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Effects of emodin on synaptic transmission in rat hippocampal CA1 pyramidal neurons in vitro

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Abstract

Rhubarb extracts provide neuroprotection after brain injury, but the mechanism of this protective effect is not known. The present study tests the hypothesis that rhubarb extracts interfere with the release of glutamate by brain neurons and, therefore, reduce glutamate excitotoxicity. To this end, the effects of emodin, an anthraquinone derivative extracted from *Rheum tanguticum* Maxim. Ex. Balf, on the synaptic transmission of CA1 pyramidal neurons in rat hippocampus were studied in vitro. The excitatory postsynaptic potential (EPSP) was depressed by bath-application of emodin (0.3–30 μM). Paired-pulse facilitation (PPF) of the EPSP was significantly increased by emodin. The monosynaptic inhibitory postsynaptic potential (IPSP) recorded in the presence of glutamate receptor antagonists (DNQX and AP5) was not altered by emodin. Emodin decreased the frequency, but not the amplitude, of the miniature EPSP (mEPSP). The inhibition of the EPSP induced by emodin was blocked by either 8-CPT, an adenosine A_1 receptor antagonist, or by adenosine deaminase. These results suggest that emodin inhibits the EPSP by decreasing the release of glutamate from Schaffer collateral/commissural terminals via the activation of adenosine A_1 receptors in rat hippocampal CA1 area and that the neuroprotective effects of rhubarb extracts may result from decreased glutamate excitotoxicity.

Keywords: Emodin; Hippocampus; EPSP; Presynaptic inhibition; Adenosine; Glutamate release

1. Introduction

Post-traumatic, as well as ischemic, brain damage is produced, at least in part, by over-activity of excitatory amino acid neurotransmission (Choi and Rothman, 1990; Meldrum and Garthwaite, 1990; Benveniste, 1991). A marked increase in the extracellular concentration of excitatory amino acid transmitters occurs in patients with post-traumatic or ischemic brain damage (Baker et al., 1993; Palmer et al., 1994). Activation of glutamate receptors, such as *N*-methyl-D-aspartic acid (NMDA) and non-NMDA receptors, causes damage to neuronal cells after ischemic or traumatic brain injury (Choi and Rothman, 1990; Meldrum and Garthwaite, 1990). It is also known that neuronal injury is often associated with a sustained elevation of intracellular Ca^{2+} that is largely a consequence of the opening of ion channels or of damage to the plasma membrane (Choi, 1988; Siesjö and Bengtsson, 1989). Moreover, oxygen free-radicals have been implicated in the pathogenesis of microvascular damage, brain edema, and cerebral ischemia (Kontos and Povlishock, 1986).

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In pathophysiological conditions, endogenous adenosine released from neurons and glia may play a neuroprotective role in the nervous system (Schubert et al., 1997; Stone, 2002; Ribeiro et al., 2003).

Recently, it has been shown that 1,3,8-trihydroxy-6-methylanthraquinone (emodin), an anthraquinone derivative from rhubarb (*Rheum tanguticum* Maxim. Ex. Balf.), has protective effects against brain disturbances induced by severe cerebral injury (Gu et al., 2000). Rhubarb extracts have been shown to modulate intracellular Ca^{2+} concentration in isolated brain cells (Lin and Jin, 1995). Emodin has been shown to inhibit lipid peroxidation in rat brain homogenates (Sato et al., 1992). To the best of our knowledge, however, the effects of rhubarb extracts on the release of neurotransmitters, including glutamate and γ-aminobutyric acid have not been studied previously in the central nervous system (CNS). The purpose of the present study is to investigate the effects of emodin on the excitatory postsynaptic potential (EPSP) and the inhibitory postsynaptic potential (IPSP) in hippocampal CA1 pyramidal neurons in vitro. The present results show that emodin inhibits the EPSP but not the IPSP by decreasing the release of glutamate from nerve terminals of the Shaffer collaterals in the rat hippocampal CA1 area. This effect appears to be mediated by adenosine A_1 receptors. Some of these results have been published in abstract form (Gu et al., 2001).

2. Methods

2.1. Brain slice preparations

The experimental protocols were approved by the Animal Research Committee of the Kurume University School of Medicine. All efforts were made to minimize suffering and the number of animals used. Sagittal brain slices including the hippocampal CA1 area were obtained from male Wistar rats (100–250 g) in a manner similar to that described previously (Takeya et al., 2002). Briefly, each rat was anaesthetized with pentobarbital sodium (nembutal, 50 mg/kg, i.p.) and killed by decapitation. Whole brains were rapidly removed and immersed for 8–10 s in a cooled artificial cerebrospinal fluid (ACSF at 4–6 °C) that was pre-bubbled with 95% O_2–5% CO_2. Brain slices (400 μm in thickness) were cut with a Vibroslice (Campden Instruments) within 1 min after the removal of the brain. The brain slice was left for 1 h to recover in oxygenated ACSF at room temperature (22–24 °C) and then transferred to the recording chamber. The composition of the ACSF was as follows (in mM): NaCl 117, KCl 4.7, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, and D-glucose 11 (299±4 mOsm). The brain slice was continuously superfused with oxygenated ACSF at the rate of 2–3 ml/min (31–32 °C). Before recordings, an incision was made on the hippocampal slice between the CA3 and CA2 regions to prevent recurrent excitation of CA1 neurons (Schwartzkroin and Prince, 1978).

