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PROLACTIN-RELEASING PEPTIDE (PrRP) PROMOTES AWAKENING AND SUPPRESSES ABSENCE SEIZURES

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Abstract—Prolactin releasing peptide (PrRP) is a recently identified neuropeptide that stimulates prolactin release from pituitary cells. The presence of its receptor outside the hypothalamic-pituitary axis suggests that it may have other functions. We present here evidence that PrRP can modulate the activity of the reticular thalamic nucleus, a brain region with prominent PrRP receptor expression that is critical for sleep regulation and the formation of non-convulsive absence seizures. Intracerebroventricular injection of PrRP (1–10 nmol) into sleeping animals significantly suppresses sleep oscillations and promotes rapid and prolonged awakening. Higher concentrations of PrRP (10–100 nmol) similarly suppress spike wave discharges seen during absence seizures in genetic absence epilepsy rats from Strasbourg, an animal model for this disorder. In concordance with these findings, PrRP suppressed evoked oscillatory burst activity in reticular thalamic slices *in vitro*.

These results indicate that PrRP modulates reticular thalamic function and that activation of its receptor provides a new target for therapies directed at sleep disorders and absence seizures. © 2002 IBRO. Published by Elsevier Science Ltd. All rights reserved.

Key words: prolactin releasing peptide, GPCR, sleep, petit-mal seizures, reticular nucleus of the thalamus, thalamic slice.

The natural ligand of the G protein-coupled receptor GPR10 (Marchese et al., 1995; also called hGR3 by Hinuma et al., 1998) and its rat homologue UHR-1 (Welch et al., 1995) was isolated as a 31-amino acid peptide (Hinuma et al., 1998). This peptide was named prolactin releasing peptide (PrRP) because it induced release of prolactin from pituitary cells through activation of the GPR10 receptors. The PrRP receptor is highly expressed not only in the anterior pituitary but also in various hypothalamic nuclei (Hinuma et al., 1998; Roland et al., 1999) and several studies have shown PrRP as an endocrine modulator of hypothalamic hormones such as FSH/LH (Seal et al., 2000), CRH (Matsumoto et al., 2000) and oxytocin (Maruyama et al., 1999b) as well as being involved in the regulation of food intake (Lawrence et al., 2000). Other studies have reported evidence supporting the notion that PrRP functions are not confined to the hypothalamus/pituitary system. Innervation by fiber tracts containing PrRP was found in the amygdala, thalamus, and in the bed nucleus of the stria terminalis (Maruyama et al., 1999a). The receptor for PrRP is present in various brain regions and receptor distribution overlaps in some, but not all, areas with that of the PrRP-positive fiber tracts (Roland et al., 1999; Ibata et al., 2000). These anatomical data suggest that the PrRP system may have broader functions in the brain that are not yet understood.

One brain area with prominent expression of PrRP receptor mRNA is the reticular thalamic nucleus (RTN) (Roland et al., 1999; Ibata et al., 2000), a layer of mainly GABAergic cells that surrounds much of the thalamus like a shell (Jones, 1985). The RTN receives collaterals from thalamocortical and corticothalamic fibers and in return modulates thalamic output through inhibitory projections (Destexhe, 2000; Murray and Guillery, 1996). Because of these interconnections, the RTN is believed to play an important role in controlling the transfer of sensory information to the cortex and also in setting the vigilance state (Steriade et al., 1986). It is of

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Abbreviations: aCSF, artificial cerebrospinal fluid; ECoG, cortical EEG; EDTA, ethylenediaminetetra-acetate; EEG, electroencephalogram; EMG, electromyograph; GAD, glutamate decarboxylase; GAERS, genetic absence epilepsy rats from Strasbourg; HSD, honestly significant difference; PrRP, prolactin releasing peptide; RTN, reticular thalamic nucleus; SWD, spike wave discharges.

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particular interest in this regard that thalamic activity can assume two alternative firing modes characterized as tonic firing and as rebound burst firing (Contreras et al., 1993). The latter sustains sleep spindles (7–14 Hz) and occurs during early sleep. The input from the RTN is believed to play a critical role in this form of synchronized activity (Steriade et al., 1985). Characteristic thalamocortical spike-and-wave discharges (SWD) also appear as a pathological marker in the non-convulsive type of epilepsy called absence seizure (petit mal), and abnormalities in the RTN-thalamic circuitry have been implicated in its pathogenesis (McCormick and Bal, 1997; Avanzini et al., 2000).

Given the critical role of the RTN neurons in the generation and control of thalamic oscillatory activity, activation of PrRP receptors in this region could potentially be used to influence sleep/wake states and absence seizures. The present study tested this idea by measuring the effects of PrRP infusion on oscillatory activity in the thalamus/RTN system in an *in vitro* slice preparation and in two *in vivo* paradigms, namely the sleep/wake states in normal rats and the electroencephalogram (EEG) seizures in the genetic absence epilepsy rats from Strasbourg(GAERS).

EXPERIMENTAL PROCEDURES

In situ hybridization and double-labeling in situ hybridization

In situ hybridization was performed as previously described (Winzer-Serhan et al., 1999). Frozen sections (20 μm) were prepared from adult Sprague–Dawley rat brains and fixed in 4% paraformaldehyde in 0.1 M PB (pH 7.4). *In situ* hybridization was carried out by incubating the sections overnight at 60°C in hybridization buffer (50% formamide, 10% dextran sulfate, 500 μg/ml tRNA, 10 mM dithiothreitol, 0.3 M NaCl, 10 mM Tris pH 8.0, 1 mM EDTA pH 8.0) with ^{35}S-labeled GPR10 sense and antisense cRNA probes (10^7 c.p.m./ml). The cRNA probes were prepared from full length GPR10 cDNA (1100 bp) template (gift from Dr. Brian O'Dowd, University of Toronto) in pcDNA3. The template was first linearized with either *Hin*dIII or *Not*I to generate the templates for the antisense or sense cRNA transcripts using SP6 or T7 RNA polymerases, respectively. The cRNA probes were generated by alkaline hydrolysis to approximately 600 bases. The sections were opposed to β-max film with ^{14}C standards of known radioactivity. Some slides were dipped in liquid Kodak NT2B emulsion and exposed for four weeks at 4°C. After development, the sections were counter-stained with Cresyl Violet.

