



Transmeningeal delivery of GABA to control neocortical seizures in rats

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Received 9 February 2007; received in revised form 10 March 2007; accepted 12 March 2007

Available online 2 May 2007

KEYWORDS

GABA;
Acetylcholine;
Neocortical seizures;
EEG;
Dura mater

Summary Transmeningeal drug delivery, using an implanted hybrid neuroprosthesis, has been proposed as a novel therapy for intractable focal epilepsy. As part of a systematic effort to identify the optimal compounds and protocols for such a therapy, this study aimed to determine whether transmeningeal gamma-aminobutyric acid (GABA) delivery can terminate and/or prevent neocortical seizures in rats.

Rats were chronically implanted with an epidural cup and an adjacent EEG electrode in the right parietal cortex. While the rat was behaving freely, a seizure-inducing concentration of acetylcholine (Ach) was applied into the cup. In a seizure termination study, either artificial cerebrospinal fluid (ACSF) or GABA (0.25, 2.5, 25 or 50 mM) was delivered into the exposed neocortical area during an ongoing seizure. In a seizure prevention study, either ACSF or 50 mM GABA was delivered into the epidural cup before the application of Ach.

Epidural delivery of 50 mM GABA completely terminated ongoing Ach-induced EEG seizures and convulsions within 17–437 s after its delivery. ACSF and lower concentrations of GABA did not produce this effect, but 25 mM GABA reduced seizure severity. However, the used GABA concentration could not prevent the development, or affect the severity, of Ach-induced EEG seizures and convulsions.

This study indicates that transmeningeal GABA delivery can be used for terminating neocortical seizures, but to achieve seizure prevention via this route either a more efficient GABA delivery method needs to be developed or other neurotransmitters/pharmaceuticals should be employed for this purpose.

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Introduction

We have previously shown that epidural pentobarbital administration can both terminate and prevent locally induced neocortical seizures in rats, and, based on these data, we proposed the development of transmeningeal pharmacotherapy of intractable focal epilepsy (Ludvig et al., 2006). Transmeningeal pharmacotherapy would employ fully implanted hybrid neuroprostheses to deliver seizure-controlling compounds across the meninges, in response to ictal and/or pre-ictal neural signals recorded in the neocortical focus or multiple foci (Ludvig and Kovacs, 2002; Ludvig et al., 2005). A prerequisite of translating this idea into clinical practice is to determine, first in animals, the ideal set of pharmaceuticals and/or neurotransmitters that upon localized delivery can exert fast and effective seizure-controlling effect.

Here we report our experiments with epidurally delivered GABA, the major inhibitory neurotransmitter in neocortex. In this region, GABA is released by at least six different types of GABAergic interneurons (Somogyi et al., 1998; Gupta et al., 2000; Calcagnotto et al., 2005) and exerts its actions primarily via GABA_A and GABA_B receptors, both formed by various combinations of multiple subunits (Smith and Olsen, 1995; Bowerly and Smart, 2006) located at postsynaptic and presynaptic sites on both pyramidal cells and interneurons (Somogyi et al., 1998; MacDermott et al., 1999; Bacci et al., 2003). Neurocytological and neurochemical data in various experimental epilepsy models and studies on resected human epileptic tissue support that GABA system dysfunction can contribute to acquired focal epilepsies (Ribak et al., 1979; Olsen and Avioli, 1997; Sherwin, 1999; Treiman, 2001; Silva et al., 2002). Enhanced inhibitory GABAergic neurotransmission contributes to the therapeutic mechanism of action of many antiepileptic drugs (AEDs), including all benzodiazepines and barbiturates, as well as valproate, vigabatrin, tiagabine, zonisamide and topiramate (Shorvon, 2005; Rogawski, 2006). However, these GABA-modulator AEDs are ineffective in medically refractory focal epilepsy (Devinsky, 1999; Oxbury et al., 2000). In part, this lack of response to oral/parenteral GABA modulators in focal epilepsy may result from failure of the agent to reach the epileptogenic zone(s) in sufficiently high concentration where their interactions with GABA receptors could make the highest impact, preventing a seizure. Localized drug delivery strategies, like using the transmeningeal route, provide a possible strategy to circumvent the problems inherent to systemic drug delivery.

