release of a neuroactive substance.

Dependence of glial Ca_i rises on stimulation parameters

In one series of experiments, the frequency, strength, or duration of tetanic stimulation was varied for further characterization of the glial Ca_i transients (Fig. 4). The Ca_i rise increases were evoked at a stimulation voltage of >10 V. In ~30% of 47 fura-2 AM ester-loaded or whole-cell-recorded cells, the stimulus-induced Ca_i rises increased almost steadily on elevating the stimulus strength to values of up to 100 V (Fig. 4*A*). In the other cells, saturation of the evoked intracellular Ca^{2+} transients was revealed between 50 and 75 V (Fig. 4*B*). In contrast to this ceiling

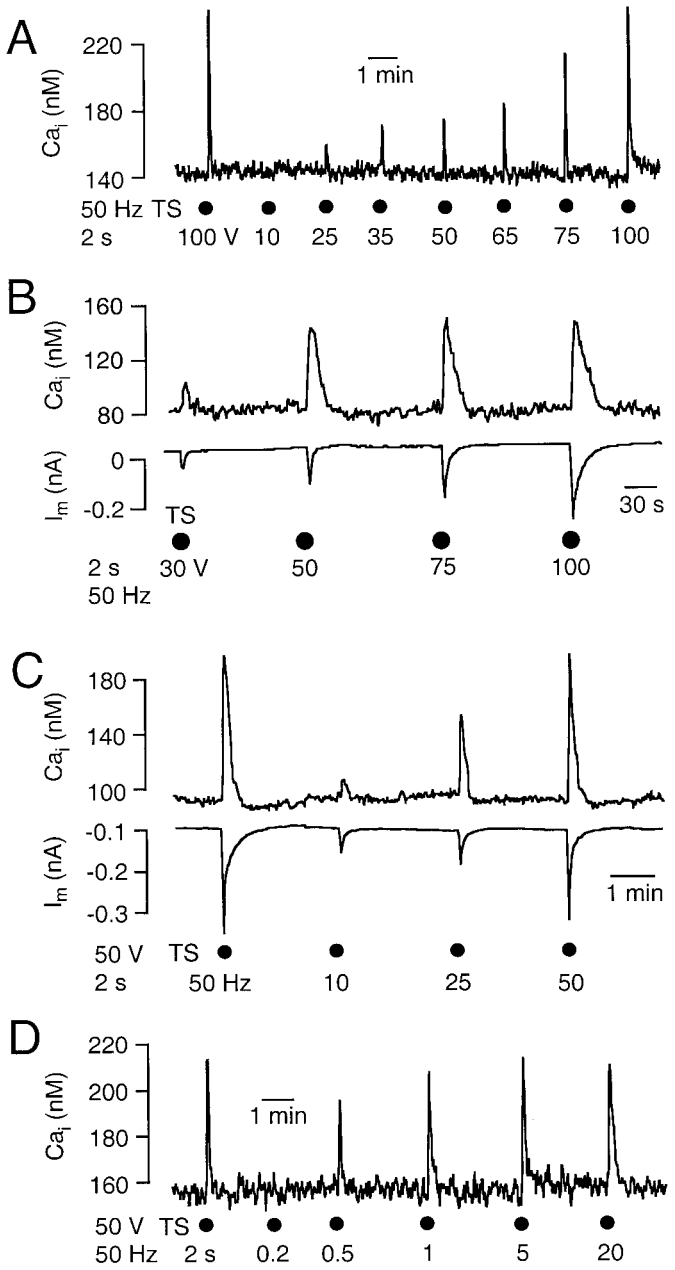

Figure 4. Dependence of activity-induced glial Ca_i transients on stimulus parameters. *A*, At stimulus strengths exceeding 10 V, tetanic stimulation at 50 Hz for 2 sec evoked a gradually increasing Ca_i transient. *B*, In contrast to the cell in *C*, stimulus-induced Ca_i rises saturated at a level of ~150 nM at stimulus intensities of >50 V, whereas the concomitant inward current steadily increased in magnitude. *C*, Stimulation within the granule cell layer at 50 for 2 sec induced noticeable Ca_i rises and inward currents at a frequency of 50 and 25 Hz, whereas stimulation at 10 Hz evoked only a small intracellular Ca^{2+} transient and concomitant inward current. *D*, On stimulation with 50 Hz at 50 V, the threshold for the glial Ca_i rise was between 10 and 25 single pulses (duration 0.2 sec).