Glutamate decarboxylase (GAD) double-labeling studies were carried out the same way except that hybridization included both ^{35}S-labeled GPR10 cRNA (2×10^7 c.p.m./ml) and digoxigenin-labeled GAD67 cRNA (0.04 μg/ml). GAD67 probe was generated from GAD67 cDNA in pBS (gift of Dr. Edward Jones) using *Bam*H1 and T7 to produce the antisense probe and *Pvu*II and T3 to produce the sense probe. Digoxigenin-UTP was added to the reaction instead of ^{35}S-UTP. All subsequent steps were done according to manufacturer's instructions (Genius kit, Roche).

EEG/electromyograph (EMG) recordings and i.c.v. injections into sleeping animals

EEG and EMG recordings were made from male Sprague–Dawley rats (300–400 g, Sasco, Oregon, WI, USA) according to the method described previously (Berridge and Foote, 1996). Rats were housed in pairs for at least seven days prior to surgery with *ad libitum* access to food and water on an 11/13 h light/dark cycle (lights on 7:00 AM). Two microliters of vehicle or PrRP31 dissolved in vehicle (1 nmol/2 μl; 10 nmol/2 μl) was infused intracerebroventricularly through a pre-implanted cannula (Berridge and Foote, 1996). Artificial extracellular fluid (147 mM NaCl, 1.3 mM $CaCl_2$, 0.9 mM $MgCl_2$, 2.5 mM KCl, 5.0 mM NaH_2PO_4; pH 7.4) plus 200 μg/ml cytochrome c served as vehicle. Infusions were made over a 2-min period using a microprocessor-controlled infusion pump (Harvard Apparatus, South Natick, MA, USA). Cortical EEG (ECoG) from the frontal cortex and EMG from the dorsal neck muscles were recorded for 60 min prior to infusion and 90 min following infusion. To be scored as a distinct epoch, the appropriate ECoG and EMG activity patterns needed to persist for a minimum of 15 s. Time spent in each state was scored and totaled for the five 30-min epochs of the observation period, two during the pre-infusion period and three during the post-infusion period. The location of the infusion needle was verified morphologically after each experiment. Data were only used for analysis when EEG recordings were electrically adequate and infusion needle placement was accurate. Statistical analysis was performed using a two-way mixed-design analysis of variance with drug treatment as the between-subjects variable and time as the within-subjects variable. Post-hoc analyses were conducted using Tukey's honestly significant difference (HSD) test.

EEG recordings and i.c.v. injection of GAERS

The ECoG was measured with conventional methods (Liu et al., 1992). In brief, six male GAERS (300–400 g) were bilaterally implanted with 4 stainless-steel electrodes at the frontal and parietal cortex, and with a stainless steel guide cannula (AP = −0.8, ML = 1.2, CV = 3 mm, with bregma as reference). Both the guide cannula and EEG electrodes were anchored to the skull using retaining screws and dental acrylic cement. After one week of recovery, a stainless steel injection cannula was introduced into the guide cannula such that it extended 2–4 mm beyond the tip. Five microliters of solution containing 0, 10, 50 or 100 nmol of PrRP31 were injected through the injection cannula over a 1-min period. The EEG was recorded continuously for the duration of the experiment while the animal was freely moving. The rats were carefully watched and were prevented from falling asleep. Seizure duration was determined as the cumulative duration of spindle wave discharge per consecutive 20-min periods (seconds of seizure activity per 20-min recording) before (reference) and at least one hour after the initial injection. Data from six animals receiving the same treatment were pooled. Statistical analysis was performed using the non-parametric Wilcoxon test.

Electrophysiology in thalamic and hippocampal slices

Horizontal thalamic slices (400 μm) were prepared from Sprague–Dawley rats (postnatal 13–15 day) using a vibratome (Leica, VT1000S) (Cox et al., 1997). The slices were transferred to a recording chamber after at least 1 h of recovery and were maintained in superfusion with artificial cerebrospinal fluid (aCSF) equilibrated with 95% O_2/5% CO_2 at 0.5 ml/min. The aCSF contained (in mM): NaCl 126, KCl 2.5, NaH_2PO_4 1.25, $CaCl_2$ 2, $MgSO_4$ 0.63, $NaHCO_3$ 26, glucose 10, bicuculline 0.01, and D-aminophosphonovaleric acid 0.1. Cytochrome c (100 μg/ml) was routinely added to the aCSF to prevent peptide adsorption to tubing; cytochrome c itself had no effect on oscillatory activity in thalamic slices. A glass recording electrode filled with 2 M NaCl was positioned in the RTN and the recording depth was adjusted so as to obtain distinct oscillatory discharges in response to stimulation of the internal capsule. Stimulation (1–15 μA) was delivered with a bipolar nichrome electrode every 20–30 s. Experiments were carried out at 34°C.