Our main objective was to test the feasibility of delivering GABA directly through the dura mater, without damaging the underlying cortical tissue, to terminate focal ictal activity, using a dose-escalating approach. An additional objective was to test whether GABA, delivered in the same fashion, can also prevent these ictal events.

Methods

Animals

Male Long-Evans rats ($n = 14$), weighing 300–400 g, were used. They were subjected to an experimental protocol that was approved by

the Institutional Animal Care and Use Committees at NYU School of Medicine (#060102-01) and SUNY Downstate Medical Center (#07-237-06).

Surgical procedures

Detailed procedures are available elsewhere (Ludvig et al., 2006). Briefly, each rat was anesthetized, placed in a stereotaxic apparatus, and a 4.5 mm diameter craniotomy was drilled in the right parietal bone according atlas of Paxinos and Watson (1998). An epidural cup was placed in the craniotomy, and two epidural screw-electrodes were placed in the skull posterior to the cup. An additional screw served as the grounding electrode. The electrodes were connected to a Mill-Max socket via insulated wires, and the entire assembly was secured to the skull with dental acrylic. Recording sessions started on the second or third postoperative day

EEG and behavioral monitoring

Throughout EEG recording, the rat was behaving freely in an electrically shielded test chamber. Movement artifacts were eliminated from the recordings by using a cable with operational amplifiers, as described (Ludvig and Tang, 2000; Ludvig et al., 2006). The electrophysiological signals were amplified (10,000 \times) and filtered (using a band-pass of 1.0–100.0 Hz). The analog data were digitized at 1000 Hz and stored with proprietary software on a PC. The behavior of the animal was monitored with a camcorder.

Epidural drug delivery

Ach (50 μ l; 274 mM) was delivered into the epidural cup, either before or after the delivery of ACSF (control solution) or a selected concentration of GABA. The used GABA concentrations (50 μ l volume; pH 7.4) were 0.25, 2.5, 25 and 50 mM, with ACSF used as solvent. The ionic composition of ACSF was as described (Ludvig and Tang, 2000). The solutions were made isotonic by the appropriate reduction of the NaCl concentration in the ACSF solvent. All solutions were delivered manually. In the seizure termination study, ACSF or GABA was delivered while the Ach solution was still in the cup and then kept in the cup together with Ach. Similarly, in the seizure prevention study, all successively delivered solutions were kept in the epidural cup until the end of data collection.

Experimental protocol

Up to 6 recording/drug delivery sessions were conducted in each of the 14 rats. Two daily sessions, separated with a 4-h interval, were conducted. Thus, the experiments were completed by the end of the 4th or 5th postoperative day. This eliminated the confounding effect of diminishing seizure responses to Ach, which often occur after repeated epidural Ach applications over longer periods (John and Ludvig, unpublished observation). Seizure termination and seizure prevention studies were both performed according to the study design shown in Fig. 1. The tested GABA concentrations for the seizure termination study were 0.25, 2.5, 25, and 50 mM, whereas for the seizure prevention study the highest GABA concentration, 50 mM, was tested. Each recording/drug delivery session started with a 10-min collection of the baseline EEG data (Phase 1). In the seizure termination study, this Phase 1 period was followed by delivering the Ach solution into the cup and keeping it there for 10 min (Phase 2). In the 20th minute of the experimental session either ACSF or a GABA solution was delivered and kept in the cup together with Ach for 15 min (Phase 3). The seizure-terminating effects of ACSF, 0.25, 2.5, and 25 mM GABA were all tested in each of 5 rats, while in 6 additional rats we specifically tested the seizure termination action of 50 mM GABA. The seizure prevention study was conducted on 5 rats (including 2 rats