of the stimulus-induced Ca_i rises at a level of 50–130 nM, the accompanying inward currents increased almost linearly over the tested range of stimulus voltage (Fig. 4*B*). As measured at 50 V (2 sec pulse train duration), robust Ca_i rises were elicited at a stimulus frequency of 10 Hz and saturated in amplitude at 50 Hz (Fig. 4*C*). Finally, >10 stimuli (50 Hz, 50 V) were necessary to detect a rise of glial Ca_i (Fig. 4*D*).

Effect of glutamatergic antagonists on glial and neuronal Ca_i transients

Previous studies showed that bath application of glutamate receptor agonists induces a Ca_i rise in Bergmann glia (Muller et al., 1992; Tempia et al., 1996; Shao and McCarthy, 1997). To determine whether glutamate receptors are involved in the stimulus-induced glial responses, the effects of glutamate receptor antagonists were investigated. The amplitude of the evoked glial Ca_i increases did not significantly change (77.3 ± 10.9 nM during control vs 74.6 ± 9.3 nM, $n = 6$) (Fig. 5*A*,*C*) after incubation in a solution that contained 1 mM of the metabotropic glutamate receptor blocker (RS)-α-methyl-4-carboxy-phenyl-glycine (MCPG) (Batchelor et al., 1996; Takechi et al., 1998) as well as 50 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) plus 100 μM 2-amino-5-phosphonovalerate (APV) to block the AMPA/kainate and NMDA types of glutamate receptors (Knopfel et al., 1991; Llano et al., 1991; Miyakawa et al., 1992; Takechi et al., 1998), respectively. In contrast, the concomitant glial depolarization was reversibly attenuated by 38.0 ± 10.2% (Fig. 5A, C).

The attenuating effect of the glutamatergic antagonists suggests that the glial membrane response to tetanic stimulation is caused partly by accumulation of extracellular K^+ as a result of neuronal K^+ efflux (Ballanyi et al., 1987; Ballanyi, 1995) attributable to the glutamate-induced depolarization of Purkinje neurons (Knopfel et al., 1991; Llano et al., 1991; Miyakawa et al., 1992; Takechi et al., 1998). The latter assumption is supported by the finding that superfusion of the solution containing 50 μM CNQX, 100 μM APV, and 1 mM MCPG abolished the synaptically evoked initial depolarization and subsequent afterhyperpolarization as well as the concomitant Ca_i increase by between 100 and 300 nM in four Purkinje neurons (Fig. 5*B*,*C*). In a further series of experiments, it was revealed that 50 μM CNQX abolished the stimulus-induced Ca_i increases that ranged from 60 to 150 nM in 15 fura-2 ester-loaded granule cells (data not illustrated).

Effects of Ba^{2+} on glial and neuronal Ca_i transients

To investigate whether stimulus-evoked neuronal K^+ release influences the glial Ca_i transient, the effects of Ba^{2+} were tested. It was shown previously that 1 mM Ba^{2+} suppresses both glial K^+ conductance and K^+ uptake in astrocytes *in situ* on stimulation of afferent nerve fibers (Ballanyi et al., 1987; Barres et al., 1990). In the present study, superfusion of Ba^{2+} (1 mM) evoked a persistent inward current of 727 ± 50 pA ($n = 5$). In this situation, the stimulus-evoked glial inward current was reversibly attenuated by 66 ± 8%, whereas the accompanying Ca_i increase was not different from control values (Fig. 6*A*,*C*). In three of these Bergmann cells, addition of the mixture of glutamate receptor blockers to the Ba^{2+}-containing solution reduced neither the evoked inward current nor the glial Ca_i rise (data not shown). In contrast to lack of Ba^{2+} effects on intracellular Ca^{2+} transients in the glial cells, the drug greatly potentiated the very moderate (32 ± 5 nM) stimulus-induced Ca_i rises in four voltage-clamped Purkinje neurons. Furthermore, Ba^{2+} increased the magnitude of the concomitant neuronal inward current and subsequent outward current by several hundred percent but did not induce a sustained inward current as revealed in the glial cells (Fig. 6*B*,*D*).