Transverse hippocampal slices (400 μm) were prepared from 16–20 days old Sprague–Dawley rats using conventional methods (Arai et al., 1996). Slices were submerged in aCSF containing (in mM): NaCl 124, KCl 3, KH_2PO_4 1.25, $CaCl_2$ 3.4, $MgSO_4$ 2.5, $NaHCO_3$ 26, glucose 10 and cytochrome c

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(100 μg/ml). The aCSF was equilibrated with 95% O_2/5% CO_2 and applied at 0.5 ml/min. Extracellular recordings were made from stratum radiatum of the field CA1 in response to activation of Schaffer-commissural fibers in the same stratum. Stimulation was delivered through nichrome bipolar electrodes every 20 s. All experiments were carried out at room temperature.

The data were digitized at 1–5 kHz (thalamic slice) and 5–10 kHz (hippocampal slice) with the Neuronal Activity Acquisition Program (Eclectek Enterprise, CA, USA). Student's paired *t*-test (pre- vs. post-peptide treatment) was employed for statistical analysis.

Drugs

Rat PrRP31 (31-amino acid peptide, synthesized by Research Genetics) was dissolved in distilled water at 1 mM and diluted 100 times in aCSF containing 100 μg/ml cytochrome c (Sigma).

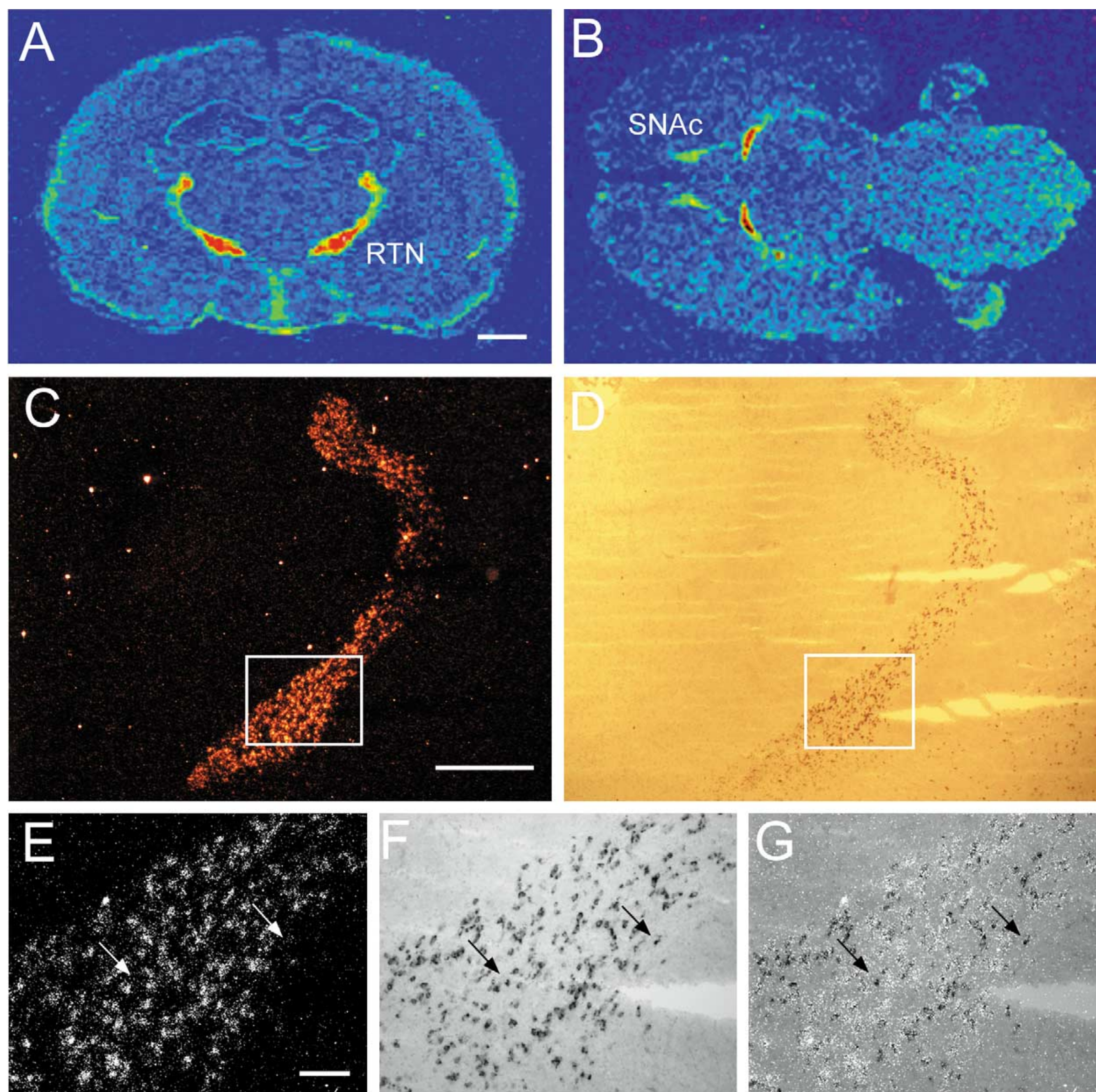

Fig. 1. GPR10 is highly expressed on GABAergic neurons of the RTN. *In situ* hybridization with ^{35}S-labeled antisense GPR10 cRNA on (A) coronal and (B) horizontal sections of adult rat brains. Adjacent sections were labeled with ^{35}S-labeled sense GPR10 cRNA as control; these sections did not show specific staining (data not shown). (C–F) Identification of cell types expressing GPR10 was assessed by double-labeling *in situ* hybridization (see Experimental procedures). (C) Emulsion dipped section visualized under dark field microscopy showing GPR10 labeling within the RTN. (D) The same section exposed to digoxigenin-labeled antisense GAD67 cRNA to identify GABAergic neurons; the section was visualized under light field. (E–F) Higher magnification of (C) and (D) showing that GPR10 is expressed in the same GABAergic neurons. (G) Overlay of emulsion grains and digoxigenin-labeled neurons shows considerable overlap in staining, although some GABAergic neurons are not PrRP receptor positive (arrows). RTN: reticular thalamic nucleus; SNAc: shell, nucleus accumbens. Scale bar = 1 mm, (A–D); 0.1 mm, (E–G).

