Acamprosate-responsive brain sites for suppression of ethanol intake and preference

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THE GLUTAMATE ANTAGONIST, acamprosate (Campral), is one of a limited number of antirelapse drugs approved by the Food and Drug Administration to help reduce relapse risk in recovering alcoholics (25, 53). Neurochemically, it is thought to act by attenuating increased glutamatergic neurotransmission associated with alcohol dependence and repeated episodes of withdrawal (53, 56). Notably, in mPer2 mutant mice with nonfunctional mPer2, elevated glutamatergic tonic, and raised ethanol intake, acamprosate depresses extracellular glutamate and drinking to wild-type (WT) levels (56). Independent of its role in the etiology of alcoholism, mPer2 is a negative transcription factor of a molecular feedback loop that gates endogenous circadian timekeeping (47, 56).

In humans, the degree of efficacy of acamprosate in treating alcohol dependence is significant, but variable. It is estimated that recovering alcoholics treated with acamprosate are ~2–3 times more prone to remain abstinent, at least in the short term vs. placebo (34, 48). Characterization of acamprosate pharmacokinetics has revealed that acamprosate’s actions may be restricted by limited transport across biological tissues and the development of tolerance (11, 53, 66). Also, little is known concerning the brain site(s) for acamprosate’s suppressive action on ethanol intake, although there is some evidence that application of acamprosate into the nucleus accumbens core (a major component of the mesolimbic alcohol reward circuitry), can suppress ethanol intake in rats by interacting with accumbal glycine receptors and possibly by activating cholinergic transmission in the ventral tegmental area (VTA) (8, 9).

To address the question of the brain site(s) of acamprosate action, intracranial constant-release acamprosate-containing microimplants were used to map brain areas responsive to the suppressive effect of acamprosate on ethanol intake and preference during relapse. We examined the effects of the acamprosate implants in ethanol-prefering mPer2 mutant vs. wild-type (WT) mice to determine whether the propensity for excessive ethanol intake affects the degree of suppressive response to the drug. The effects of acamprosate treatment on the daily locomotor activity rhythm were also assessed to explore possible circadian rhythm-related actions of mPer2 mutation and drug treatment (3). Brain areas targeted with acamprosate in this study represent major alcohol reward [VTA, peduculopontine tegmentum (PPT), and nucleus accumbens (NA)] and circadian-related brain areas [intergeniculate leaflet (IGL) and suprachiasmatic nucleus (SCN)] (22, 26, 33, 37, 52, 61). These circadian areas were included because of the suspected role of circadian signaling in modulating ethanol reward and craving (3, 4, 47, 49, 56, 62, 63), and as such, are potential targets for acamprosate action.

MATERIALS AND METHODS

Animals

Adult, 8-wk-old homozygous mPer2 mutant male mice (strain: B6.129S7-MPER2tm1Brd/J) and wild-type (WT) mice [strain: B6(Cg)-Tyr−/−J] were produced from breeding pairs obtained from the Jackson Laboratory (Bar Harbor, ME). Both strains are back-crossed to C57BL/6. Animals were singly housed in polycarbonate cages under a 12:12-h light-dark photoperiod (LD) at a light intensity of 270 lux in a temperature-controlled vivarium (23°C) with food (Prolab 3000, PMI Feeds, St. Louis, MO) and water provided ad libitum. The experiments followed the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Kent State Institutional Animal Care and Use Committee.
Acamprosate Implants

Bilateral acamprosate microimplants (1.25 mm × 0.75 mm; prepared from a homogeneous mixture of 300 mg acamprosate and 1 g beeswax [or beeswax alone for blank control implants]) were stereotaxically targeted to the lateral margin of the selected drug reward and circadian brain areas (stereotaxic coordinates derived from 1) VTA: AP = −3.08 mm from bregma, L = −0.75 mm from midline, and H = −4.00 mm from dura; 2) PPT: AP = −4.72 mm from bregma, L = −0.80 mm from midline, and H = −3.00 mm from dura; 3) NA: AP = +1.70 mm from bregma, L = −0.75 mm from midline, and H = −4.00 mm from dura; 4) IGL: AP = −2.30 mm from bregma, L = −2.40 mm from midline and H = −3.00 mm from dura; 5) SCN: AP = −0.46 mm from bregma, L = −0.03 mm from midline, and H = −5.50 mm from dura; and 6) Hippocampus: AP = −2.70 mm from bregma, L = −2.25 mm from midline, H = −2.00 mm from dura). For implantation, animals were anesthetized with pentobarbital sodium (Nembutal: 35.0 mg/kg) and pretreated with Marcaine (0.25% bupivacaine, 0.05 ml sc) and atropine (0.09% atropine, 0.10 ml ip) to manage localized pain and reduce respiratory occlusion, respectively. The microimplants were extruded from a stainless-steel 26-gauge cannula directly into the targeted brain regions. Following implantation, the skin was sutured with stainless-steel wound clips, and the animals were given 3 days of postoperative recovery prior to experimentation.