2.2. Electrophysiological recordings

Conventional intracellular recordings were made from hippocampal CA1 pyramidal neurons with glass microelectrodes filled with 3 M potassium acetate (70–120 MΩ). Membrane potential of hippocampal CA1 pyramidal neurons was recorded with an Axoclamp-2A amplifier (Axon Instruments, Inc.) and continuously monitored through the Scope mode of PowerLab (AD Instruments, Inc.). Input resistance of hippocampal neurons was measured by electrotonic potentials produced by passing hyperpolarizing current pulses with duration of 200 ms. Data were digitized online and stored by AxoData 2.0 software (Axon Instruments Inc.) via the ITC-16 computer interface with A/D converter (InstruTECH Corp.). Postsynaptic potentials were evoked by a bipolar stimulation electrode placed on the stratum radiatum in the hippocampal CA2 region (Stimulator: Nihon Kohden SEN-7103). To isolate the EPSP from the fast IPSP, hippocampal slices were superfused with ACSF containing either bicuculline (15 μM) alone or combination of bicuculline (5 μM) and picrotoxin (50 μM). The intensity of the test stimulus was chosen to yield an EPSP that was half the size necessary to activate an action potential (5–10 V for 200 μs). In case of analyzing the paired-pulse facilitation of the EPSP, CGP55845 (1 μM), a $GABA_B$ receptor antagonist, was added to the superfusing solution to block the slow IPSPs. Monosynaptic IPSPs were evoked by direct stimulation of the hippocampal CA1 area in the presence of 6,7-dinitroquinoxaline-2,3-dione (DNQX, 20 μM) and (±)-2-amino-5-phosphonopentanoic acid (AP5, 40 μM), where the EPSP was completely blocked. Unless otherwise stated, sample traces of synaptic potentials shown in the figures represent averages from six consecutive events. Miniature EPSPs (mEPSPs) were recorded at −70 mV in the presence of tetrodotoxin (TTX, 1 μM) and bicuculline (15 μM). Events were accepted for analysis, when they had amplitudes of >0.2 mV, had a monophasic rising phase and decayed to base line in an approximately exponential fashion in 20 ms. The mEPSP was analyzed with mini-analysis software (Synaptosoft). For statistical comparison, we used the Kolmogorov–Smirnov (K-S) test applied to cumulative probability distribution of data. In addition to electrophysiological recordings, a double-beam spectrophotometer (Model U-2001, Hitachi) was used to measure the absorption characteristics of the emodin dissolved in the ACSF. Spectral absorbance measurements were made by scanning over the wavelength range 230–600 nm. Experimental values

are presented as the mean ± standard error of the mean (SEM). Statistical analysis was performed using AxoGraph 4.5 analyses software. Data were analyzed by Student's *t*-test. $P < 0.05$ was accepted as statistically significant.

2.3. Drugs

Drugs were purchased from the following sources: picrotoxin was from Wako Pure Chemical Ind. (Osaka, Japan). (−)-Bicuculline methiodide, DNQX, AP5, 8-cyclopentyl-1,3-dimethylxanthine (8-CPT), adenosine deaminase (type V), emodin and picrotoxin were purchased from Sigma-Aldrich Fine Chemicals (St. Louis, MO, USA). TTX was from Alomone Labs Ltd. (Jerusalem, Israel). Emodin was dissolved in dimethylsulfoxide (DMSO) and added to the ACSF. The final concentration of DMSO (0.1%) had no direct effect on hippocampal CA1 pyramidal neurons. The other drugs were dissolved in the ACSF.

3. Results

3.1. Effects of emodin on the EPSP and the IPSP

The resting membrane potential and input resistance of hippocampal CA1 pyramidal neurons were −69 ± 1 mV ($n = 59$) and 40 ± 4 MΩ ($n = 59$), respectively. During the experiments, the membrane potential of these neurons was initially held at −68 to −70 mV by injecting DC current into the cells. Fig. 1A shows an example of the effect of emodin (30 μM) on the EPSP in a hippocampal CA1 pyramidal neuron. Stimulation of the Schaffer collaterals (5–10 V for 200 μs) evoked an EPSP in hippocampal CA1 pyramidal neurons superfused with ACSF containing bicuculline (15 μM) (Fig. 1A). The averaged amplitude of 48 consecutive EPSPs was 5.8 ± 0.2 mV in this neuron. Bath-application of emodin (30 μM) produced no visible change in the membrane potential and input resistance of hippocampal CA1 pyramidal neurons (data not shown), but depressed the amplitude of the EPSP to 2.3 ± 0.1 mV (Fig. 1A). The emodin-induced depression of the EPSP reached a peak within 20 min and recovered within 20–40 min after removal of emodin from the perfusion solution. Pooled data showed that emodin (30 μM) reduced the amplitude of the EPSP to 37 ± 2.6% ($n = 17$) of control in hippocampal CA1 pyramidal neurons (Fig. 1B). Since the concentration of bicuculline (15 μM) could produce non-specific effects (Seutin et al., 1997), we further investigated the effects of emodin in the presence of lower concentration of bicuculline combined with picrotoxin. The effects of emodin (30 μM) determined in the presence of bicuculline (5 μM) and picrotoxin (50 μM) were not different from those