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RESULTS

Expression of GPR10 on GABAergic neurons of the RTN

The PrRP receptor mRNA has previously been shown to be highly expressed in the RTN (Hinuma et al., 1998; Roland et al., 1999). In order to examine if the receptor is expressed in the principal GABAergic cells of this region, a double-labeling *in situ* hybridization experiment was performed using digoxigenin-labeled GAD67 and ^{35}S-labeled PrRP receptor riboprobes. In accordance with the earlier studies, we found that the PrRP receptor is prominently expressed in the RTN (Fig. 1A, B), and its pattern of expression coincided with the 'shell' pattern seen for GAD67 (Fig. 1C, D). Higher magnification showed that PrRP receptor and GAD67 mRNAs are found within the same cells and that nearly all of the PrRP receptor-positive cells are also GAD67 positive (Fig. 1E–G). Some of the GAD-positive cells did not show detectable expression of PrRP receptors (Fig. 1E–G, arrows), but those cells seemed less abundant and it thus appears likely that the PrRP receptor is present on most of the principal GABAergic neurons of the RTN.

Effects of PrRP on sleep/wake states

In a first test whether PrRP can modulate activity in the RTN, we examined its effect on slow wave sleep in animals. PrRP was injected intracerebroventricularly at various doses (1–10 nmol) into sleeping rats. Experiments were carried out during the light cycle when the rats spend the majority of time sleeping. EEG and EMG were recorded for 150 min in three groups, each consisting of six rats, that received either vehicle, 1 nmol PrRP, or 10 nmol PrRP. Analyses were conducted across five consecutive 30-min epochs; vehicle or PrRP were injected at the end of the second epoch. Vehicle-treated rats spent a substantial portion of the entire testing period asleep with characteristic EEG/EMG recording (Berridge and Foote, 1996). Infusions of PrRP elicited dose-dependent increases in the EEG/EMG indices of waking (Fig. 2A); PrRP significantly increased total time spent awake (treatment, $F_{(2,15)}=24.4$, $P<0.001$; time, $F_{(4,60)}=19.5$, $P<0.001$; treatment×time, $F_{(8,60)}=8.4$, $P<0.001$; Fig. 2B) and accordingly decreased total time spent asleep (slow-wave sleep+REM sleep). Post-hoc analyses indicate that total time spent awake was significantly increased during post-infusion epochs in rats treated with 10 nmol PrRP with only a trend at the 1 nmol dose. The increase in awake time at the higher dose was mirrored in the near-complete suppression of slow-wave sleep (treatment, $F_{(2,15)}=20.7$, $P<0.001$) (Fig. 2C).

Effects of PrRP on absence seizure formation in GAERS

Absence seizures are characterized by SWD that are similar to the spindle wave oscillations seen during sleep and that originate, like the latter, from the reticular thalamus. In certain genetic rat strains, such as the Wistar strain called GAERS (Danober et al., 1998), such seizure activity occurs spontaneously. Their seizure episodes are characterized by behavioral arrest and recurrent EEG activity exhibiting bilateral synchronous SWD and they are for this reason considered to be an animal model for absence seizures (Marescaux et al., 1992). These animals were used to examine if PrRP is effective in controlling SWD during these seizures. The EEG was recorded throughout the course of the experiment and the time spent in SWDs was cumulated over 20-minute intervals from the initial i.c.v. infusion of either aCSF control or one of three peptide concentrations (10, 50 and 100 nmol). Vehicle-treated rats exhibited recurrent SWD throughout the recording session (Fig. 3A) that did not differ significantly from the activity during the 20-min period preceding the injections (mean cumulated duration of SWD: 340 to 367 s). Injection of PrRP suppressed the EEG seizures as shown in Fig. 3B, C. At the 10-nmol dose, effects were statistically significant but short-lasting; SWD duration was reduced by about 50% during the first 20 min and returned to baseline activity after 40 min. More potent and sustained effects were observed at the higher peptide concentrations. Seizure activity during the first 20 min after injection was reduced by $76\pm22\%$ ($n=6$) at 50 nmol PrRP and by $99\pm1.0\%$ ($n=6$) at 100 nmol PrRP. In both cases, SWD duration was still reduced by more than 50% one hour after the injection.

Effects of PrRP on oscillations in the reticular thalamic slice system

The *in vivo* studies demonstrated that i.c.v. application of PrRP promotes wakefulness in normal animals and suppresses absence seizures in GAERS. These experiments were not designed to address which brain regions are responsible for mediating the peptide's effects, but the well-known role of the RTN in both of these *in vivo* paradigms and the high expression of the PrRP receptor in this region made it seem likely that PrRP directly influenced reticular thalamic activity. To test this proposition, we employed a thalamic slice preparation which has been shown to maintain sufficient reciprocal synaptic connections to generate oscillatory activity (Cox et al., 1997) not unlike the sleep oscillations and the epileptic slow wave discharges. A single stimulation pulse given to the input fibers in the internal capsule triggered repetitive burst firing in the RTN which typically consisted of 6–10 bursts at a frequency of 3–5 Hz (Fig. 4). Application of PrRP (1–20 μM) reduced the number of these oscillatory bursts in a concentration-dependent manner (Fig. 4A–D). The onset of the reduction occurred within 5 min after injection, but washout of the effect was relatively slow and variable. The average reduction at 10 μM was $28\pm5\%$ ($P<0.005$, $n=12$) and slightly larger effects (33%) were seen in two experiments at 20 μM. At these concentrations PrRP does not cause generalized disturbances in synaptic transmission as shown with slices from the hippocampus, a region in which the PrRP receptor is not expressed. In this prep-

