Potentiation of Ethanol Effects in Cerebellum by Activation of Endogenous Noradrenergic Inputs

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ABSTRACT

We previously found that beta adrenergic agonists such as norepinephrine and isoproterenol potentiate the depressant actions of ethanol (EtOH) on cerebellar Purkinje neurons. Furthermore, antagonism of the beta adrenergic effects of endogenously released catecholamines with timolol reduced EtOH-induced depressions of neuronal activity in that brain area. In the present study, we further investigated the hypothesis that activity of the endogenous noradrenergic innervation to the cerebellar cortex can potentiate this EtOH action. We investigated the interaction of synthetically released catecholamines on EtOH-induced depressions of cerebellar Purkinje neurons in three different experiments: (1) endogenous catecholamine release was facilitated by applying the catecholamine uptake inhibitor desmethylimipramine, (2) activity of the noradrenergic innervation of the cerebellar cortex from locus ceruleus was increased by causing acute withdrawal from 7 days of chronic morphine treatment with the opiate antagonist naloxone, and (3) the noradrenergic innervation of the cerebellum was activated directly by electrical stimulation of the locus ceruleus. We found that all three conditions potentiated EtOH-induced depressions in the cerebellum and that this potentiation of ethanol effects could be antagonized by the systemic administration of the beta adrenergic antagonist propranolol. Furthermore, morphine withdrawal also caused potentiation of the depressant effects of phencyclidine, which are known to be regulated by the endogenous catecholamine innervation in this brain area. Taken together with our previous data demonstrating a beta adrenergic facilitation of EtOH actions in this brain area, the present results suggest that the activity of endogenous noradrenergic synapses can regulate the depressant effects of EtOH on cerebellar Purkinje neurons.

ABBREVIATIONS: DMI, desmethylimipramine; LC, locus ceruleus; EtOH, ethanol; GABA, y-aminobutyric acid; ANOVA, analysis of variance; NE, norepinephrine; i.p., intraperitoneally; PCP, phencyclidine; s.c., subcutaneously.

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of the LC mimics the electrophysiological effects of locally applied NE or cAMP analogs on Purkinje neurons (Siggins et al., 1971a,b; Hoffer et al., 1973; Moises and Woodward, 1980; Moises et al., 1981), and these effects of LC stimulation are eliminated both by the blockade of NE synthesis and storage and by destruction of the NE innervation of cerebellum with 6-hydroxydopamine (Hoffer et al., 1973). Thus, input from this pathway might well mediate the endogenous beta adrenergic regulation of EtOH mechanisms in cerebellar Purkinje neurons suggested above.

Not only are the catecholamine pathways from the LC excited by electrical stimulation but also withdrawal induced by the mu opiate receptor antagonist naloxone after chronic morphine treatment has been reported to cause increased synaptic release of NE (Rossetti et al., 1993), as well as activation of LC neurons (Rasmussen et al., 1990; Akaoka and Aston-Jones, 1991). These latter studies report that rats receiving chronic morphine injection over 7 days developed excitation of LC neurons for more than 3 h after the systemic injection of naloxone. Furthermore, microinjection of another opiate antagonist, methylnaloxonium directly into the LC of morphine-dependent rats induced behavioral signs of withdrawal (Maldonado et al., 1992). These data, together with the observation that neither mu opioid receptors nor mu receptor mRNA is present in the cerebellar cortex (Mansour et al., 1994), suggest that the activation of noradrenergic mechanisms in the cerebellum during morphine withdrawal results from increased input from the LC innervation of that brain area. Thus, if endogenous NE can regulate acute ethanol actions in the cerebellum, this beta adrenergic effect might be accentuated during naloxone-induced morphine withdrawal.