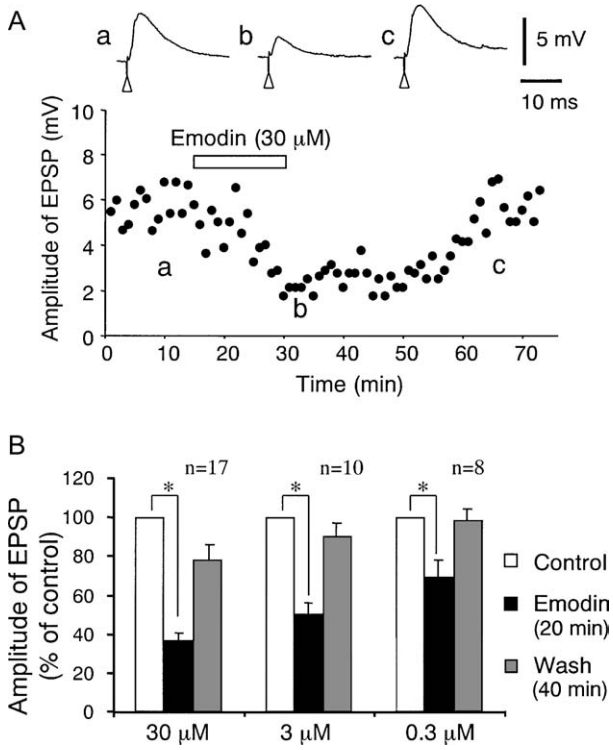

Fig. 1. The effect of emodin on the monosynaptic EPSPs obtained from hippocampal CA1 pyramidal neurons by stimulation of the Schaffer collaterals. (A) Example of the effect of emodin (30 μM) on the EPSP in a hippocampal CA1 pyramidal neuron. In the upper traces, each record is the average of six EPSPs. Open triangles indicate the time of Schaffer collateral stimulation. Records (a)–(c) were obtained at the time indicated by the respective letters in the scatter plot (lower graph). The amplitude of the evoked EPSP was reduced by bath-application of emodin within 15–20 min. Lower graph shows a scatter plot of the EPSP amplitudes. Each data point was obtained by average of six EPSPs. The horizontal bar indicates the period of the application of emodin. (B) Concentration-dependent effect of emodin (0.3, 3.0, 30 μM) on the EPSP. Ordinate represents the relative amplitude of the EPSP. The number of experiments (*n*) at each concentration is shown at the top. The amplitude of the EPSP before application of emodin was taken as 100%. Vertical lines on columns indicate SEM. The asterisks indicate statistical significance ($P < 0.05$).

obtained in the presence of bicuculline (15 μM). Pooled data showed that emodin (30 μM) reduced the amplitude of the EPSP to 44 ± 6.5% ($n = 8$) of control in the presence of bicuculline (5 μM) and picrotoxin (50 μM). Fig. 1B shows the concentration dependence of the effect of emodin (0.3–30 μM) on the EPSP. The evoked EPSP was recorded before (open column), 20 min after bath application of emodin (closed columns), and 40 min after removal of emodin (shaded columns). At concentrations of 3 μM and 0.3 μM, emodin depressed the amplitude of the EPSP to 50 ± 5.3% ($n = 10$) and 69 ± 8.3% ($n = 8$) of control, respectively. The EPSP was followed by a slow IPSP, which is mediated by $GABA_B$ receptors, in about 65% of the neurons tested. The slow IPSP recorded in the present experimental conditions was blocked by

DNQX, a glutamate receptor antagonist, indicating that the slow IPSP is multisynaptic. The average amplitude of the slow IPSPs was reduced from 2.8±0.5 mV (n=11) to 0.6±0.2 mV (n=11) after treatment of emodin (30 μM).

Monosynaptic IPSPs were evoked by single focal stimulation (15 V for 200 μs) applied near the recorded neurons during superfusion with ACSF containing DNQX (20 μM) and AP5 (40 μM) to block the EPSP. Fig. 2 shows the effect of emodin (30 μM) on the monosynaptic fast IPSP (f-IPSP) in a hippocampal CA1 pyramidal neuron. Statistical data from eight experiments show that emodin (30 μM) did not significantly change the amplitude of the f-IPSP (P>0.1, data not shown). These results suggest that emodin has no effect on inhibitory synaptic transmission in the hippocampal CA1 pyramidal neurons.