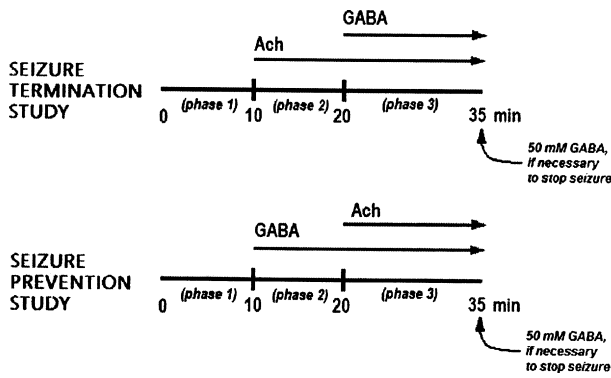


Figure 1 Schematic illustration of the experimental designs used in this study. In the seizure termination study, either 0, 0.25, 2.5, 25 or 50 mM GABA was delivered, with ACSF as the control test solution representing 0 mM. In the seizure prevention study, the tested GABA solution was always 50 mM, and each GABA delivery session was 4 h later followed by a control session where ACSF was delivered instead of GABA.

also used in the seizure termination experiments). In the seizure prevention study, the Phase 1 baseline data collection period was followed by delivering either ACSF or 50 mM GABA solution into the cup and keeping it there for 10 min (Phase 2). In the 20th minute of the experimental session Ach was delivered and kept in the cup together with the initially delivered test solution for 15 min (Phase 3). In the termination and prevention studies where seizure activity persisted until the 35th minute, 50 mM GABA was added to the epidural solutions and kept in the epidural cup for 10 min. This procedure provided us with additional data on the seizure-terminating effect of 50 mM GABA. From these additional experiments only those 50 mM GABA data were introduced into statistical analysis, which were obtained in experiments using ACSF as the test solution. This eliminated the possible confounding effects of using a lower concentration of GABA prior to the delivery of the 50 mM GABA solution.

Histology

Since no histological studies were necessary for verifying the locations of the epidural cup and electrodes, these studies were limited and aimed only to reveal possible tissue reactions at the drug delivery site. Four rats were euthanized with 120 mg/kg pentobarbital, i.p., followed by the transcardial perfusion of phosphate-buffered saline (PBS) and subsequently a 10% formalin solution. The formalin-fixed brain was removed, and a 3 mm thick block that included the epidural drug delivery site was cut out and embedded in paraffin. From this block, 20 μ m thick sections were prepared and stained with (a) hematoxylin and eosin (H&E), (b) cresyl violet, and (c) glial fibrillary acidic protein immunohistochemistry (GFAP, 1:500; Dako, Carpinteria, CA) counterstained with hematoxylin.

Data analysis

Fast Fourier Transform (FFT) was used to assess the EEG power of the recorded waves, as described (Ludvig et al., 2006). Briefly, the total spectral intensity (power) of the 13–20 (“low-beta”) frequency band was computed for each consecutive 20-s recording period. We selected low-beta power for analysis because this frequency was a useful indicator of EEG seizure activity and severity in the Ach seizure model (Ludvig et al., 2006). Thus, the average spectral intensity (μV^2) of this band in Phases 1, 2 and 3 were calculated. In addition, data playback was used for determining the duration of

each distinct seizure episode. Using these values, seizure duration ratio was calculated for each Phase, by dividing the total duration of EEG seizure episodes (s) by the length of the Phase (s). This yielded a value ranging from 0 (=no seizure) to 1 (=seizure throughout the entire Phase). For simplicity, the term seizure duration ratio is used throughout the text, but it should be kept in mind that this ratio characterized the EEG seizures only. Data playback was also used to identify the onset-times and termination-times of the Ach-induced EEG seizures, of which onset- and termination-times could be determined reliably. The behavioral data were analyzed with the notes made during the experiments.

Statistical analyses using the SPSS 11.0 and Prism 4.0 programs were performed on two dependent variables, average spectral intensity and seizure duration ratio. We used the common logarithms (log) of the average spectral intensity data to eliminate the confounding effects of the unavoidably large inter-individual variability in these values (Motulsky, 1995). The dependent variables were subjected to one of the following analyses: paired *t*-test, one-way ANOVA with Tukey’s post-hoc test, or factorial repeated measures ANOVA with least significance difference (LSD) test for pairwise comparisons, as appropriate.

Results

Baseline data

Epidural Ach delivery induced EEG seizures associated with behavioral symptoms in all rats and upon each delivery. The average latency for EEG seizure onset ($n=26$) was 64.1 ± 12.1 s (mean \pm S.E.M.). The accompanying behavioral symptoms included oral automatisms and contralateral forelimb and/or hindlimb convulsions, similar to those described previously (Ludvig et al., 2006).