In the present study, we investigated EtOH effects in the cerebellum under conditions of elevated endogenous noradrenergic input. We approached this question by studying EtOH-induced depressions of Purkinje neurons while elevating synaptic NE levels using three different experimental paradigms: (1) endogenously released NE was elevated by blocking reuptake with desmethylimipramine (DMI); (2) the NE innervation of the cerebellum was acutely activated by inducing withdrawal with naloxone in morphine-dependent rats; and (3) the noradrenergic input to the cerebellum was activated by electrical stimulation of the LC. We found that all three approaches to elevating endogenous catecholamine activity in the cerebellum potentiated the depressant effects of EtOH in this brain area.

Materials and Methods

Male Sprague-Dawley rats, initially weighing 200 g, were housed in the Laboratory Animal Care Center with a 12-h light/dark cycle, and food and water were provided ad libitum. Most animals weighed between 250 to 400 g at the time of recording, but morphine-tolerant animals were slightly older (350–450 g) because of the time required to establish tolerance.

The experiments reported here were carried out in accordance with the Declaration of Helsinki and with the “Guide for the Care and Use of Laboratory Animals” as adopted and promulgated by the National Institutes of Health.

Electrophysiology. Animals were anesthetized with 1.25 g/kg urethane and placed in a stereotaxic frame. Body temperature was...
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monitored by a rectal thermistor probe and maintained at 37°C by a heating pad. The cisterna was opened at the foramen magna to maintain an effective dose because of the short duration of action of this opiate antagonist.

For electrical stimulation of the LC, a bipolar stimulation electrode was stereotaxically placed 12 mm caudal of bregma and 1.2 to 1.5 mm lateral of midline. The electrode was angled 20° from vertical and was lowered 4.0 to 5.0 mm below the brain surface ventrally. The placement of the stimulation electrode was characterized by the stimulation-evoked depression of the spontaneous firing of a cerebellar Purkinje neuron. For this verification of LC electrode placement, a 40-V stimulation was delivered in a 10-Hz, 4-s train of 200-μs monophasic pulses. The interaction of LC activation with EtOH responses in the cerebellum was evaluated by reducing the stimulus parameters to 30 V and applying it as a prolonged 6-Hz train of 200-μs monophasic pulses. Control stimulations were made by raising the electrode into the brain tissue overlying the LC.

Chronic Morphine Treatment and Morphine Withdrawal. Twenty-eight adult male Sprague-Dawley rats were divided into two groups. Tolerance and dependence were induced in one group by administering two doses of morphine (40 mg/ml in 0.9% saline, pH 7.0) each day at 9 AM and 4 PM so that each animal received a morphine dose of 40 mg/kg s.c. on the first day, 60 mg/kg s.c. on the second day, and 80 mg/kg s.c. from day 3 to day 7, as has been previously described (Eidelberg and Bond, 1972). Similar injection volumes of 0.9% saline were given s.c. to the control rats for the same 7-day period. On the morning of the 8th day, morphine withdrawal was induced by administering 1 mg/kg naloxone s.c. (repeated hourly in longer experiments), and the animals either were tested for behavioral sensitivity to ethanol-induced ataxia during morphine withdrawal by monitoring loss of righting response or were investigated electrophysiologically to determine changes in neuronal sensitivity to EtOH before and during morphine withdrawal.

TABLE 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage of Maximal Time to Raise Right Hind Foot</th>
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<tbody>
<tr>
<td></td>
<td>1st Day of Chronic Treatment</td>
</tr>
<tr>
<td>2 injections of morphine daily</td>
<td>99.0 ± 1.0*</td>
</tr>
<tr>
<td>2 injections of saline daily</td>
<td>11.1 ± 19.7</td>
</tr>
</tbody>
</table>

Maximal time on hot pad: 40 s.

*p < .0001, ANOVA.