3.2. Effects of emodin on paired-pulse facilitation (PPF) of the EPSP

When the Schaffer collaterals were stimulated by a pair of electrical pulses with 50–100 ms intervals, repeated every 10 s, the amplitude of the second EPSP was larger than that of the first EPSP (Fig. 3A control) in hippocampal CA1 pyramidal neurons treated with bicuculline (15 μM) and CGP55845 (1 μM). Since NMDA receptor-mediated EPSPs in these neurons, when present, lasted longer than the interstimulus interval, it is possible that a component of the facilitation was the summation of the tail end of the first EPSP with the initial part of the second EPSP. However, in most of the cells that showed PPF there was no evidence of a slow component in the first EPSP (see Fig. 3A). The amplitude ratio of the second to the first EPSP was >1 in each pair. Fig. 3A shows an example of the effect of emodin (30 μM) on the PPF. Although emodin (30 μM) depressed both first and second EPSPs, the magnitude of the depression of the first EPSP was larger than that of the second. In this neuron, the averaged amplitude ratio was 1.1 and 1.5 in the absence and the presence of emodin (30 μM), respectively (Fig. 3A). Fig. 3B shows pooled data for the effect of emodin on the PPF. Emodin (30 μM) increased the ratio of the PPF from 1.3±0.07 (n=6) to 1.6±0.08 (n=6) at inter pulse interval of 100 ms. Thus, emodin (30 μM) produced 25±2.7% (n=6) enhancement of the PPF ratio (P<0.05). These data suggest that emodin decreases the probability of glutamate release from presynaptic nerve terminals.

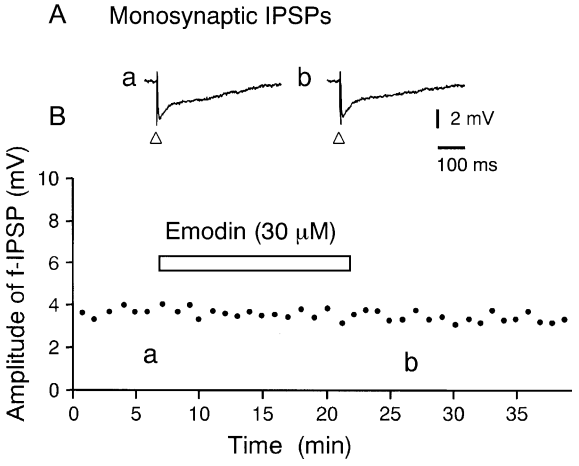

Fig. 2. The effect of emodin (30 μM) on monosynaptic IPSPs. (A) Monosynaptic IPSPs were recorded from a hippocampal CA1 pyramidal neuron superfused with an ACSF containing DNQX (20 μM) and AP5 (40 μM). Sample records (a) and (b) were taken before and 20 min after application of emodin (30 μM), respectively, at the times indicated by the letters in the scatter plot in (B). Each trace represents the average of six IPSPs. Open triangles indicate the time of local stimulation. (B) Scatter plot of the amplitude of the f-IPSP. Horizontal bar indicates the period of the application of emodin (30 μM). Each data point was obtained by average of six IPSPs.

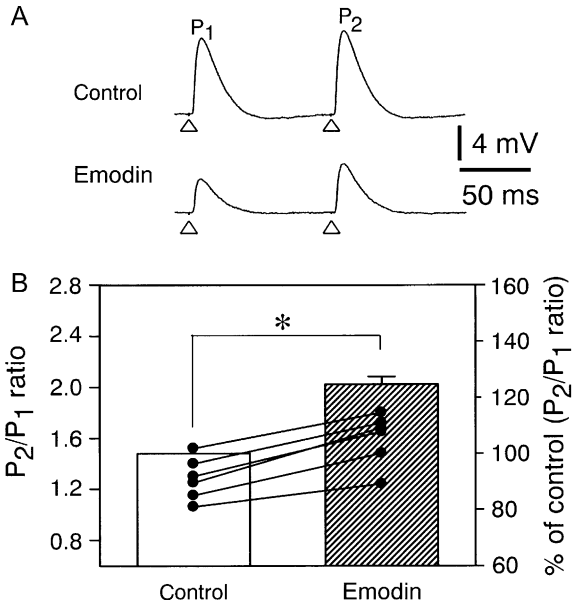

Fig. 3. Effects of emodin (30 μM) on two consecutive EPSPs evoked by paired-pulse stimuli with interval of 100 ms repeated every 10 s in the presence of CGP55845 (1 μM). (A) Sample records (average of six consecutive pairs) of EPSP pairs obtained before emodin (control) and during application of emodin (30 μM). P_1 and P_2 indicate first and second EPSPs, respectively. (B) Pooled data for the effect of emodin (30 μM) on the P_2/P_1 ratio. Open and hatched columns represent the data obtained before and during application of emodin (30 μM), respectively. Values are presented as means±SEM. Asterisk indicates that the difference between the two means is statistically significant (P<0.05).