Effects of Ca^{2+} release blockers and adrenoceptor antagonists on glial Ca_i rises

Because glutamate receptors are apparently not involved in the glial Ca^{2+} signals, we investigated whether these are caused by activation of afferent nerve fibers from noradrenergic locus coer-

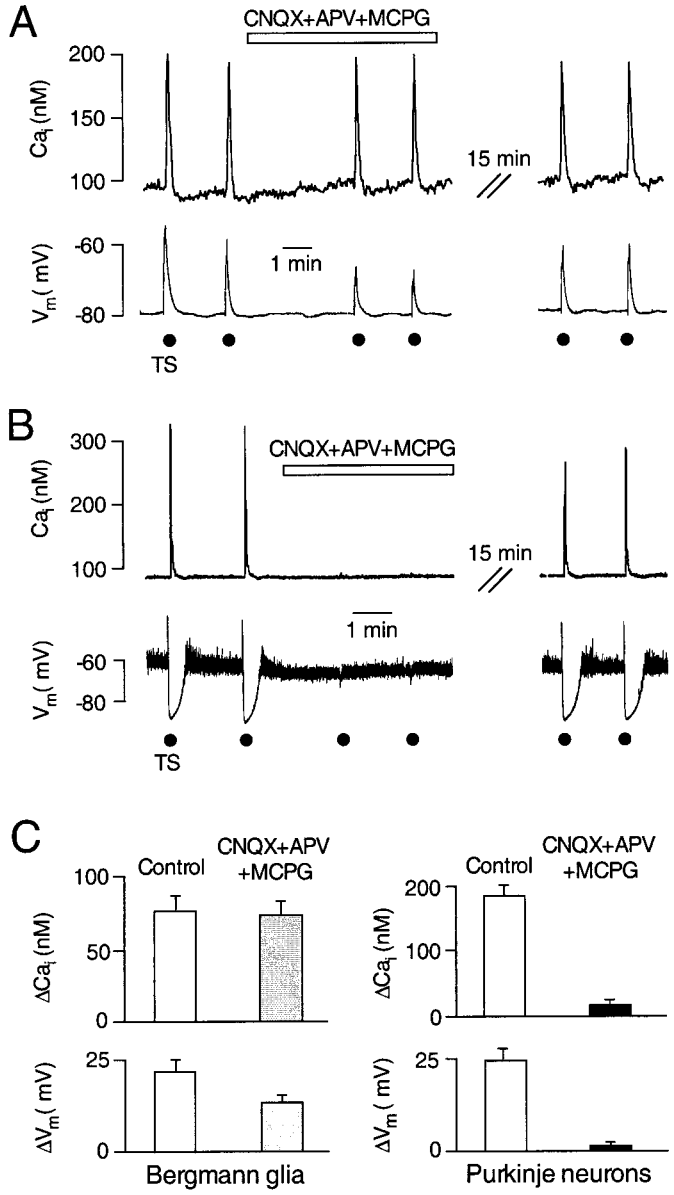

Figure 5. The evoked glial Ca_i rise does not depend on synaptically released glutamate. *A*, Block of glutamate receptors on bath-application of 50 μM CNQX, 100 μM APV, and 1 mM MCPG did not reduce the amplitude of the glial Ca_i increase attributable to tetanic stimulation (*TS*), whereas the accompanying membrane depolarization was reversibly reduced (most likely because of decreased neuronal K^+ release that typically induces a glial depolarization). *B*, The glutamate receptor blockers abolished both the stimulus-evoked depolarization (and subsequent post-tetanic hyperpolarization) and Ca_i rise in a whole-cell recorded Purkinje neuron. *C*, Statistical analysis of the effects of the glutamate blockers on the potential (V_m) and Ca_i response of six Bergmann glial cells and four Purkinje neurons (means ± SEM).