Fig. 2. Bar graphs summarizing (A) the potentiation of EtOH-induced inhibitions of neuronal firing observed on 48 cerebellar Purkinje cells in 21 animals during the facilitation of endogenous, synaptic norepinephrine levels with the catecholamine uptake antagonist DMI and (B) the antagonism of these DMI effects on seven neurons by the beta adrenergic antagonist propranolol. Both EtOH and DMI were applied from the same multibarrel micropipette used to record neuronal firing. A, EtOH-induced depressions of neuronal firing were significantly larger on the same neurons during the DMI application than they were during the control epoch (p < .0001, paired t test; t = -6.81, df = 47). B, propranolol (PRO) was administered i.p. (10 mg/kg) and caused a significant antagonism of this DMI effect (p < .001, paired t test; t = 6.22, df = 6). For this graph, the DMI potentiation of EtOH-induced inhibitions (with and without propranolol) is represented as a percentage of the control EtOH-induced depression in the absence of DMI.

integrated over 1-s time intervals and displayed as ratemeter records on a strip-chart recorder. These data were then digitized and analyzed by computer to determine percent responses to local drug applications compared with predrug baseline firing. Some data were collected and analyzed digitally using a DataWave Technologies (Longmont, CO) Experimenter's WorkBench package on a personal computer. Each neuron was required to exhibit a stable firing rate during predrug and postdrug periods, and drug antagonist responses were acceptable only if they were repeatable and reversible.

Using methods that we have described in detail, EtOH (750 mM, pH 7.0), DMI (0.1 mM, pH 7.0; Sigma Chemical Co., St. Louis, MO), and phencyclidine (PCP) ([1-(1-phenylcyclohexyl)piperidine, 1 mM, pH 7.0; NIDA, Washington, DC) were applied locally from micropipettes by micropressure ejection. Locally applied drugs were dissolved in 165 mM NaCl. Drug concentrations are typically diluted 10× to 1000× as they diffuse from the tip of the micropipette into the tissue with pressure ejection (Gerhardt and Palmer, 1987). Ejection pressure was regulated with a pneumatic valve, and the timing of drug applications was controlled by a computer-regulated crystal clock circuit. We used previously described controls for local anesthesia and pH effects as well as for artificial responses to pressure-ejected drugs (Palmer et al., 1986). In addition, 10 mg/kg propranolol HCl (5 mg/ml in 0.9% saline, pH 7.0, Sigma) was administered i.p., and 1 mg/kg naloxone (1 mg/ml in 0.9% saline, pH 7.0, Sigma) was injected s.c. The naloxone injections were repeated once per hour to maintain an effective dose because of the short duration of action of this opiate antagonist.

For electrical stimulation of the LC, a bipolar stimulation electrode was stereotaxically placed 12 mm caudal of bregma and 1.2 to 1.5 mm lateral of midline. The electrode was angled 20° from vertical and was lowered 4.0 to 5.0 mm below the brain surface ventrally. The placement of the stimulation electrode was characterized by the stimulation-evoked depression of the spontaneous firing of a cerebellar Purkinje neuron. For this verification of LC electrode placement, a 40-V stimulation was delivered in a 10-Hz, 4-s train of 200-μs monophasic pulses. The interaction of LC activation with EtOH responses in the cerebellum was evaluated by reducing the stimulus parameters to 30 V and applying it as a prolonged 6-Hz train of 200-μs monophasic pulses. Control stimulations were made by raising the electrode into the brain tissue overlying the LC.
Fig. 3. Ratemeter records showing the potentiation of EtOH- and PCP-induced depressions of Purkinje neuron firing during naloxone-induced morphine withdrawal. Depressions of neuronal firing caused by local applications of both PCP and EtOH in chronic saline-treated (morphine naïve) controls (A) were unaltered 10 to 30 min after 1 mg/kg naloxone s.c., which was used to precipitate withdrawal in morphine-tolerant animals. However, the EtOH and PCP effects on neurons from animals chronically treated with morphine (B and C) were markedly potentiated after the same naloxone treatment.
The effectiveness of the chronic morphine treatment was assessed by testing animals from both control and morphine-treated groups for their sensitivity to the noxious stimulus delivered by a hot-plate test (Lin et al., 1993b; Wang and Lee, 1993). Animals were placed into an observation chamber consisting of clear Plexiglas walls and a metal floor that was maintained at 55.0 ± 0.5°C. The day before testing, animals were placed on the nonfunctioning hot-plate for 1 min. The rats were brought to the test room 2 h before testing, and the ambient temperature during testing was 27 ± 1°C. The latency of licking a hind paw or jumping off the plate (vigorous lifting of both rear paws) was taken as the measure of nociceptive threshold (Maixner et al., 1982; Lin et al., 1993b). Cutoff time, the point at which the animal was removed from the hot-plate even if it had not yet responded, was set at 40 s to prevent tissue injury. Each animal was tested before and 20 min after the morning morphine or saline-control treatment on days 0 and 7 of the chronic treatment paradigm.