3.3. Effects of emodin on the sensitivity of glutamate receptors

The effect of emodin on the sensitivity of postsynaptic glutamate receptors was examined in neurons of the

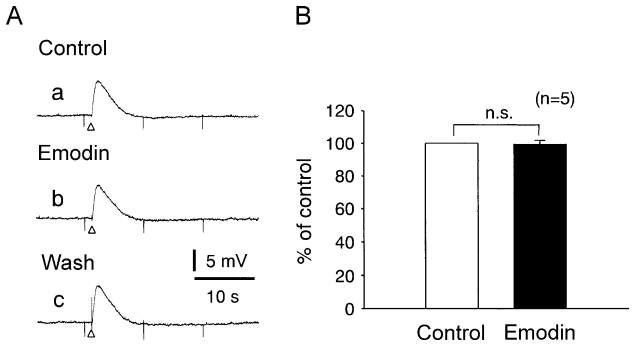

Fig. 4. Effect of emodin on the sensitivity of glutamate receptors at postsynaptic membrane in CA1 pyramidal neurons. (A) The effects of emodin (30 μM) on the glutamate-induced potentials produced by pressure pulses (140 kPa for 10 ms) applied, at the time indicated by the open triangles, through a large-bore micropipette filled with 100 mM glutamate. Records (a) and (b) were obtained before and 15 min after application of emodin. Record (c) was obtained 30 min after wash. (B) Pooled data for the effect of emodin on the glutamate-induced potentials. Open and closed columns represent data in the absence and the presence of emodin, respectively. The amplitude of the control response is indicated as 100%. Vertical lines indicate the SEM. The number in parentheses shows the number of experiments. The n.s. indicates no statistical significance.

CA1 pyramidal layer. Glutamate was directly applied to the stratum radiatum layer of the recorded neuron by a single pressure pulse through a large-bore micropipette (filled with 100 mM glutamate-containing ACSF) in the presence of TTX (1 μM). The glutamate-induced potential was not altered by emodin (30 μM) (Fig. 4A). Pooled data showed that the amplitude of the glutamate-induced potential was 99±3% ($n=5$) of control in the presence of emodin (30 μM) (Fig. 4B). These results suggest that emodin does not change the sensitivity of postsynaptic glutamate receptors on CA1 pyramidal neurons.

3.4. Effects of emodin on mEPSPs

The evidence that agonists (or transmitters) can increase the frequency of mEPSP without changing their amplitudes suggests presynaptic facilitation of excitatory synaptic transmission (Thompson et al., 1993). The mEPSPs were recorded from hippocampal CA1 pyramidal neurons in the presence of TTX (1 μM). Bicuculline (15 μM) was also added to the perfusing solution to suppress the IPSP and mIPSPs. Under these experimental conditions, spontaneously occurring mEPSPs were recorded as subthreshold depolarizing events at an average membrane potential of −70 mV. The average amplitude of the mEPSP was 0.58±0.07 mV ($n=5$) in an ACSF containing TTX (1 μM) and bicuculline (15 μM). Fig. 5 shows the effects of emodin on the amplitude (A) and frequency (B) of mEPSPs in a hippocampal CA1 pyramidal neuron. The cumulative amplitude distribution of mEPSPs was plotted in the absence and the presence of emodin (30 μM). Emodin (30 μM) did not change the cumulative distribution of the mEPSP amplitude (Fig. 5A). The mean amplitude of the mEPSPs was 0.56±0.06 mV ($n=5$) in the presence of emodin (30 μM). These data were statistically not different from those obtained in the absence of emodin (0.55±0.07 mV, $n=5$, $P>0.1$). Emodin had no effect on the time course of the mEPSP as shown in the traces of digitally averaged mEPSPs (Fig. 5D). The mean frequency of mEPSPs decreased from 0.66±0.03 Hz ($n=5$) to 0.34±0.02 Hz ($n=5$) in the presence of emodin (30 μM, $P<0.05$). These results suggest that emodin reduces the spontaneous release of glutamate from glutamatergic nerve terminals in the hippocampal CA1 area.