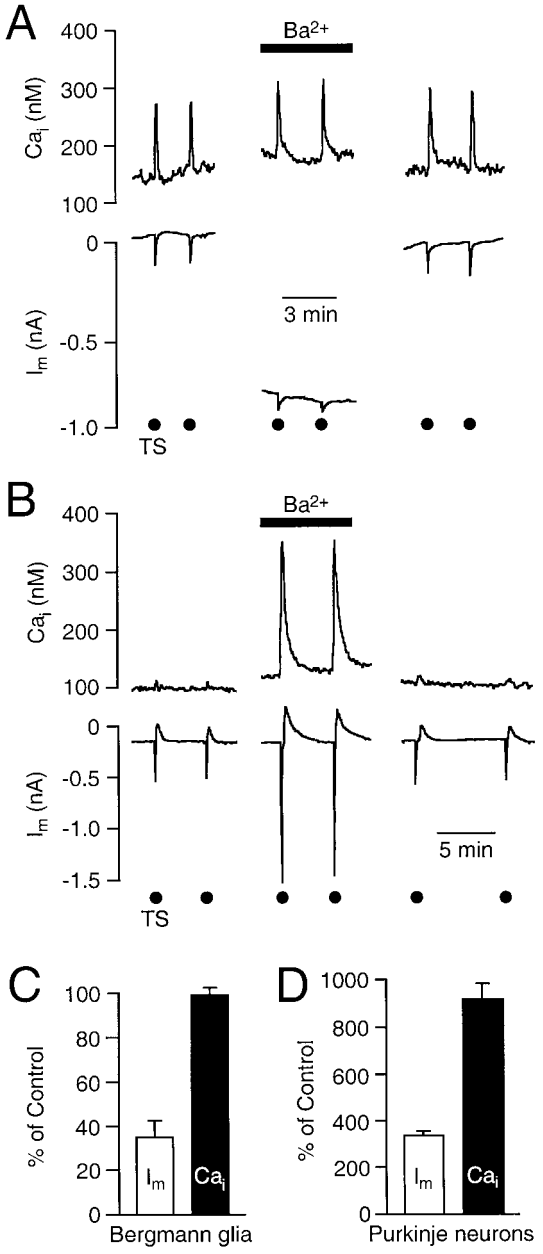

Figure 6. Effects of Ba^{2+} on stimulus-evoked glial and neuronal responses. *A*, Bath application of 1 mM Ba^{2+} led to a sustained inward current in a Bergmann glial cell. In this situation, the stimulus-evoked Ca_i rise was not reduced, whereas the accompanying inward current was attenuated by >50%. *B*, Ba^{2+} led to a prominent potentiation of stimulus-evoked Ca_i rise as well as of inward current and subsequent outward current in a Purkinje neuron. Note that the drug did not affect resting current as seen in the glial cell. *C, D*, Statistical analysis of the effects of Ba^{2+} on stimulus-evoked inward current and Ca_i in five glial cells (*C*) and four Purkinje neurons (*D*).

uleus neurons (Foote et al., 1983; Bickford-Wimer et al., 1991; Woodward et al., 1991). The α_1 adrenoceptor blocker prazosin (5 μM) abolished the Ca_i response in 8 of 11 Bergmann glial cells (Fig. 7*A*,*C*), whereas the concomitant inward current was 95.1 ± 5.7% of control (data not shown). As shown in Figure 7*A*, prazosin blocked the responses to NE (10 μM), but bath-applied ATP (1 mM) could still induce a Ca_i rise similar to those evoked by NE and electrical stimulation under control. The evoked Ca_i increase was reduced by neither the α_2 receptor antagonist yohimbine nor the β receptor blocker propranolol (5 μM each) (Fig. 7*C*). The stimulus-evoked inward current was not significantly changed by propranolol (99.3 ± 12.1% of control, $n = 5$), whereas yohimbine attenuated the inward current to 85.5 ± 7.0% of control ($n = 5$; data not shown). It was demonstrated earlier that activation of α_1 adrenoceptors of Bergmann glia by agonist application induces Ca^{2+} release from IP_3-sensitive intracellular stores (Kirischuk et al., 1996a; Shao and McCarthy, 1997). Ac-