Morphine analgesia was calculated as the percentage of maximum possible effect from response latencies normalized to the maximum possible effect (Lin et al., 1993b; Wang and Lee, 1993) as follows:

\[
\% \text{MPE} = \frac{(\text{Postmorphine latency} - \text{predrug latency})}{(\text{Cutoff time} - \text{predrug latency})} \times 100
\]

**Ethanol-Induced Ataxia.** Six control and six chronically morphine-treated animals were used in a preliminary behavioral investigation to determine the feasibility of the more time-consuming electrophysiological study. For this experiment, morphine tolerant and control animals were treated with 1 mg/kg naloxone s.c. and 10 min later were given a single ethanol dose of 3 g/kg b.wt. i.p. as a 15% (w/v) solution in 0.9% saline. Each animal was then tested for duration of loss of the righting response (sleep time in min) to the EtOH administration, and additional naloxone doses (1 mg/kg s.c.) were administered at 1-h intervals after the first treatment to maintain morphine withdrawal.

Our protocol for estimating sleep time was similar to that used in the selective breeding of the LS and SS mice and has been published.
in detail elsewhere (Palmer et al., 1987a). Briefly, rats were given a single i.p. ethanol injection and tested for the duration of loss of the righting response while supported on plastic V-shaped troughs. To be counted as recovered, each rat was required to right itself in the trough three consecutive times within a 1-min period. All sleep time measurements were conducted between 8 AM and noon. All 12 rats were tested simultaneously, and room temperature was maintained at 27°C to minimize the hypothermic reaction to ethanol (Moore and Kakihana, 1978; Malcolm and Alkana, 1981).

Statistics and Experimental Design. Although several neurons can be sampled per animal when all drugs are delivered locally from multibarrel micropipettes, only one cell was studied in each animal in conjunction with the systemic administration of either naloxone or propranolol. In the case of both drugs, EtOH data was collected from a given cell both before and after systemic drug administration. Then, 10 min (naloxone) or 30 min (propranolol), respectively, were allowed for drug absorption before data collection after systemic drug administration. Statistical significance was determined for each experiment using either a paired t test or a one-way analysis of variance (ANOVA) followed by a Student-Newman-Keuls multiple comparisons test, as indicated in the text.

Results

Potentiation of EtOH by Blocking the Reuptake of Endogenous NE with DMI. Spontaneous synaptic levels of endogenously released catecholamine were facilitated with the NE uptake inhibitor DMI, and any subsequent beta adrenergic potentiation of EtOH effects on Purkinje neurons was monitored. The local pressure application of EtOH from micropipettes caused repeatable slowing of the spontaneous firing of cerebellar Purkinje neurons, and the subsequent pressure application of DMI from another barrel of the same pipette consistently potentiated these EtOH-induced depressions (Fig. 1). This effect was reversible and repeatable on a given neuron and was significant ($p < .0001$, paired $t$ test; $t = -6.81$, df = 47) over the 48 neurons studied in 21 animals (Fig. 2A). This interaction is likely mediated by a beta adrenergic mechanism because the observed DMI-induced potentiation of these EtOH effects was significantly attenuated when repeated 30 to

![Ratemeter record from a Purkinje neuron showing potentiation of EtOH-induced depressions by electrical stimulation (E.S.) of the LC. Electrical stimulation alone caused depression of the cell firing (A). Local applications of EtOH also depressed neuronal firing (B), and electrical stimulation, which was reduced to threshold intensity for altering spontaneous neuronal firing, markedly potentiated the EtOH-induced depressions (C).](image-url)
60 min after the systemic administration of propranolol (10 mg/kg, i.p.) on all seven cells studied in seven animals (Fig. 2B; *p < .001, paired t test; t = 6.22, df = 6). The depression of Purkinje neuron spontaneous firing by DMI, which is likely mediated by the alpha adrenergic actions of NE (Granholm and Palmer, 1988), was unaltered by the propranolol treatment.