3.5. Effects of 8-CPT and adenosine deaminase on the depression of the EPSP induced by emodin

Adenosine inhibits excitatory but not monosynaptic-inhibitory synaptic transmission in the hippocampus (Kamiya, 1991; Yoon and Rothman, 1991; Thompson et al., 1992). Since emodin suppressed the EPSP without affecting the monosynaptic IPSPs, we investigated the effect of the adenosine A_1 receptor antagonist, 8-CPT (10 μM) and of the enzyme adenosine deaminase (0.8 IU/ml), on the actions of emodin. Bath application of 8-CPT increased the amplitude of the EPSP by ~24%. In the presence of 8-CPT, the effect of emodin on the amplitude of the EPSP was markedly attenuated (Fig. 6). Emodin (30 μM) depressed the amplitude of the EPSP to 85±3% ($n=5$) of control in the presence of 8-CPT (10 μM). This contrasts with the depression of the EPSP amplitude by emodin to 37±2.6% in the absence of 8-CPT. The difference between these two results is statistically significant ($P<0.01$). In addition, bath application of the enzyme adenosine deaminase

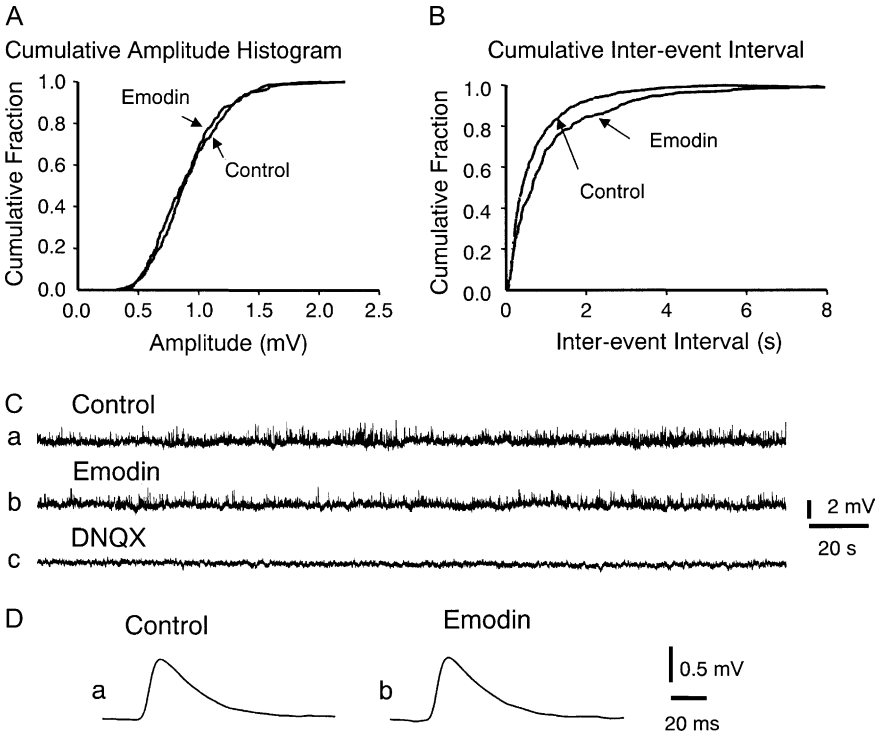

Fig. 5. Effect of emodin on the mEPSPs in hippocampal CA1 pyramidal neurons. To block evoked and spontaneous IPSPs, the standard solution contained TTX (1 μM) and bicuculline (15 μM). (A) Graph shows the cumulative distributions of the amplitude of mEPSPs before and during application of emodin (30 μM). (B) Graph shows the cumulative inter-event interval distribution of mEPSPs in control conditions and during application of emodin (30 μM). Data in (A) and (B) are from the same neuron. The resting membrane potential was held at −63 mV. Emodin had no effect on the amplitude but decreased the frequency of the mEPSPs. (C) Sample voltage traces in control conditions (a), in the presence of emodin (30 μM) (b), and in the presence of DNQX (20 μM) (c). (D) Averaged traces of mEPSPs in control conditions (a) and in the presence of emodin (b). Note that the time course of mEPSPs was not altered by emodin.

(0.8 IU/ml) (Arrigoni et al., 2001) blocked the emodin-induced suppression of EPSP (Fig. 7A). Emodin (30 μM) depressed the amplitude of the EPSP to 86±12% (n=3) of control in the presence of the enzyme adenosine deaminase (Fig. 7B). Spectral absorbance measurements were made by scanning over the wavelength range 230–600 nm. The absorbance spectrum of emodin showed three peaks at the wavelength of 254, 306, and 489 nm (Fig. 7C). After addition of adenosine deaminase (0.8 U/ml) for 1 h, the absorbance spectrum of emodin was not affected (Fig. 7C). On the other hand, the identical treatment of adenosine deaminase shifted the absorbance spectrum of adenosine to the left. The wavelength of maximum absorbance of adenosine shifted from 260 nm to 248 nm in the presence of adenosine deaminase (Fig. 7D). These data, taken together, suggest that extracellular endogenous adenosine may contribute to the emodin-induced depression of the EPSP in rat hippocampal pyramidal neurons in vitro.