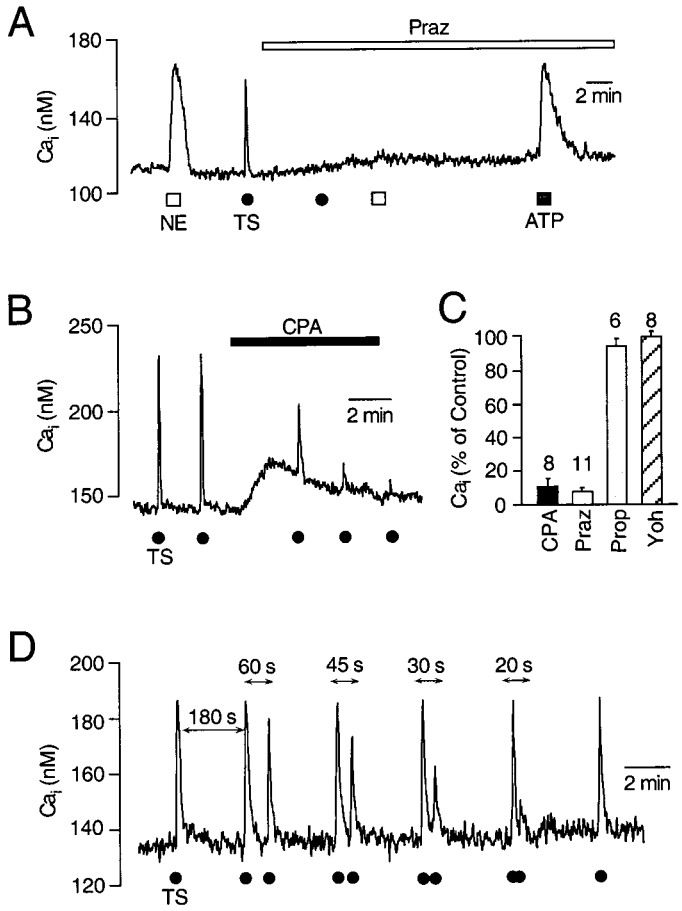

Figure 7. The activity-related glial Ca_i rise is caused by α_1 adrenoceptor-mediated release of Ca^{2+} from intracellular stores. *A*, The rises of glial Ca^{2+} caused by tetanic stimulation (*TS*) and bath-applied NE (10 μM) were both abolished by addition of the α_1 adrenoceptor blocker prazosin (*Praz*, 5 μM), whereas 1 mM ATP still evoked a Ca_i rise. *B*, The glial Ca_i transient was also suppressed after incubation with the Ca^{2+} store pump blocker cyclopiazonic acid (*CPA*, 30 μM). *C*, Statistical analysis of the effects of Praz, CPA, or 5 μM of the α_2 and β adrenoceptor blockers yohimbine (*Yoh*) or propranolol (*Prop*) on the stimulus-induced Ca_i rise. *Numbers* correspond to measured cells. *D*, A decrease in the interstimulus interval led to progressive reduction of the glial Ca_i rise.

cordingly, pretreatment of slices with the Ca^{2+} uptake blocker cyclopiazonic acid (CPA; 30 μM) suppressed the synaptically evoked Ca_i rise of the Bergmann glial cells (Fig. 7*B*,*C*), whereas the accompanying inward current was not affected (data not shown). That the electrically evoked Ca_i rise is caused by intracellular Ca^{2+} release is also suggested by the observation that the peak of the response remained unaffected at stimulus intervals of >1 min, whereas it was progressively attenuated at shorter intervals, and no response was observed at a stimulus interval of <15 sec (Fig. 7*D*). This time course is similar to that of refilling of IP_3-sensitive Ca^{2+} stores after depletion with NE, ATP, or histamine in these cells (Kirischuk et al., 1995, 1996a; Shao and McCarthy, 1997).