**Morphine Tolerance and Potentiation of EtOH-Induced Ataxia During Opiate Withdrawal.** The effectiveness of the chronic morphine treatment used in this experiment was assayed by determining the analgesia caused by acute morphine treatment using a tail-flick test. Twenty rats were chronically treated twice daily with injections of either morphine or a control saline solution (see Materials and Methods). The animals that were chronically treated with morphine for 7 days developed tolerance to the analgesic effect of an acute morphine injection (Table 1). This effect was significant over the 10 rats studied (ANOVA: *p < .0001; F = 16.1, df = 39, followed by a Newman-Keuls multiple range test: *p < .001, q = 8.193), whereas morphine-induced analgesia after 7 days of chronic morphine treatment was not significantly different from that caused by a control saline injection either before morphine treatment or after 7 days of chronic saline treatment.

As a preliminary study to determine the feasibility of using opiate withdrawal to investigate endogenous NE influences on EtOH electrophysiology, we determined the effect of opiate withdrawal on EtOH-induced ataxia using a “sleep time” behavioral test (Palmer et al., 1987a). Withdrawal was induced acutely by the s.c. administration of 1 mg/kg naloxone, an opiate antagonist, and supplemental s.c. injections of 1 mg/kg naloxone were made once an hour thereafter. Three of the six chronic morphine-treated animals, which were withdrawing from morphine effects in this fashion, died after the administration of systemic EtOH (3 g/kg i.p.). The mean loss of righting response to a systemic EtOH dose for the remaining three animals was significantly longer from that of the six animals that were chronically treated with control saline injections and then given the same naloxone treatment before behavioral testing (215.7 ± 11.72 versus 162.5 ± 12.3 min, *p < .05, t test; t = 2.718, df = 7).

**Potentiation of EtOH-Induced Neuronal Depressions During Opiate Withdrawal.** Activation of noradrenergic nerve terminals by naloxone-induced morphine withdrawal was used to study the influence of this catecholamine pathway on EtOH effects in the cerebellum. The average responsiveness of a single cerebellar Purkinje neuron to the depressant effects of locally applied EtOH was determined in vivo before and 10 to 30 min after 1 mg/kg s.c. naloxone administration in each of 16 animals. PCP, which is principally a noradrenergic uptake inhibitor in this preparation (Marwaha et al., 1980; Palmer et al., 1987b), was applied to the same cells to assess the effectiveness of the noradrenergic pathway activation during opiate withdrawal. Eleven of the animals were chronically treated with morphine and five were chronically administered equivalent injections of a control saline solution. In control animals chronically treated with saline, naloxone administration did not alter the neuronal depressions caused by local applications of either PCP (*n = 5) or EtOH (*n = 5) on any of the five control cells studied. Typical ratemeter records are illustrated in Fig. 3A; however, the depressant neuronal effects of EtOH, when locally applied to Purkinje neurons in animals chronically treated with morphine, were potentiated by the naloxone treatment (Fig. 3, B and C). This effect is likely to be associated with increased synaptic catecholamine release in the cerebellum because the effects of PCP, an indirect noradrenergic agonist on Purkinje neurons, were also potentiated on...
the same cells during the naloxone-induced withdrawal in these animals. Thus, morphine withdrawal caused statistically significant increases (paired t tests) in the depressions of Purkinje neuron firing caused by local applications of both EtOH ($p < .01, n = 7$; $t = 4.28, df = 6$; Fig. 4, A versus B) and PCP ($p < .001, n = 11$; $t = 6.93, df = 10$; Fig. 4, C versus D).