In Vitro Acamprosate Release

The daily rate of acamprosate release from the microimplants was determined in vitro by incubating the implants in 1.5 ml physiological saline at 37°C over 27 days. Each day, the saline medium was decanted and replenished with new medium. The daily samples were stored frozen prior to acamprosate analysis by UV spectrometry (Varian Cary 300 Bio; Varian, Santa Clara, CA) using an absorbance calibration curve generated at a wavelength of 201 nm. Release of acamprosate was asymptotic, reaching a relatively steady-state release rate of ~50 ng·day⁻¹·implant⁻¹ by day 6 of incubation. This profile of acamprosate release is shown in Fig. 1.

Histological Evaluation of Acamprosate Implant Sites

Animals were deeply anesthetized with Nembutal and intracardially perfused with 100 ml of 4% buffered paraformaldehyde (pH = 7.3). The brains were extracted and immersion-fixed in 4% paraformaldehyde for 24 h, followed by immersion in 30% sucrose for 24 h at 4°C. Cryostat SCN and hippocampal sections (40 μm-thick) were stained with cresyl violet. VTA, PPT, IGL, and NA (40 μm-thick) sections were incubated with rabbit polyclonal IgG antibodies to tyrosine hydroxylase for localization of dopaminergic neurons in the VTA and NA (Santa Cruz Biotechnology, Santa Cruz, CA), choline acetyltransferase for localization of cholinergic neurons in the PPT, and glutamate decarboxylase for localization of GABA neurons in the IGL (Millipore, Billerica, MA). Staining was visualized using Vectastain Elite ABC kit with 3,3-diaminobenzidine tetrahydrochloride as chromagen (Vector Laboratories, Burlingame, CA). Representative micropellet photomicrographs and overall micropellet placements are presented diagrammatically in Fig. 2.

Circadian Activity Measurements

General circadian locomotor activity was measured using overhead passive infrared motion detectors interfaced with a computerized data acquisition system (Clocklab, Coulbourn Instruments, Whitehall, PA). Data were collected in 1-min bins, and activity onset associated with lights-off [designated as zeitgeber time (ZT) 12] was defined by the initial 6-min period that 1) coincided with an intensity of activity that exceeded 10% of the maximum rate for the day, 2) preceded by at least 4 h of activity quiescence, and 3) followed by at least 60 min of sustained activity. Activity offset was defined as a final period of activity that 1) immediately preceded by at least 60 min of activity and 2) was followed by at least 4 h of inactivity. Alpha (measured in hours) represented the length of the nighttime activity period between activity onset and offset. Total activity across alpha was calculated from normalized activity bout durations (measured in min) × activity bout numbers derived from the Clocklab data acquisition system.

Experimental Protocol

WT and mPer2 mutant mice (n = 12, each strain) received free-choice (15%) ethanol/water for 3 wk. The ethanol was withdrawn for 3 wk and then reintroduced to facilitate relapse. Ethanol preference was calculated as the percentage of daily ethanol intake relative to total fluid intake measured in 50-ml plastic, graduated vials to the nearest 0.25 ml (Fisher Scientific; Pittsburgh, PA). Four days before ethanol reintroduction, blank or acamprosate-containing microimplants were implanted into brain areas listed above (n = 6, for each area). Five weeks following ethanol reintroduction, animals were euthanized with pentobarbital overdose, and brains were immediately extracted to verify implant sites. General circadian activity was recorded during ethanol withdrawal and ethanol reintroduction.