DISCUSSION

α_1 adrenoceptor mediation of activity-induced Ca_i rises

A number of recent studies have established that neuronal activity in peripheral (Kriegler and Chiu, 1993; Lev-Ram and Ellisman, 1995; Robitaille, 1995) and central (Dani et al., 1992; Porter and McCarthy, 1996; Carmignoto et al., 1998; Newman and Zahs, 1998) nervous tissue induces a Ca^{2+} signal in adjacent glial cells. However, stimulus-evoked responses *in situ* involve complex interactions of various cell types that are mediated by a diversity of neuroactive substances that potentially act on a multitude of glial receptors. Accordingly, identification of the mechanisms that are responsible for activity-related Ca^{2+} signals of central glia was so far successful only in few studies. These reports indicated a crucial role of metabotropic glutamate receptors in such glial activity (Dani et al., 1992; Porter et al., 1996; Pasti et al., 1997). Our results show for the first time that nerve activity produces a rise of intracellular Ca^{2+} in central nervous glia that is mediated by NE and does not involve glutamate receptors. The finding that the evoked responses are similar to those during bath application of NE, and that both types of Ca_i transients are abolished by prazosin, strongly suggests that activity-induced interstitial accumulation of noradrenaline results in activation of α_1 adrenoceptors on the Bergmann glial cells. Previous work using agonist application showed that NE-induced Ca_i rises in Bergmann glia (Kirischuk et al., 1996a; Shao and McCarthy, 1997) and astrocytes (Brune and Deitmer, 1995; Duffy and MacVicar, 1995; Finkbeiner, 1996; Porter and McCarthy, 1996, 1997) are caused by intracellular Ca^{2+} release from IP_3-sensitive stores. The depressing effect of both the Ca^{2+} uptake blocker CPA and the stimulus intervals of <1 min on the evoked Ca_i increases are in accordance with these previous findings on the mechanism and kinetics of α_1 adrenoceptor-mediated glial Ca^{2+} increases.

Source of interstitial NE

The observed complete block of stimulus-evoked membrane or Ca_i responses of both Purkinje and granule neurons on inhibition of glutamate receptors [see also Knopfel et al. (1991); Takechi et al. (1998)] suggests that also the remaining neurons of the cerebellar slice, such as stellate or basket cells, are functionally switched-off. However, in this situation the α_1 adrenoceptor-mediated glial Ca_i rise was not attenuated. This indicates that the proposed activity-evoked interstitial NE transient is not caused by an indirect effect associated with activation of the neuronal network within the slice, but rather is caused directly by action potential-induced axonal release. NE-containing locus coeruleus nerve fibers are distributed throughout the cerebellum as in other regions of the forebrain (Foote et al., 1983). It is assumed that their target might be glial cells rather than neurons (Stone and Ariano, 1989; Salm and McCarthy, 1992; Ridet et al., 1993; Paspalas and Papadopoulos, 1996). Electrical stimulation of locus coeruleus with parameters similar to those in the present study was demonstrated to elicit a rise of interstitial NE in the cerebellum *in vivo* (Bickford-Wimer et al., 1991). The concentration of released NE, which could amount to several micromoles, was found to increase linearly in the voltage range of 10–50 V in the latter report. In the present study, the stimulus-evoked glial Ca_i rises steadily increased in magnitude in the same range of stimulation voltage as used *in vivo* until the Ca^{2+} responses saturated (attributable to maximal release from stores) in most cells at stimulus strengths of between 50 and 100 V.

Lack of glutamate effects

The lack of effects of the ionotropic and metabotropic antagonists clearly shows that glial glutamate receptors are not involved in the Ca_i response of Bergmann cells during activation of afferents within the granule cell layer. This result was somehow surprising,

because stimulation of climbing fibers in this region of the cerebellar slices results in synaptic release of glutamate (Knopfel et al., 1991; Llano et al., 1991). Furthermore, agonist-induced activation of Ca^{2+}-permeable AMPA/kainate receptors of Bergmann cells was found to evoke a noticeable Ca_i transient (Muller et al., 1992; Tempia et al., 1996). However, prolonged activation of these glial AMPA/kainate receptors by bath application of a high concentration (1 mM) of kainate was necessary in these studies to evoke a Ca_i transient of a magnitude comparable with that induced by electrical stimulation. On the basis of the established small fractional Ca^{2+} influx through this type of glutamate receptor (Burnashev et al., 1992; Tempia et al., 1996), it is possible that Ca^{2+} influx during the short period of synaptic activation is not sufficient to produce a major rise of glial Ca_i. That NMDA and metabotropic glutamate receptors are not involved in the activity-induced Ca_i transient is indicated by the lack of effects of the antagonists APV and MCPG in the present study. It has also been shown previously that activation of NMDA receptors (Muller et al., 1993; Shao and McCarthy, 1997) or of metabotropic glutamate receptors with t-ACPD (Kirischuk et al., 1996b; A. Kulik and K. Ballanyi, unpublished observations) does not induce a rise of intracellular Ca^{2+} in Bergmann glia.