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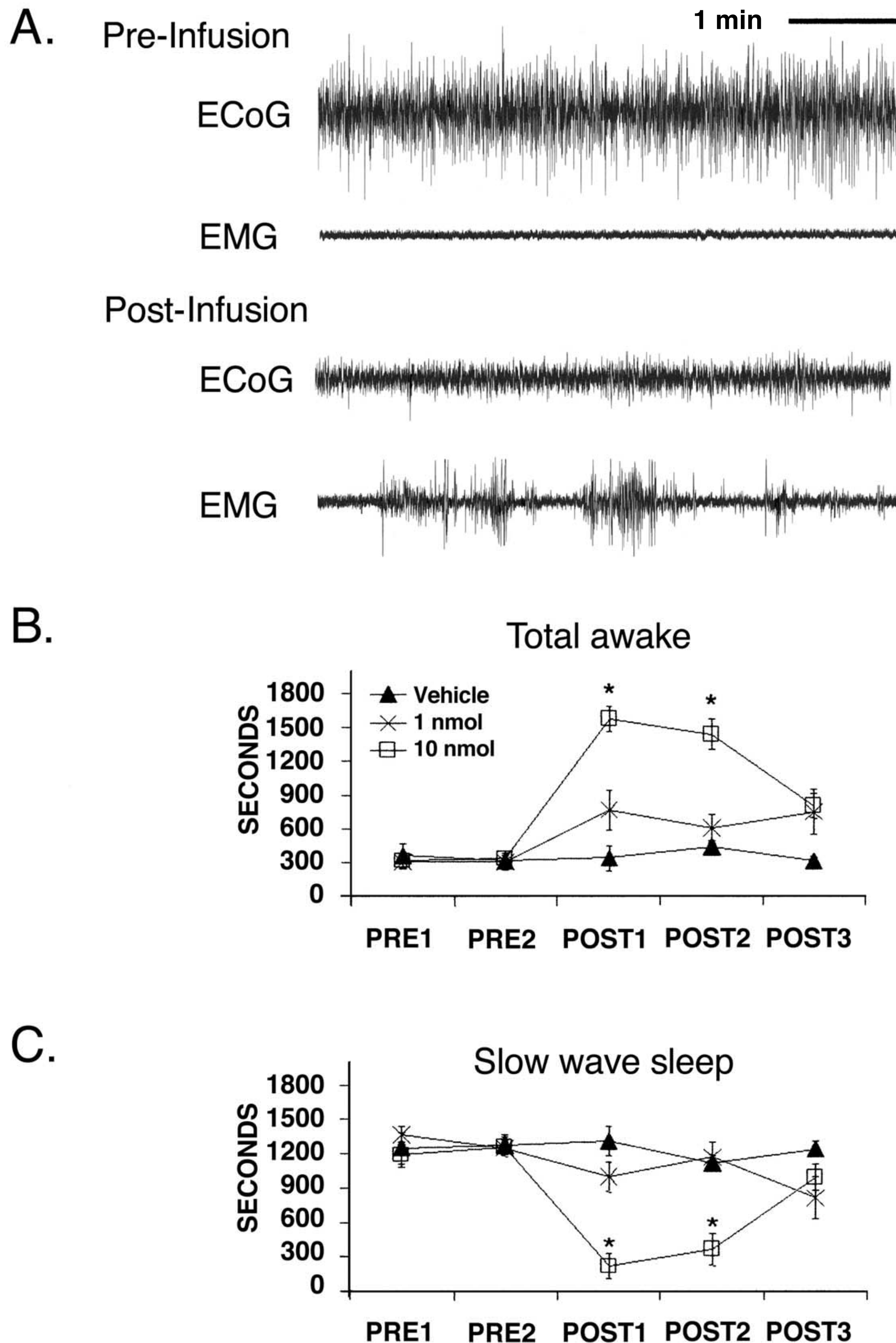

Fig. 2. PrRP induces waking and suppresses slow wave sleep. (A) Representative EEG/EMG traces of pre- and post-injection effects of PrRP (10 nmol) on sleeping animals. (B, C) Effects of varying concentrations of PrRP infused into the lateral ventricle on (B) total time spent awake and (C) slow wave sleep. Symbols represent mean ± S.E.M. (six animals) of the time (s) spent in the two different behavioral state categories per 30-min epoch. PRE1 and PRE2 represent pre-infusion epochs. POST1–POST3 represent post-infusion epochs. Vehicle-treated rats spent a substantial proportion of the testing period asleep. Total time spent awake was significantly increased during the POST1 and POST2 epochs in the 10 nmol group. $^{*}P < 0.01$; $^{+}P < 0.05$ (Tukey's HSD test) compared to vehicle-treated animals.