Potentiation of EtOH-Induced Neuronal Depressions During Electrical Stimulation of the LC. Purkinje neuron responsiveness to local EtOH applications was determined before and during activation of noradrenergic afferents to the cerebellum by electrical stimulation of the LC in vivo. The stimulating electrode was stereotaxically placed in the LC so that electrical stimulation caused depressions of the Purkinje neuron being recorded (Fig. 5A), and the stimulus voltage was then reduced until no large change in spontaneous activity was observed. LC stimulation caused potentiation of the depressant effects of locally applied EtOH (Fig. 5, B and C), and this effect was statistically significant ($p < .001$, paired t test; $t = 5.53, df = 53$) among the 54 neurons studied in 29 rats (Fig. 6A). The potentiation of EtOH effects in the cerebellum by the activation of LC inputs to that brain area was probably mediated by a beta adrenergic mechanism because this effect was antagonized by the systemic administration of 10 mg/kg propranolol i.p. (Fig. 7) on all six neurons studied (Fig. 6B; ANOVA: $p < .001; F = 11.4, df = 23$, followed by a Newman-Keuls multiple range test: $p < .05$, $q = 7.067$). The LC-induced depression of Purkinje neuron firing, which is primarily mediated by the alpha adrenergic actions of NE (Granholm and Palmer, 1988), was unaltered by the propranolol treatment (Fig. 7).

**Discussion**

We previously reported that the depressant effects of locally applied EtOH on cerebellar Purkinje neurons are partially antagonized by the beta adrenergic antagonist timolol (Lin et al., 1994). These data suggested that the endogenous noradrenergic input to the cerebellum might regulate EtOH effects in that brain area. In the present study, we tested this hypothesis by monitoring the effect of elevating endogenous catecholamine synaptic activity on EtOH-induced depressions of Purkinje neurons. We used three different approaches to investigate this interaction: (1) the spontaneous level of synaptically released catecholamine was elevated with local applications of the catecholamine uptake antagonist DMI; (2) the activity of the input from the LC to the cerebellar cortex was elevated by eliciting opiate withdrawal; and (3) the noradrenergic innervation of cerebellar Purkinje neurons was directly activated by electrical stimulation of the LC. EtOH-induced depressions of Purkinje neuron firing were potentiated in all three experiments. These data support the hypothesis that endogenous activity of the catecholamine innervation of Purkinje neurons from the LC can regulate EtOH mechanisms in these cells.

A beta adrenergic mechanism probably mediates the potentiation of EtOH-induced depressions observed in this study because systemic applications of the beta adrenergic antagonist propranolol antagonize the ability of both DMI and electrical stimulation of the LC to potentiate the actions of EtOH in the present experiments. Furthermore, acute opiate withdrawal caused potentiation of not only EtOH actions but also the depressant effects of locally applied PCP. The responsiveness of cerebellar Purkinje neurons to PCP-induced depressions is known to be dependent on, and indicative of, catecholamine synaptic activity in that brain area (Marwaha et al., 1980; Palmer et al., 1987b) and, thus, is an indication of increased catecholamine synaptic input to these cells with naloxone-induced opiate withdrawal in the current experiments. Although DMI application and LC stimulation also caused direct depressions of spontaneous activity, these effects were apparently not mediated by a beta adrenergic mechanism because they were not prevented by the same propranolol administrations that blocked the observed potentiations of EtOH effects. These findings are consistent with our previous observation that the depressant effects of LC pathway activation on cerebellar Purkinje neurons are mediated by an alpha adrenergic mechanism of action (Granholm and Palmer, 1988).