Seizure termination study

The Ach-induced EEG seizures were accompanied with a significant increase in the log average spectral intensity (“power”) of low-beta EEG waves (Fig. 2). Indeed, factorial repeated measures ANOVA (factor 1: Phase [Phases 1, 2, 3]; factor 2: test solution [GABA 0, 0.25, 2.5 and 25 mM]) for these data yielded significant main effect for Phase ($F_{2,8}=72.44$; $p<0.001$) with pairwise comparisons showing that the “Ach-only” Phase 2 power values were consistently and significantly higher than the corresponding values obtained during Phase 1 ($p<0.01$). Furthermore, pairwise comparisons showed that 25 mM GABA significantly decreased the power of 13–20 Hz waves ($p<0.01$), from a Phase 2 value of 1.24 ± 0.21 ($n=5$) to a Phase 3 value of 0.84 ± 0.20 ($n=5$). This Phase 3 power value for 25 mM GABA was statistically not different ($p=0.14$) from the normal, “pre-Ach” Phase 1 power of 0.64 ± 0.22 ($n=5$; Fig. 2). At the same time, ACSF, 0.25, and 2.5 mM GABA exerted no statistically significant influence on the Ach-induced power increase (Fig. 2).

In the same experiments, the seizure duration ratio data yielded a different picture. Thus, none of the applied test solutions, including 25 mM GABA, changed the Ach-induced seizure duration ratio significantly: the Ach-induced seizures were maintained in Phase 3. Accordingly, factorial repeated measures ANOVA (factor 1: Phases [2, 3]; factor 2: test solution [GABA 0, 0.25, 2.5 and 25 mM]) for this dataset yielded

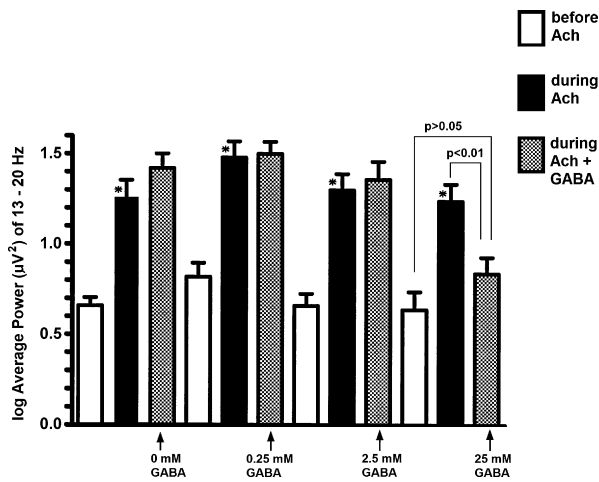


Figure 2 Average power (spectral intensity) of 13–20 Hz EEG waves before epidural Ach administration, during Ach-induced seizures, and during the presence of either ACSF (0 mM GABA) or a selected concentration of GABA in the epidural cup, as indicated. Bars represent mean \pm S.E.M. Asterisks indicate seizure-related power values significantly higher ($p < 0.05$) than the power of 13–20 Hz waves before Ach exposure. Note the concentration-dependent reducing effect of GABA on seizure-related power increase.

no significant main effect for Phase ($F_{1,4} = 4.54$; $p = 0.10$) and none of the post-hoc analyses showed significant difference between the Phase 2 and Phase 3 seizure duration ratio data. Thus, even when 25 mM GABA was delivered into the epidural cup following Ach, which decreased the seizure duration ratio from 0.70 ± 0.08 ($n = 5$) to 0.38 ± 0.18 ($n = 5$), this decrease was not significant statistically ($p = 0.73$).