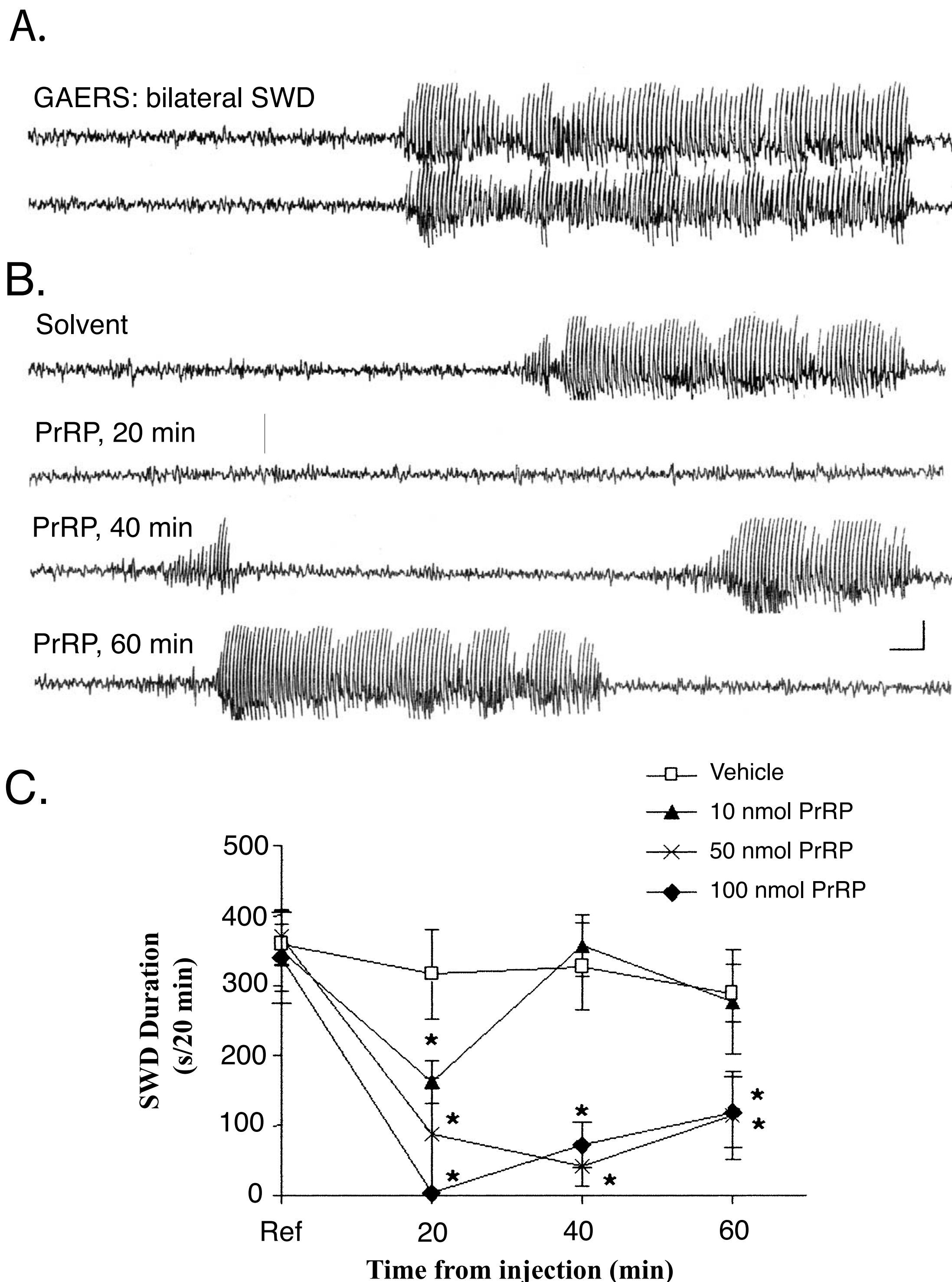

Fig. 3. PrRP suppresses absence seizure activity in GAERS. (A) Representative EEG with SWD in a GAERS: bilateral frontoparietal SWD before injection. (B) Changes in EEG pattern after i.c.v. injection of PrRP (100 nmol). Calibration: 200 μV/1 s. (C) Cumulative duration of SWD per 20 min in GAERS (mean ± S.E.M.) before injection (Ref) and 20, 40, and 60 min after injection of vehicle or 10, 50, or 100 nmol PrRP. $*P<0.001$.

aration, PrRP at 10 μM had no detectable effect on amplitude (4.6 ± 5.7%, $n=6$) or half-width (2.5 ± 1.6%) of synaptic responses (Fig. 4E). Taken together, these results suggest that PrRP specifically suppresses thalamic oscillations and that it does so through the PrRP receptor on the principal RTN neurons.

DISCUSSION

The present study showed that PrRP has an inhibitory action on rhythms generated in the RTN and that it can thereby influence processes such as sleep and absence seizures. These effects are likely to be mediated by recep-