Statistics

A repeated-measures ANOVA was used to compare raw values of ethanol intake and preference between strain (mPer2 mutant vs. WT) during ethanol introduction and reintroduction and raw values of water intake between strain, treatment, or brain area. A one-way ANOVA was used to compare circadian activity measures between strain. Student- Newman-Keuls post hoc analysis was executed where appropriate. Paired t-tests were used to compare ethanol intake, preference, and circadian activity measures prior to and during theoretical constant acamprosate (or blank) release over 4 wk. SPSS 16.0 (Chicago, IL) was used to analyze the data. In all cases, the level of significance was P < 0.05.

RESULTS

Strain-related differences in ethanol intake, preference, and circadian activity. Baseline levels of ethanol intake and preference were 1.9- and 1.3-fold greater, respectively, in mPer2 mutant vs. WT mice (intake: F1,22 = 56.4; preference: F1,22 =
66.9; both, \( P < 0.01 \)). Nocturnal activity onset was 2.1 ± 0.4 h earlier in the \( mPer2 \) mutant mice compared with WTs (ZT 10.4 ± 0.2 vs. ZT 12.5 ± 0.6, respectively; \( F_{1,22} = 42.1; P < 0.01 \)). Alpha was extended by 1.7 ± 0.1 h in the \( mPer2 \) mutant vs. WT mice (\( F_{1,22} = 10.2; P < 0.01 \)), and the \( mPer2 \) mutants were 1.4 times more active across the nocturnal active period compared with WTs (total activity: \( F_{1,22} = 16.8 \); bout duration: \( F_{1,22} = 48.1 \); both, \( P < 0.01 \)).
Acamprosate Suppresses Ethanol Intake and Preference in Brain Reward Areas

Ventral tegmental area. Acamprosate implants in the VTA of mPer2 mutants (calculated over total days of significant acamprosate effect) suppressed ethanol intake and preference from pretreatment levels by 38.7 ± 7.8% and 18.7 ± 4.3%, respectively, during ethanol reintroduction (both, P < 0.01; Figs. 3 and 4). In WT mice, this treatment suppressed ethanol intake and preference by 60.5 ± 11.8% and 49.6 ± 9.6%, respectively (both, P < 0.01). Higher values of ethanol intake and preference were observed in mPer2 mutant mice vs. WT mice throughout the acamprosate treatment (intake: F1,22 = 15.6; preference: F1,22 = 18.3; both, P < 0.01). Blank implants in mPer2 mutants and WT mice did not change ethanol intake or preference from baseline (both, P > 0.05).

Pedunculopontine tegmental area. Acamprosate implants in the PPT of mPer2 mutants suppressed ethanol intake and preference from pretreatment levels by 16.5 ± 7.5% and 14.2 ± 3.4%, respectively, during ethanol reintroduction (both, P < 0.01; Figs. 3 and 4). In WT mice, this treatment suppressed ethanol intake and preference by 45.6 ± 12.4% and 41.0 ± 3.8%, respectively (both, P < 0.01). Higher values of ethanol intake and preference were observed in mPer2 mutant mice vs. WT mice throughout the acamprosate treatment (intake: F1,22 = 22.4; preference: F1,22 = 17.2; both, P < 0.01). Blank implants in mPer2 mutants and WT mice did not change ethanol intake or preference from baseline (both, P > 0.05).

Nucleus accumbens. Acamprosate implants in the NA of mPer2 mutants did not change ethanol intake or preference from pretreatment levels during ethanol reintroduction (both, P > 0.05; Figs. 3 and 4). In WT mice, this treatment suppressed ethanol intake and preference by 27.2 ± 11.2% and 22.0 ± 5.4%, respectively (both, P < 0.01). Higher values of ethanol intake and preference were observed in mPer2 mutant mice vs. WT mice throughout the acamprosate treatment (intake: F1,22 = 16.3; preference: F1,22 = 18.8; both, P < 0.01). Blank implants in mPer2 mutants and WT mice did not change ethanol intake or preference from baseline (both, P > 0.05).

Acamprosate Acts Within Circadian Areas to Suppress Ethanol Intake and Preference

Suprachiasmatic nucleus. Acamprosate implants in the SCN of mPer2 mutants suppressed ethanol intake and preference from pretreatment levels by 36.3 ± 11.9% and 28.8 ± 4.7%, respectively, during ethanol reintroduction (both, P > 0.05; Figs. 5 and 6). In WT mice, this treatment suppressed ethanol intake and preference by 48.7 ± 17.9% and 41.1 ± 5.9%, respectively (both, P < 0.01). Higher values of ethanol intake and preference were observed in mPer2 mutant mice vs. WT mice throughout the acamprosate treatment (intake: F1,22 = 40.0; preference: F1,22 = 9.4; both, P < 0.01). Blank implants in mPer2 mutants and WT mice did not change ethanol intake or preference from baseline (both, P > 0.05).