4. Discussion

In the present study, we examined the effects of emodin, an anthraquinone derivative, on EPSPs and IPSPs in the hippocampal CA1 pyramidal neurons. Bath application of emodin depressed the amplitude of the EPSP without significantly changing the resting membrane potential or input resistance. Emodin directly inhibited excitatory synaptic transmission in the hippocampal CA1 area, because (1) depression of the EPSP by emodin was recorded while the f-IPSP was blocked by bicuculline and (2) emodin did not affect the f-IPSP recorded in the presence of AP5 and DNQX. It has been shown that the mEPSP represents the random release of single neurotransmitter packets (Chavez-Noriega and Stevens, 1994; Behr et al., 2000). Changes in mEPSPs frequency provide information about possible changes in the presynaptic release process, while changes in the amplitude of the miniature potentials reflect alterations in postsynaptic receptor properties. We determined whether a presynaptic mechanism is involved in the emodin-induced depression of the EPSP in hippocampal CA1 pyramidal neurons. Emodin did not alter the amplitude of exogenous glutamate-induced potentials. Emodin decreased the frequency of mEPSPs but did not alter their amplitude. These results suggest that emodin presynaptically reduces the excitatory synaptic transmission in hippocampal CA1 pyramidal neurons without affecting the sensitivity of glutamate receptors at the

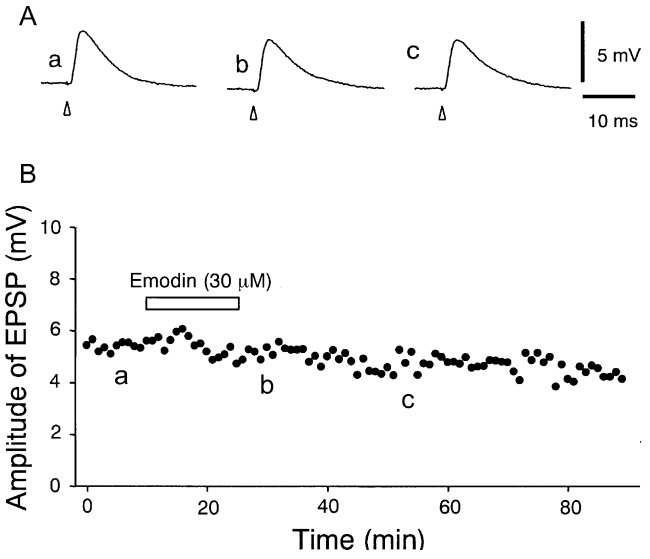

Fig. 6. Effect of 8-CPT on the emodin-induced depression of the EPSPs. (A) Sample traces of EPSP obtained at the time indicated by the respective letters in the scatter plot in (B). Emodin (30 μM) was superfused in the presence of 8-CPT (10 μM), an adenosine A_1 receptor antagonist. Each record is the average of six EPSPs. (B) Time-course of the effect of emodin in the presence of 8-CPT. Data points are averages of six responses. Period of drug application is indicated by the horizontal bar.