Although our results exclude a major contribution of glutamate to the Ca_i signal of the Bergmann glia during stimulation of afferents within the granule cell layer, activation of glutamate receptors might contribute to the accompanying inward current. We found that Ba^{2+} significantly reduced but did not abolish the stimulus-evoked inward current of the Bergmann cells at a concentration sufficient to block (inwardly rectifying) glial K^+ channels (Ballanyi et al., 1987; Barres et al., 1990; Reichelt and Pannicke, 1993). This indicates that a noticeable portion of the glial membrane response is not caused by a depolarizing effect of glutamate-induced K^+ release from neurons or nerve fibers (Ballanyi, 1995). The finding that combined application of CNQX, APV, and MCPG did not further reduce the evoked inward current in the presence of Ba^{2+} indicates that glutamate receptors play no obvious role in the response of Bergmann glia under the stimulation conditions of the present study. The remaining component of the activity-related membrane current is possibly mediated by electrogenic glutamate uptake into the Bergmann glial cells (Bergles et al., 1997; Clark and Barbour, 1997; Linden, 1997).

In response to synaptically released glutamate, changes of intracellular Na^+, K^+, and pH caused by activation of glial glutamate transport or K^+ uptake might induce a signal pathway within Bergmann glia that is independent of intracellular Ca^{2+} (Ballanyi et al., 1987; Ballanyi, 1995; Tsacopoulos and Magistretti, 1996; Clark and Barbour, 1997). Furthermore, the finding that prazosin did not affect the stimulus-evoked responses in some (3 of 11) cells indicates that other mechanisms of Ca^{2+} signaling are also functional in Bergmann glia *in situ*. In this context, it is important to note that a recent study showed the existence of microdomains for possible neuron–glia interaction in this type of glial cell (Grosche et al., 1999). In the latter report, it was demonstrated that electrical stimulation in the region of the molecular layer elevates Ca_i in small compartments within Bergmann glia. Also in the present study, it was found that the Ca_i rises, induced by stimulation within the granule cell layer, were not always uniformly distributed in the soma and dendritic appendages. These observations are in line with the hypothesis by Grosche et al. (1999) that a Bergmann glial cell consists of hundreds of independent compartments that are capable of autonomous interactions with the particular group of synapses that they ensheath.

Functional relevance

In glia of various tissues, it was found that a rise of intracellular Ca^{2+} induces a diversity of responses, such as membrane depolarization by block of K^+ channels, release of neurotransmitters, proliferation, or stimulation of aerobic metabolism (Subbarto and Hertz, 1990; Muller et al., 1992; Tsacopoulos and Magistretti, 1996; Pfrieger and Barres, 1997; Araque et al., 1998; Verkhratsky et al., 1998). Furthermore, it becomes more and more established that stimulation of glia evokes, in reverse, dynamic changes of neuronal morphology and function, and that activity-related glial Ca_i rises are pivotal for such feedback modulation (Tsacopoulos and Magistretti, 1996; Pasti et al., 1997; Pfrieger and Barres, 1997; Araque et al., 1998; Kang et al., 1998; Newman and Zahs, 1998). The observation that glutamatergic antagonists did not affect the evoked Ca_i rises suggests that NE-containing varicosities mediate Ca^{2+} signaling that is independent of glutamatergic processes constituting the major excitatory input to the Purkinje neurons (Knopfel et al., 1991; Llano et al., 1991). A series of studies have shown that NE exerts various modulatory effects on Purkinje neurons (Foote et al., 1983; Bickford-Wimer et al., 1991). At present it seems that the complex interaction of NE with adrenoceptor subtypes determines whether this neurotransmitter attenuates or potentiates excitatory or inhibitory inputs to these cerebellar neurons (Kirischuk et al., 1996c; Jeng and Wang, 1998). Some questions associated with the pharmacology of the influence of NE on Purkinje neurons (Foote et al., 1983; Parfitt et al., 1988; Woodward et al., 1991; Jeng and Wang, 1998) might be answered in future studies devoted to analyzing the possible feedback effect of glial α_1 adrenoceptors on these cerebellar neurons.

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