In the seizure termination experiments employing 50 mM GABA, significant differences were found between the low-beta EEG power values collected in the three experimental phases (one-way ANOVA: $F_{2,28} = 14.46$, $p < 0.01$). Tukey's post-hoc tests revealed that the log average spectral intensity of low-beta EEG waves in Phase 1, 0.68 ± 0.07 ($n = 15$), significantly ($p < 0.01$) increased to 1.13 ± 0.10 ($n = 15$) in Phase 2. This seizure-related power increase was totally eliminated in Phase 3 when 50 mM GABA was added to the epidural cup. Thus, in Phase 3 the logarithm of average spectral intensity was 0.56 ± 0.07 ($n = 15$), which was significantly ($p < 0.01$) lower than in the preceding "Ach-only" Phase, while not different statistically ($p > 0.05$) from the corresponding normal value generated before Ach delivery (Fig. 3).

In contrast to lower concentrations of GABA, 50 mM GABA not only restored the normal spectral intensity of 13–20 Hz waves, but also had a robust effect on the seizure duration ratio. Accordingly, as shown in Fig. 4, the seizure duration ratio of 0.63 ± 0.06 ($n = 15$) during the "Ach-only" Phase 2 was significantly ($t_{15} = 7.93$; $p < 0.01$) reduced to as low as 0.11 ± 0.04 ($n = 15$) in the "Ach + GABA" Phase 3. Indeed, this GABA solution terminated both EEG and behavioral seizures within 196.46 ± 43.56 s (min: 17s; max: 437s; $n = 15$) after its administration. This remarkable effect is demonstrated on Fig. 5, which illustrates the entire course of a seizure termination experiment, and on the EEG recordings of Fig. 6.

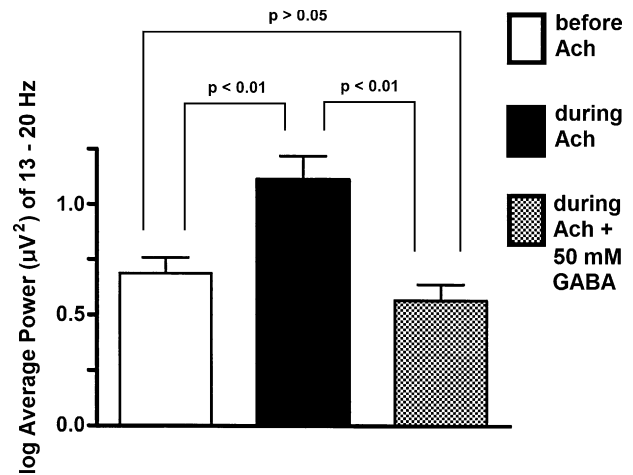


Figure 3 Average power (spectral intensity) of 13–20 Hz EEG waves before epidural Ach administration, during Ach-induced seizures, and during the presence of 50 mM GABA in the epidural cup, as indicated. Bars represent mean \pm S.E.M. Note the Ach-induced significant power increase and the elimination of this seizure-related EEG effect by the used GABA solution.

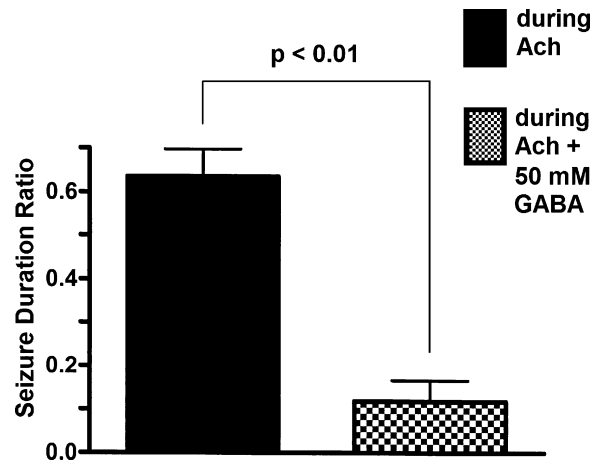


Figure 4 Seizure duration ratio during Ach-induced seizures, and during the presence of 50 mM GABA in the epidural cup, as indicated. Bars represent mean \pm S.E.M. Compare this figure with Fig. 3; note the significant and marked reduction in EEG seizure activity after the epidural delivery of this GABA solution. (The still measurable seizure duration ratio after GABA was due to the latency time of the development of its seizure-terminating action.)