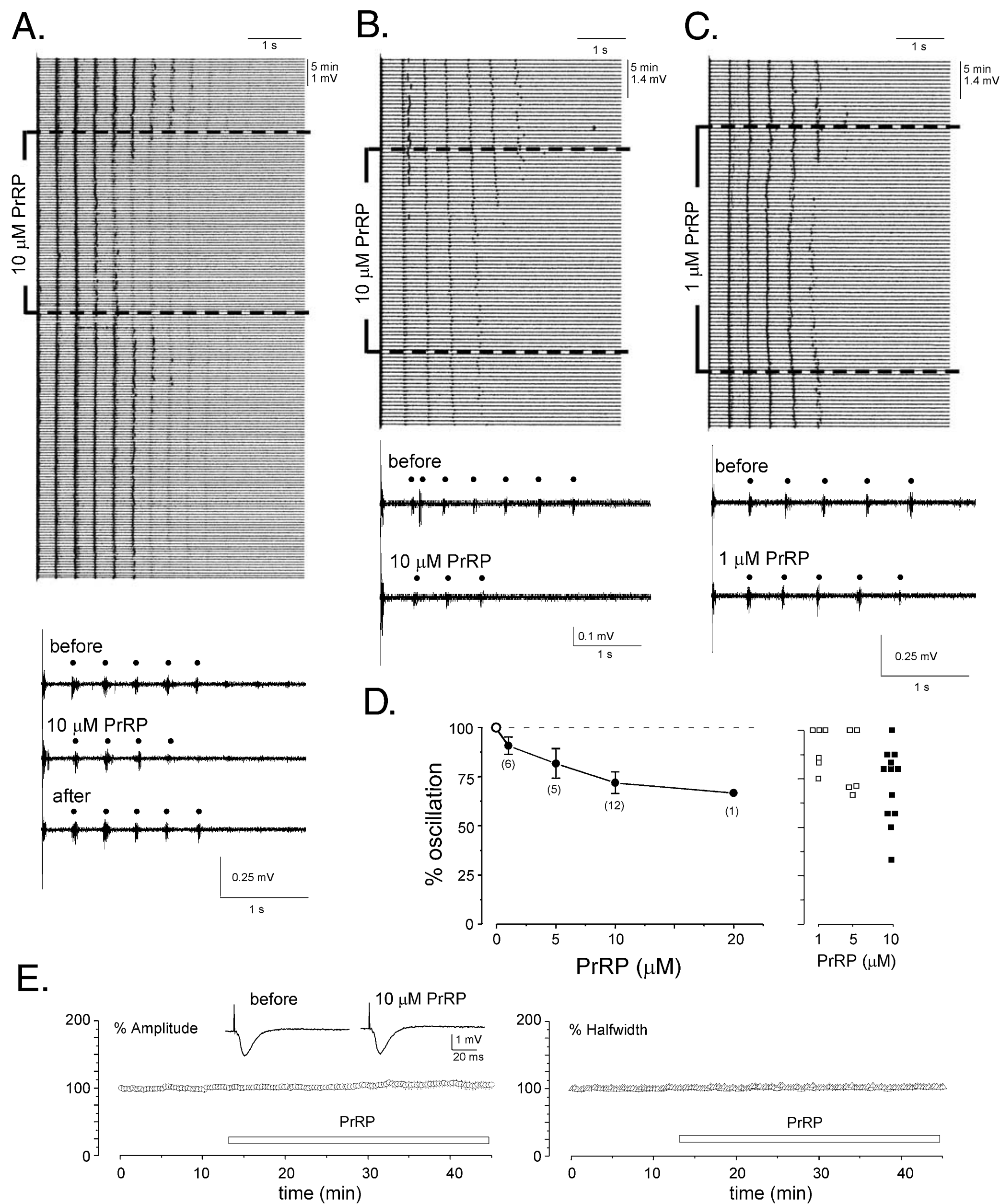

Fig. 4. PrRP suppresses thalamic oscillations *in vitro*. Oscillatory activity was recorded from the RTN in response to stimulation of the internal capsule at 30-s intervals. Each block contains 100 or 195 sweeps lasting 5 s. After establishing a stable baseline for 10–15 min, PrRP was applied to the recording chamber for about 20–25 min as indicated with dashed lines (A–C, top). The traces shown underneath each block were taken immediately before and during PrRP application. The stimulus artifact was truncated. The burst discharges counted for analysis are indicated with dots. (A) Representative experiment with 10 μM PrRP. Note that some recovery was observed upon washout of the peptide. The number of the burst discharges was reduced from six to four (67%) in the presence of PrRP. (B) Another representative experiment with 10 μM PrRP. The burst discharge indicated with a triangle originated from a cell (or cells) adjacent to the recording electrode, which is not participating in lasting slow oscillatory activity (bottom). PrRP reduced the number of bursts from 6 to 3 (50%). (C) Representative experiment with 1 μM PrRP. This concentration of PrRP reduced the intensity of the burst discharge but not the number of bursts. (D) Summary. Concentration–response relations of PrRP's effect on thalamic oscillation. % Oscillation denotes the number of burst discharges immediately before washout of the peptide relative to that of baseline oscillations. The number in parentheses indicates the number of experimental cases. The graph on the right shows the distribution of the PrRP effects at the lower three concentrations, each point representing a single experiment. (E) Effects of PrRP on synaptic transmission in field CA1 of the hippocampus. Transverse hippocampal slices were prepared from young rats (16–20 days) and maintained submerged in aCSF. After establishing a stable baseline, 5–10 μM PrRP was applied as indicated with horizontal bars. The amplitude and the halfwidth of each response was normalized to that of the baseline response and plotted against time. The symbols represent the mean and S.E.M. of six experiments. Representative traces taken immediately before and during application of 10 μM PrRP are shown at the top.

tors in the RTN itself because oscillations in thalamic slices were similarly affected by the peptide. We also confirmed previous findings (Hinuma et al., 1998; Roland et al., 1999) that mRNA for this receptor is prominently expressed in this structure and further clarified with double-labeling *in situ* hybridization that the receptor is present on the principal GABAergic neurons of the RTN. The receptor most likely is located postsynaptically in dendrites and soma of these cells, as binding of radiolabeled PrRP was found in the RTN rather than in the target regions of their axons (Roland et al., 1999).

Effects of i.c.v. injection of PrRP on sleep/wake states

When injected into animals during their sleep phase, PrRP induced a robust change in the EEG/EMG patterns. Slow wave sleep characterized by high amplitude, low frequency waves and a silent EMG was replaced within minutes by normal waking patterns, and the animals exhibited visible behavioral indices of waking. Conversion to the awake state was nearly complete at a dose of 10 nmol and lasted for well over an hour. These findings may seem at variance with a recent study by Zhang et al. (2000, 2001) who reported an increase in REM and non-REM sleep following chronic injection of PrRP. Differences in the experimental design are likely to account for this disparity. The present study examined the animals for 90 min following acute injection of PrRP and the tests were carried out during the light period when animals are normally asleep. Zhang et al. (2000) used chronic injection over 10 h, starting during the dark cycle, and scored behavioral changes over an extended 12-h period. If acute treatment with PrRP promotes awakening as indicated by our observations, the increase in sleep parameters seen in their study may have resulted from a 'rebound' increase in REM or non-REM such as following sleep deprivation (Endo et al., 1997). In molecular terms, chronic infusion of PrRP may have caused progressive desensitization of the receptor and hence an inability of the PrRP system to maintain an adequate vigilance state during the later part of their sessions. More detailed studies concerning the timing of PrRP administration and changes in sleep patterns will be required to resolve this issue.