LC stimulation has been previously reported not only to cause depression of spontaneous Purkinje neuron firing (Siggins et al., 1971a; Hoffer et al., 1973; Granholm and Palmer, 1988) similar to that observed in the present study but also to potentiate Purkinje neuron responses to afferent input (Moises and Woodward, 1980; Moises et al., 1981). Of particular interest in those studies is the finding that the depressant
effects of GABA on these cells is potentiated by LC stimulation through a beta adrenergic mechanism of action. This effect is mimicked by the local application of the beta adrenergic agonists at doses that have little effect on spontaneous activity (Waterhouse et al., 1982; Sessler et al., 1989; Lin et al., 1993a) and involves a cAMP/protein kinase A second messenger system (Siggins et al., 1971b; Sessler et al., 1989; Cheun and Yeh, 1992). We (Lin et al., 1993a) and others (Lee et al., 1995) previously found that EtOH potentiation of the depressant effects of GABA on cerebellar Purkinje neurons also involves a beta adrenergic mechanism of action, and we recently reported that EtOH influences the cAMP regulation of GABA responsiveness in these same cells (Freund and Palmer, 1997). Perhaps the potentiation of EtOH-induced depressions by LC afferent activation observed in the present study involves a similar interaction of EtOH with the beta adrenergic facilitation of endogenous GABA mechanisms in this brain area. We did previously find that EtOH-induced depressions of Purkinje neuron activity involve activation of the GABA_A receptor mechanism (Freund et al., 1993). We reported that beta adrenergic agonists only partially antagonize this effect, which suggests that EtOH-induced depressions are not dependent on this catecholamine mechanism (Lin et al., 1994). However, in the same study, we report that beta adrenergic agonists will routinely potentiate EtOH depressions on Purkinje neurons. In the present study, we find that the activation of endogenous noradrenergic nerve terminals in the cerebellum by LC stimulation causes a similar effect.

During this study, we collected preliminary evidence that LC activation during acute opiate withdrawal not only potentiates EtOH-induced depressions of cerebellar Purkinje neurons but also results in the prolongation of EtOH-induced behavioral ataxia measured by “sleep time.” These data are consistent with previous studies indicating that the sensitivity of cerebellar Purkinje neurons to the depressant effects of EtOH closely correlates with sensitivity to EtOH-induced behavioral ataxia (Palmer et al., 1987a). The observed changes in EtOH sleep time likely were not due to influences of chronic morphine treatment on EtOH metabolism because we previously found that the same chronic morphine treatment did not result in any changes in EtOH clearance from the blood compared with EtOH-naive or saline-treated control rats (Wang and Lee, 1993). These data suggest that the activity of LC afferents not only facilitates the cerebellar actions of EtOH but also influences some related EtOH-induced behaviors. Indeed, we found not only that both ethanol-induced electrophysiological and behavioral responses are potentiated during morphine withdrawal but also that some morphine-withdrawing animals died after a systemic EtOH dose (3 g/kg) that was never lethal in control animals. These are preliminary data and require conformation; however, they imply that acute replacement of opiate with alcohol in opiate-addicted patients could be potentially dangerous.

The present data suggest that the endogenous catecholamine innervation to the cerebellum can regulate the EtOH responsiveness of Purkinje neurons. Thus, the expression of those EtOH actions that are influenced by this beta adrenergic mechanism may vary among behavioral states depending on the associated activity of the innervation from the LC. Furthermore, the neuronal effects of EtOH on a given cerebellar neurotransmitter mechanism, such as GABA_A responsiveness, also appear to depend on the activity of this noradrenergic innervation (Lin et al., 1993a; Lee et al., 1995). Clearly, the actions of EtOH on isolated neurotransmitter mechanisms in a simple system lacking this innervation would not accurately reflect the role of that mechanism in mediating the cellular actions of EtOH in the cerebellum in vivo. Indeed, we recently found that postsynaptic differences in beta adrenergic responsiveness between cerebellar Purkinje neurons from LAS and HAS rats, which were selectively bred for low and high behavioral responsiveness to alcohol treatment, respectively, explain the differential expression of both EtOH-sensitive neuronal responses to GABA as well as rapid acute neuronal tolerance to EtOH effects between these two rat lines (Pearson et al., 1997). The characterization of such influences will require further investigation; however, it is clear that the LC innervation influences EtOH mechanisms in this brain area in vivo.

References