Seizure prevention study

The experiments that used 50 mM GABA as a pretreatment prior to Ach delivery showed that this procedure did not prevent the Ach-induced power increase in the 13–20 Hz frequency band. Thus, the log average spectral intensity during the sole presence of GABA in the epidural cup was 0.42 ± 0.09 ($n = 5$), which increased significantly ($p < 0.01$) to 0.80 ± 0.07 ($n = 5$) after the delivery of Ach. This effect was similar to that of ACSF, as the log average spectral intensity during the sole presence of ACSF in the cup was 0.50 ± 0.11 ($n = 5$), which increased significantly ($p < 0.05$) to 1.18 ± 0.18 ($n = 5$ tests) upon Ach delivery. Indeed, the fac-

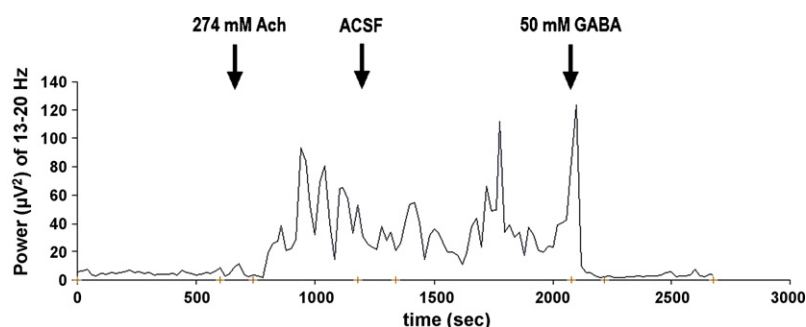


Figure 5 Fast Fourier Transform (FFT) analysis of the power (spectral intensity) of 13–20 Hz waves during the course of a seizure termination experiment, where the effect of ACSF on Ach-induced seizures was tested and 50 mM GABA was used to stop the ongoing seizures. Horizontal axis: recording session time; vertical axis: power of the examined frequency band. Arrows indicate the time points of the successive Ach, ACSF and GABA deliveries into the epidural cup. Note the clear increase in the power of 13–20 Hz waves upon Ach administration, the maintenance of this state during ACSF exposure, and the return of normal pre-Ach values after GABA delivery.

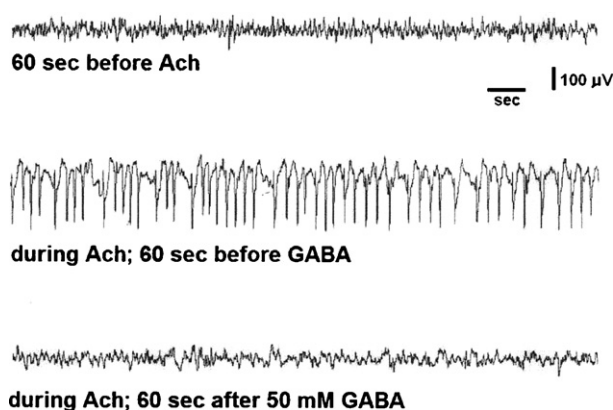


Figure 6 Parietal cortical EEG recordings from a freely behaving rat before Ach administration, after the epidural administration of Ach, and after the addition of 50 mM GABA into the epidural cup. Calibrations and drug delivery schedules are as indicated. Note the restoration of normal, pre-seizure EEG activity within 60 s after GABA delivery.

torial repeated measures ANOVA (factor 1: Phases [1, 2, 3]; factor 2: test solution [GABA and ACSF]) showed significant main effect for Phase ($F_{2,8} = 7.38$; $p = 0.015$). Importantly, pairwise comparisons demonstrated no significant differences between the Phase 1 and Phase 2 power values regardless of whether GABA or ACSF was present in the epidural cup during Phase 2.

In the same experiments, the seizure duration ratio data showed that GABA pretreatment did not prevent the Ach-induced seizures. Accordingly, the Phase 3 seizure duration ratio following 50 mM GABA pretreatment was 0.38 ± 0.10 ($n = 5$), which was statistically not different ($t_4 = 1.96$, $p = 0.12$) from the corresponding value obtained in Phase 3 following ACSF pretreatment (0.60 ± 0.09 , $n = 5$; Fig. 7).