PrRP directly modulates oscillatory activity in the reticular thalamus in vitro

PrRP suppression of SWD in the GAERS animals exhibited a time course similar to that seen in the sleep/wake experiments. The duration of slow wave discharge was reduced by about 50% at the 10-nmol dose, and injections of at least 50 nmol were needed for effective suppression of seizure activity. This indicates that a higher degree of PrRP receptor occupancy may be needed to suppress seizures than to reduce sleep states. Overall, the concentration range and the time course of the PrRP effect in the *in vivo* experiments were comparable to those in other studies. For instance, Matsumoto et al. (2000) found that 10–20 nmol PrRP i.c.v. transiently increased plasma ACTH levels with a time profile comparable to that reported here and that it induced c-fos expression in the hypothalamus. Nonetheless, the effects observed here most likely were exerted through a direct action on the receptors in the RTN rather than through extrinsic pathways or modulation of ascending modulatory systems. The reason is that oscillatory discharges in thalamic slices, in which activity is disconnected from those external influences, were reduced by PrRP in a manner that strongly resembled the effects on EEG activity in intact animals. The effect in slices was concentration dependent with an approximately 30% reduction in the number of oscillatory bursts at 10–20 μM, a concentration that was estimated to be similar in magnitude to that attained in the brain after i.c.v. injections of 10 nmol peptide. The lack of effect in hippocampal slices appears to rule out the possibility that the concentrations of PrRP employed in the present study disturb excitatory synaptic transmission in a non-selective manner. Taken together, our results suggest that PrRP suppresses oscillatory discharges in the thalamus, presumably through activation of PrRP receptors in the RTN, and that the same effect accounts for the regulation of sleep/wake cycles and for the suppression of absence seizures in GAERS animals. The possibility can of course not be dismissed that effects on other brain structures contributed to the observed *in vivo* actions. However, regions which play a prominent role in sleep-wake regulation such as the preoptic nucleus and the suprachiasmatic nucleus have only moderate to low levels of PrRP receptor expression (Roland et al., 1999; Ibata et al., 2000; unpublished data) and there is no evidence that those areas play a role in the formation of absence seizures. Similarly, PrRP receptor expression is almost undetectable in the locus coeruleus (unpublished data) which is pivotal in regulating arousal. A direct action of PrRP on the RTN thus remains perhaps the most plausible explanation.

Cellular mechanisms for PrRP's action on the reticular thalamic neurons

PrRP most likely modulates the GABAergic output from the RTN that is critical for maintaining thalamic network activity. At the cellular level, PrRP receptor activation has been described to initiate various biochemical events via coupling to G_q, including an increase in intracellular calcium and arachidonic acid release (Hinuma et al., 1998), but the mechanisms whereby PrRP suppresses oscillatory burst activity remains to be investigated. Generation of oscillatory spindle wave discharges is critically dependent on the intrinsic membrane properties of the thalamic cells, and in particular on potassium conductances (I_h, I_K) (Luthi et al., 1998). Activation of various G protein-coupled receptors in the RTN through NE, ACh, and 5HT suppresses a potassium current (Ik), resulting in a depolarization of the membrane potential and the abolition of low threshold calcium spikes that are critical for maintaining oscillatory rhythms (26). Certain locally released neuropeptides such as CCK regulate spindle wave activity in a similar manner (Cox et al., 1997; Lee and McCormick,

1997). Modification of these or similar conductances by PrRP is thus a likely possibility.

Does the PrRP system have a physiological role?

Although exogenously applied PrRP evidently is able to modulate thalamic activity, the question remains if such activity is regulated physiologically by endogenously released peptide. Immuno-histochemical studies so far have failed to detect PrRP fiber terminals in the vicinity of the RTN, except for a high density in the mediodorsal thalamic nucleus, a region which, interestingly, does not show receptor expression (Maruyama et al., 1999a; Ibata et al., 2000). Two discrete nuclei, the nucleus tractus solitarius in the brainstem and the dorsomedial hypothalamus, contain PrRP mRNA and immunoreactivity (Lee et al., 2000; Chen et al., 1999; Maruyama et al., 1999a) but there are currently no indications from the literature of retrograde or anterograde tract tracing studies to demonstrate connections from either of these regions to the RTN (Morales et al., 2000). An alternatively possibility would be that PrRP reaches the RTN through the cerebrospinal fluid after being released elsewhere, for instance from the abundant PrRP immunoreactive fibers seen near the ependymal cells of the third and lateral ventricles (Iijima et al., 1999). Future studies will have to determine if any of these sources release PrRP in concentrations sufficient to reach and activate the receptors in the RTN.

CONCLUSIONS

We have shown that PrRP is capable of modulating thalamic network activity. Although the physiological significance of this system remains to be determined, targeting the receptor of PrRP may provide a new avenue for the management of absence seizures, and possibly, for sleep disorders. Recent efforts to find new pharmacological approaches have focused on $GABA_B$ receptor and NMDA receptor antagonists, both of which inhibited EEG seizure in GAERS animals and evoked oscillations in thalamic slices (Liu et al., 1992; von Krosigk et al., 1993; Peeters et al., 1989). However, these receptors are widely distributed throughout the brain and blocking them is likely to result in general disturbance of synaptic plasticity (Collingridge and Singer, 1990; Davies et al., 1991) and cognitive function. In contrast, PrRP receptors because of their restricted distribution in the brain may allow for a much more focused intervention.

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