Intergeniculate leaflet. Acamprosate implants in the IGL of mPer2 mutants did not change ethanol intake or preference from pretreatment levels during ethanol reintroduction (both, P > 0.05; Figs. 5 and 6). In WT mice, this treatment suppressed ethanol intake and preference by 45.0 ± 19.6% and 28.8 ± 7.8%, respectively (both, P < 0.01). Higher values of ethanol intake and preference were observed in mPer2 mutant mice vs. WT mice throughout the acamprosate treatment (intake: F1,22 = 22.1; preference: F1,22 = 16.5; both, P < 0.01). Blank implants in mPer2 mutants and WT mice did not change ethanol intake or preference from baseline (both, P > 0.05).

Acamprosate does not affect ethanol intake and preference in the hippocampus. Acamprosate implants in the hippocampus of mPer2 mutant and WT mice did not change ethanol intake or preference from pretreatment levels during ethanol reintroduction (all P > 0.05; Figs. 5 and 6). Higher values of ethanol intake and preference were observed in mPer2 mutant mice vs. WT mice throughout the acamprosate treatment (intake: F1,22 = 95.4; preference: F1,22 = 67.5; both P < 0.01). Blank implants in mPer2 mutants and WT mice did not change ethanol intake or preference from baseline (both P > 0.05).

Effects of acamprosate on water intake. Pretreatment levels of water intake did not differ between mPer2 mutant and WT mice (96.0 ± 1.8 ml·kg⁻¹·day⁻¹ and 96.2 ± 1.6 ml·kg⁻¹·day⁻¹, respectively; F1,22 = 1.1; P > 0.05). Across all brain areas of implant and strain, there was a main effect for treatment, such that mice with acamprosate implants consumed more water compared with mice with blank implants (123.1 ± 4 ml·kg⁻¹·day⁻¹ and 103.8 ± 3.5 ml·kg⁻¹·day⁻¹, respectively; F1,22 = 46.5; P < 0.01). There were significant interactions for brain area × treatment (F2,33 = 14.1), brain area × strain (F2,33 = 17.5), and brain area × treatment × strain (F2,33 = 2.9). Within acamprosate treatment groups, there was a significant effect of strain, such that WT mice consumed more water compared with mPer2 mutants (125.9 ± 4.3 ml·kg⁻¹·day⁻¹ and 112.5 ± 3.3 ml·kg⁻¹·day⁻¹, respectively; F1,10 = 66.5; P < 0.01) and a significant effect for brain area of implant, such that mice with acamprosate implants in reward and circadian brain areas consumed more water compared with mice with acamprosate implants in the hippocampus (132.0 ± 3.1 ml·kg⁻¹·day⁻¹ and 94.8 ± 3.6 ml·kg⁻¹·day⁻¹, respectively; F5,10 = 8.5; P < 0.05, post hoc analyses). Water intake did not differ between blank treatment groups (P > 0.05).

Effects of acamprosate on circadian locomotor activity. Acamprosate and blank implants in all targeted brain areas of mPer2 mutant and WT mice did not change the phase angle of entrainment to the LD cycle or alpha. However, there was a 1.3-fold increase in the amplitude of nighttime circadian activity rhythms from pretreatment levels in WT mice with acamprosate implants in the VTA and NAc (P < 0.05). Acamprosate implants in the SCN of mPer2 mutant and WT mice increased the amplitude of nighttime circadian activity from pretreatment levels by 1.2- and 2.3-fold, respectively (both P < 0.05; Fig. 7).