Histological study

In all four examined rats, inflammation consisting of lymphocytic infiltration was seen in the subarachnoid area

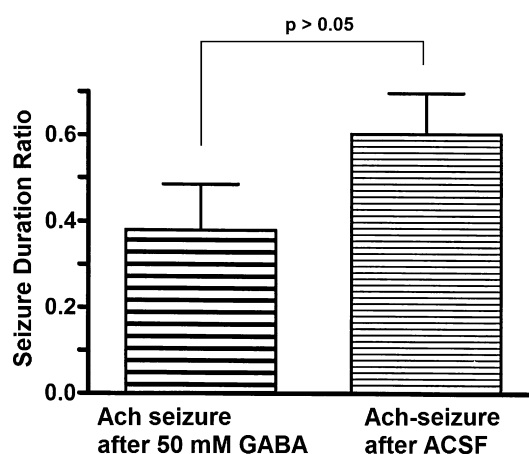


Figure 7 Seizure duration ratio during Ach-induced neocortical seizures after epidural pretreatment with 50 mM GABA and ACSF, as indicated. Bars represent mean \pm S.E.M. Note the lack of statistically significant difference between GABA and ACSF pretreatments.

of the right neocortical region underlying the epidural cup. Reactive astrocytosis was observed in all cases to varying degrees, with more extensive astrogliosis in the area of the cup (Fig. 8). No obvious reduction in cell number was observed on either H&E or Nissl stained sections.

Discussion

It was found that epidural application of GABA can terminate focal neocortical seizures in rats. This effect was concentration-dependent, as 0.25 and 2.5 mM GABA, just as ACSF, were ineffective in altering the course of these seizures, while 25 mM GABA could decrease EEG seizure severity (marked reduction in seizure-related increase in the power of 13–20 Hz activity) and 50 mM GABA completely stopped the EEG and behavioral seizures. The inability of GABA to readily cross the blood-brain barrier (Krantis, 1984) suggests that the seizure-terminating effect of this compound was primarily due to its diffusion across the exposed

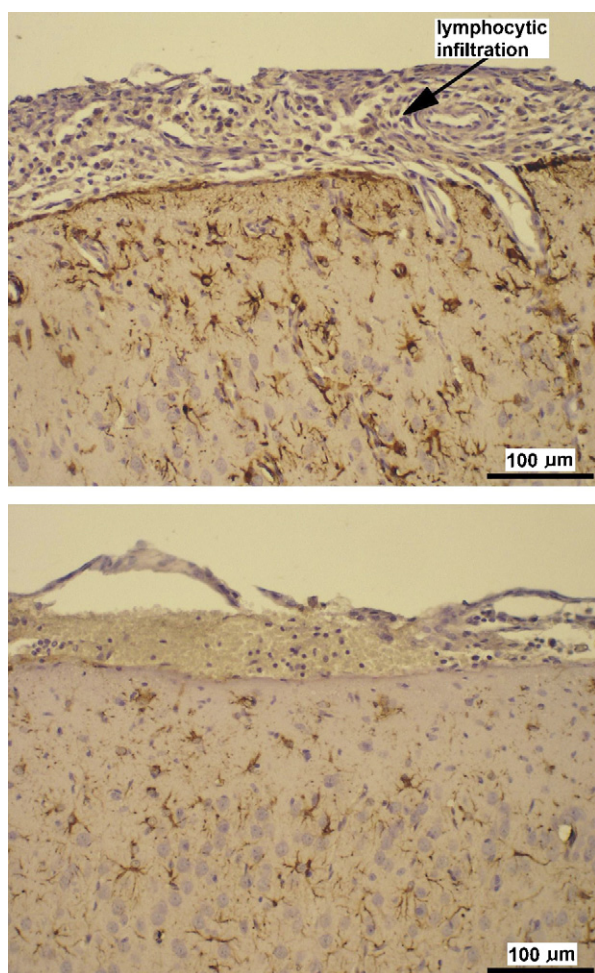


Figure 8 Photomicrograph of the right neocortical area underlying the epidural cup (upper panel) and the contralateral neocortex (lower panel), as labeled with GFAP immunohistochemistry and hematoxylin counterstain. Coronal sections are shown. Note that the surface of the brain exposed to the epidural cup is marked by the accumulation of lymphocytes and polymorphonuclear leukocytes, while the underlying cortical tissue is characterized by extensive astrogliosis.