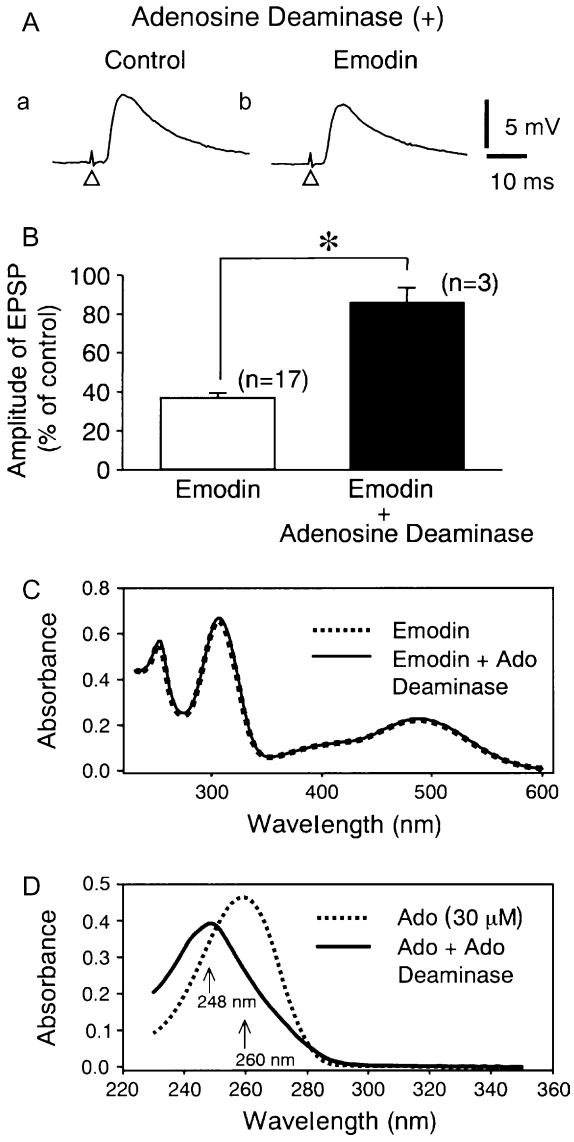

Fig. 7. Effect of adenosine deaminase on the emodin-induced depression of the EPSPs.(A) Sample records (a) and (b) were taken before and 20 min after application of emodin (30 μM) in the presence of adenosine deaminase (0.8 IU/ml), respectively. Each trace represents the average of six EPSPs. (B) Pooled data for the effect of emodin on the EPSP in the absence (open column) and in the presence of adenosine deaminase (closed column), respectively. The number in parentheses shows number of experiments. Vertical lines on columns indicate SEM. The asterisk indicates statistical significance ($P<0.05$). (C) Graph shows the absorption spectrums of emodin (30 μM) before (dotted line) and after treatment of adenosine deaminase (0.8 IU/ml) for 1 h (solid line). (D) Graph shows the absorption spectrums of adenosine (30 μM) before (dotted line) and after treatment of adenosine deaminase (0.8 IU/ml) for 1 h (solid line). The upward arrows indicate the wavelength of maximum absorbance.

postsynaptic membrane. If neurotransmitters (or agonists) depress the release probability, the paired-pulse ratio would be increased (see Manabe et al., 1993; Debanne et al., 1996). We tested the effect of emodin on PPF in the hippocampal CA1 pyramidal neurons in the presence of CGP55845, a $GABA_B$ receptor antagonist, which blocks the slow IPSPs. Although emodin decreased the amplitude of both first and second EPSP, it produced a significant enhancement of the ratio of the PPF of the EPSP. Since the amount of PPF is inversely proportional to release probability (Debanne et al., 1996; Thomson, 2000), emodin may directly reduce the release probability of glutamatergic synaptic vesicles in hippocampal neurons. It has been reported that adenosine depresses excitatory synaptic transmission while having no effect on inhibitory synaptic transmission in the rat CNS in vitro (Kamiya, 1991; Lambert and Teyler, 1991; Yoon and Rothman, 1991; Hasuo et al., 1992; Thompson et al., 1992). The inhibitory action of adenosine is mediated by presynaptic mechanisms since adenosine decreased the frequency of mEPSCs without affecting the distribution of their amplitudes (Scanziani et al., 1992; Scholz and Miller, 1992). In the present study, we showed that the effects of emodin on excitatory and inhibitory synaptic transmission are comparable to those of adenosine. Moreover, the effects of emodin were markedly attenuated by 8-CPT, an adenosine A_1-receptor antagonist or by the enzyme adenosine deaminase. The lack of depression of the EPSPs induced by emodin in the presence of adenosine deaminase is not due to destruction of emodin itself by adenosine deaminase since the absorption spectrum of emodin was not altered by treatment of adenosine deaminase. These results suggest that depression of glutamate release mediated by adenosine receptors may be involved in the effects of emodin on

the hippocampal CA1 pyramidal neurons. However, the mechanism of release of adenosine and/or accumulation of extracellular endogenous adenosine by emodin is unknown. Further experiments are needed to clarify this. However, the activation of adenosine receptors has been reported to be involved in the neuroprotection from damages generated by mechanical or hypoxic/ischemic insults (Schubert et al., 1997; Stone, 2002). Taken together, the present data suggest that emodin, an extract of rhubarb, may have neuroprotective action via the endogenous adenosine in the rat hippocampal CA1 area.

Although brain injury resulting from head trauma is associated with high mortality, little progress has been made in the development of effective pharmacological agents to protect brain-injured patients. The excitatory amino acid glutamate plays an important role in the pathogenesis of neuronal damage and death in acute cerebral ischemia, which is known as "excitotoxicity" (Choi and Rothman, 1990; Olney, 1990; Benveniste, 1991). Excitotoxicity is caused by an excessive release of glutamate, which in turn overstimulates postsynaptic glutamate receptors. Although the molecular mechanisms of glutamate receptor-mediated excitotoxicity are uncertain, there is general agreement that it is, in large part, Ca^{2+}-dependent (Sattler and Tymianski, 2001). The central role of Ca^{2+} in mediating cell damage is indicated by clinical trials of Ca^{2+} antagonists, dihydropyridine and nimodipine in head-injury patients (Robinson and Teasdale, 1990; Kakarieka et al., 1994). In animal models of cerebral ischemia and traumatic brain injury, antagonists for NMDA, AMPA and mGluR1 receptors protect against acute brain damage and delayed behavioral deficits (Hayes et al., 1988; Chen et al., 1991; Lyeth et al., 2001). The present study showed that emodin blocked Ca^{2+}-dependent release of glutamate from the Schaffer collaterals in the hippocampal CA1 area. It has been reported that rhubarb extracts decrease intracellular concentration of Ca^{2+} in isolated brain cells (Lin and Jin, 1995). The present results, together with previous reports, suggest that the protective effect of rhubarb extracts against brain disturbances induced by severe cerebral injury (Gu et al., 2000) may be mediated by inhibition of excitotoxicity.

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