DISCUSSION

Acamprosate has been used clinically since 1989 as a pharmacological agent to help promote abstinence in alcohol-dependent patients. In the majority of clinical trials, this drug was shown to have a statistically significant effect over placebo in controlling relapse, at least during the initial period of abstinence (34, 48). In rodents, acamprosate’s overall suppressive effect on ethanol intake is consistent across studies, despite wide differences in drug and/or ethanol treatment regimens. For example, in C57 mice subjected to the drinking-in-the-dark protocol to enhance nighttime ethanol intake, systemic administration of acamprosate (300
Fig. 3. Line graphs show percent change of ethanol intake ± SE from pretreatment levels in mice receiving acamprosate (ACAMP) or no acamprosate (BLANK) from microimplants in reward areas [ventral tegmental area (VTA), peduculopontine tegmentum (PPT), and nucleus accumbens (NAc)] during simulated relapse. *Significant difference from pretreatment levels ($P < 0.05$). *Significant difference between treatment groups for any given day ($P < 0.05$). Bar graphs show means ± SE for the time point of maximal suppression of ethanol intake by acamprosate microimplants in WT vs. mPer2 mutant (PER2 KO) mice. *Significant difference between strains ($P < 0.05$).
Fig. 4. Line graphs denote percent change of ethanol preference ± SE from pretreatment levels in mice receiving acamprosate (ACAMP) or no acamprosate (BLANK) from microimplants in reward areas (VTA, PPT, and NAc) during simulated relapse. *Significant difference from pretreatment levels (P < 0.05). *Significant difference between treatment groups for a given day (P < 0.05). Bar graphs show means ± SE for the time point of maximal suppression of ethanol preference by acamprosate microimplants in WT vs. mPer2 mutant (PER2 KO) mice. *Significant difference between strains (P < 0.05).
Fig. 5. Line graphs show percent change of ethanol intake ± SE from pretreatment levels in mice receiving acamprosate (ACAMP) or no acamprosate (BLANK) from microimplants in circadian areas [suprachiasmatic nucleus (SCN), intergeniculate leaflet (IGL), and also hippocampus (HP)] during simulated relapse. *Significant difference from pretreatment levels ($P < 0.05$). *Significant difference between treatment groups for a given day ($P < 0.05$). Bar graphs denote means ± SE for the time point of maximal suppression of ethanol intake by acamprosate microimplants in WT vs. mPer2 mutant (PER2 KO) mice. *Significant difference between strains ($P < 0.05$).
Fig. 6. Line graphs show percent change of ethanol preference ± SE from pretreatment levels in mice receiving acamprosate (ACAMP) or no acamprosate (BLANK) from microimplants in circadian areas (SCN, IGL, and HP) during simulated relapse. *Significant difference from pretreatment levels ($P < 0.05$). *Significant difference between treatment groups for a given day ($P < 0.05$). Bar graphs show means ± SE for the time point of maximal suppression of ethanol preference by acamprosate microimplants in WT vs. mPer2 mutant (PER2 KO) mice. *Significant difference between strains ($P < 0.05$).
mg/kg ip) suppresses nighttime ethanol intake by 20% (26). Also in C57 mice, acamprosate (300 mg/kg ip) reduces overall daily ethanol intake under free-choice by up to 70% (3). In ethanol-preferring clock gene (mPer2) mutant mice, acamprosate (200 mg/kg and 300 mg/kg ip) suppresses free-choice ethanol intake by 60–77% (3, 56). In ethanol-prefer-
ring rats, acamprosate (200 mg/kg ip) suppresses ethanol intake by 20–40% (8, 59). Finally, as shown here, brain stimulation with acamprosate-containing microimplants in selected reward and circadian areas of C57 WT and mPer2 mutant mice reduces free-choice ethanol intake during in-
duced relapse by 40–50%. The commonality of effects of acamprosate observed at the behavioral level between hu-
mans and rodents suggests that defining the central sites and neurophysiological actions of this drug from basic animal studies is useful for understanding the nature of its clinical action in humans.

Acamprosate Action in Brain Reward Areas

To our knowledge, the present study is the first to explore the effects of centrally administered acamprosate in modulat-
ing ethanol drinking behavior. In the first series of experiments, bilateral acamprosate implants were targeted to brain reward areas implicated in modulating ethanol intake and craving. These areas included the VTA and NA of the mesocorticolum-
sic system and the PPT of the mesopontine system. As re-
vealed in rodent models of alcoholism, lesioning or pharma-
cologic manipulation of the VTA (18, 20), the NAc (10, 14), or the PPT (52) alters ethanol intake. In our experiments, the most pronounced response to acamprosate was in the VTA and PPT, where acamprosate decreased ethanol intake by 60% and preference by 40% in WT mice. At present, there is little direct neurochemical evidence for an action of acamprosate in these regions. However, such action, at least in the VTA, is plausible given evidence that ethanol activation in this area stimulates dopamine release in the NAc (36), which is also affected by acamprosate via modulation of VTA ACh receptors (8). Also, acamprosate inhibits NMDA (glutamatergic) receptor response in mesencephalic neurons, presumably containing elements of the VTA (1). Further, antagonism of NMDA receptors within the VTA has also been shown to reduce reward-seeking be-
haviors (31). Finally, acamprosate reduces behavioral ethanol withdrawal (2, 55), ameliorates glutamate neurotoxicity, and therefore, may limit the drive to drink (15, 28, 35) by acting in part through metabotropic glutamate type 5 receptors (mGlu5) (28, 38), which are also present in the VTA (46).

Acamprosate implants in the NAc of WT mice also signifi-
cantly suppressed ethanol intake and preference (both by ~20%), but to a lesser extent than in the other reward areas. Nevertheless, this suppression is significant with respect to previous observations that strychnine administration into the NAc of ethanol-prefering rats (to inhibit glycine receptors) completely blocked acamprosate suppression of ethanol intake. Also, previous studies have shown that the administration of acamprosate in the NAc increases levels of accumbal extracel-
lar dopamine (8, 9) and dopamine reuptake transporters (11). Further, acamprosate dampens ethanol and NMDA-induced accumbal dopamine release (6, 44, 57). These results, to-
gether with those of the present study, strongly implicate the NAc as a target for acamprosate (8, 9), which is consistent with the role of the NAc in contributing to alcohol intake (43). It is notable that the degree of response to acamprosate in all three reward areas in mPer2 mutants was less robust than in WTs. It is possible that the enhanced drive for ethanol in mPer2 mutants rendered them less susceptible to acamprosate action. This is consistent with our previous study showing that intraperitoneal injection of acamprosate was somewhat less effective in suppressing ethanol intake in mPer2 mutants compared with WTs (3).

It should be noted that the present results do not rule out possible actions of acamprosate in other brain regions involved with ethanol drive and reward. One such area is the striatum/ caudate nucleus, particularly since systemic acamprosate re-
duces extracellular glutamate in the ventral striatum of mPer2 mutant mice (56). Interestingly, one area in which acamprosate did not suppress drinking and preference is the hippocampus. This is surprising since the hippocampus participates in rein-
statement of alcohol seeking via operant conditioning, and
such seeking can be prevented by the activation of metabotropic glutamate receptors, which are affected by acamprosate (51, 65). Nevertheless, this negative result helps support the issue of anatomical specificity of the acamprosate-releasing implants.

**Acamprosate action in brain circadian areas.** In the second series of experiments, acamprosate was targeted to brain sites involved in regulating circadian timing. Recent studies have shown that the circadian timing system regulates ethanol seeking and consumption as reflected in the distinct daily rhythm in ethanol intake that peaks at the beginning and end of the night in rodent models of alcoholism (3, 4, 16, 49, 62) and in the additional circadian phase of ethanol intake that aligns with a 2-h phase-advance in nighttime activity onset in mPer2 mutant vs. wild-type mice (3). This strain difference in circadian entrainment in mPer2 mutant vs. wild-type mice is further noted in this study. In mammals, behavioral and physiological circadian rhythms are generated and maintained by the SCN of the anterior hypothalamus (12, 17, 40). Major photic and nonphotic regulatory inputs to the SCN arise from the retina and the IGL, respectively (7, 21, 22, 26, 30). The circadian phase of alcohol intake could be regulated by signaling from the SCN to the VTA (37) and/or by clock gene activity within the reward areas (47, 63). In view of findings that 1) the SCN clock is directly disrupted by ethanol (4, 5, 45, 49, 50) and 2) the SCN and IGL possess transmitter systems sensitive to acamprosate, including ACh, glutamate, and glycine (29, 41, 58), these circadian regulatory areas represent potential substrates for acamprosate’s modulation of ethanol intake. Results from the present study bear out this hypothesis, as acamprosate implants targeted to the SCN and IGL inhibited ethanol intake in WT mice to a similar degree as acamprosate implants in the VTA and PPT. Also, unlike other targeted regions, acamprosate in the SCN suppressed ethanol intake and preference in WTs and mPer2 mutants similarly. It is notable that actogram analysis of circadian locomotor activity revealed that acamprosate suppression of ethanol intake was associated with few effects on circadian rhythm parameters, which were limited to an increase in the nighttime rhythm amplitude, and, more importantly, did not perturb rhythm stability, phase angle of entrainment to the LD cycle, and the duration of nocturnal activity (alpha). This indicates that the suppression of alcohol intake is not a direct consequence of altered circadian timekeeping.

**Acamprosate implants: site specificity, release kinetics, tolerance, and postdeprivation rebound.** The constant-release acamprosate microimplants were originally designed to map hypothalamic sites of action of another small molecule, melatonin, in mice (23, 24). The in vitro release profile is similar for acamprosate and melatonin, both having a similar molecular mass (181 and 232 g/mol, respectively). The melatonin implants had differential effects within various hypothalamic nuclei over a 7-wk period (23), and autoradiographic analysis of 3H-melatonin incorporated into the implants showed that spread of label was ~0.2 mm, indicating reasonable site specificity of implant effect. Site specificity of acamprosate action is highlighted diagrammatically in Fig. 2. In this regard, the extent of acamprosate suppression of ethanol intake and preference in mice with mistargeted acamprosate microimplants was substantially less than in mice that received accurately targeted implants (10–15% vs. 45–60%, respectively).

The in vitro release of acamprosate from the implants is asymptotic, with near-constant output of ~50 ng·day^{-1}.ml^{-1} incubation medium apparent by day 7. This concentration (0.3 μM) is similar to that in the cerebrospinal fluid of recovering alcoholic patients taking the medication (0.5–1.5 μM) (64). In the brain, the concentration of acamprosate at the point of release from the implant would likely be considerably higher, probably within the range suitable for modulating activated NMDA receptor activity associated with ethanol withdrawal (13, 42). It is apparent that in the SCN, IGL, and PPT, the suppressive action of the acamprosate began to steadily decrease by ~8 days of implantation, suggestive of developing drug tolerance, as drug release is constant at this time. In the VTA and NAc, the reduction in response to acamprosate began to occur by ~20 days. In studies on acamprosate tolerance, the suppressive actions of acamprosate on voluntary ethanol intake were reversed by the second week of daily systemic administration of acamprosate in alcohol-preferring rat strains (11, 53), which is in line with the present observations.

In previous studies (32, 39, 54, 60), it has been observed that repeated episodes of withdrawal induce a postdeprivation elevation in ethanol intake, and promote a more compulsive vs. controlled state of ethanol intake, reminiscent of drinking in relapsed alcoholics. Thus, one methodological limitation of the present study is that is does not discriminate between compulsive vs. controlled state of ethanol intake, reminiscent of drinking in relapsed alcoholics. However, the concentration of acamprosate in the SCN and IGL was similar for acamprosate and melatonin, both having a similar molecular mass (181 and 232 g/mol, respectively). The in vitro release of acamprosate from the implants is apparent by day 7. This concentration (0.3 μM) is similar to that in the cerebrospinal fluid of recovering alcoholic patients taking the medication (0.5–1.5 μM) (64). In the brain, the concentration of acamprosate at the point of release from the implant would likely be considerably higher, probably within the range suitable for modulating activated NMDA receptor activity associated with ethanol withdrawal (13, 42). It is apparent that in the SCN, IGL, and PPT, the suppressive action of the acamprosate began to steadily decrease by ~8 days of implantation, suggestive of developing drug tolerance, as drug release is constant at this time. In the VTA and NAc, the reduction in response to acamprosate began to occur by ~20 days. In studies on acamprosate tolerance, the suppressive actions of acamprosate on voluntary ethanol intake were reversed by the second week of daily systemic administration of acamprosate in alcohol-preferring rat strains (11, 53), which is in line with the present observations.

**Perspectives and Significance**

In summary, this study presents the first demonstration of brain sites responsive to the suppressive effect of acamprosate on ethanol intake and preference. Specifically, these experiments have revealed that reward and circadian brain areas are responsive to acamprosate and that acamprosate’s actions are modulated by the mPer2 clock gene. Thus, genetic and/or other factors that contribute to a higher drive for ethanol intake could affect the therapeutic efficacy of acamprosate treatment.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

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