meninges into the underlying cortical region. Indeed, the behavioral seizures were characterized by contralateral forelimb/hindlimb convulsions, indicating seizure activity in the motor cortex under the anterior portion of the epidural cup. Studies with autoradiography, dynamic positron emission tomography (PET), and/or integrative optical imaging methods will be necessary to elaborate the dynamics of GABA diffusion, and of the diffusion of other agents, across the meninges into the underlying neocortical tissue, including the tissue buried in the folds of sulci. Such studies would have gone well beyond the scope of the present work, but their critical importance is recognized by us.

The seizure-terminating effect of epidural GABA administration is consistent with the finding of Yokoi et al. (1993) that 100 mM GABA applied onto the pia mater suppressed interictal-type focal EEG spikes in immobilized rats. Our data are also consistent with those previously reported

by Brailowsky's group that intracortical (intraparenchymal) infusion of GABA in high ($100 \mu\text{g}/\mu\text{l} = 970 \text{ mM}$) concentration reduced motor seizures in amygdala-kindled rats and blocked the epileptiform EEG and behavioral responses to intermittent light stimulation in photosensitive baboons (Fukuda et al., 1987; Brailowsky et al., 1989). Using yet another method, Gernert et al. (2002) also showed that transplantation of genetically engineered GABA-producing cells into the piriform cortex delayed, albeit did not prevent, seizure development. The novelty of transmeningeal GABA application is that it allows the delivery of GABA into large neocortical regions in a controlled fashion and without placing foreign bodies into the brain.

The seizure prevention study showed that GABA, at least at the used concentration (50 mM) and delivery parameters, did not prevent the development of Ach-induced neocortical seizures. This was reflected in both the average spectral intensity data and the seizure duration ratio data obtained during the Ach-seizures, as neither of these variables was significantly affected by prior GABA pretreatment. This result was unexpected. Thus, a given GABA solution can be efficient in terminating an ongoing neocortical seizure, while inefficient in preventing the development of the same type of epileptiform activity. In our experiment, the 10-min GABA pretreatment could down-regulate/desensitize the local inhibitory GABA receptors. This could permit the generation of epileptiform discharges upon Ach exposure, despite the large quantity of GABA molecules in the extracellular environment. Short-term exposure of GABA receptors to GABA reduces their responsiveness to this neurotransmitter, likely contributing to GABA-withdrawal-induced hyperexcitability (Casasola et al., 2002) and the failure to achieve antiepileptic effect with some intracerebral GABA delivery methods (Nilsen and Cock, 2004). Other explanations can be weighed only after this study is repeated with other delivery parameters, with pairing the delivered GABA with uptake and metabolism inhibitors (e.g., tiagabine, vigabatrin), and with a repertoire of cellular and molecular techniques.

The histological data help to understand our previous observation that repeated epidural Ach applications for more than a week often lead to less severe seizures. It seems this phenomenon is partially due to the inflammatory response to the use of the epidural cup. Thus, lymphocytic infiltration around the meninges might hamper the transmeningeal passage of Ach and thus reduce its epileptogenic potential upon epidural delivery. This inflammatory response should be attenuated in transmeningeal drug delivery tests in animals and in future transmeningeal pharmacotherapy techniques to achieve adequate cortical drug penetration.

In summary, this study confirmed that transmeningeal drug delivery can stop focal neocortical seizures. This supports the idea that localized intracranial drug administrations in general, and transmeningeal drug administrations in particular, offer an effective strategy to treat intractable focal epilepsy (Eder et al., 1997; Ludvig, 2000; Stein et al., 2000; Litt et al., 2001; Ludvig and Kovacs, 2002; Ansel et al., 2004; Nilsen and Cock, 2004; Ludvig et al., 2005, 2006; Thompson, 2005; Turner et al., 2005). We note that due to the thickness of the dura mater in humans, and due to the possibility of withdrawal seizures following contin-

uous, long-lasting infusions (e.g., GABA infusions; Fukuda et al., 1987; Brailowsky et al., 1990), clinically useful transmeningeal drug therapy will likely employ on-demand, controlled drug deliveries via subdurally placed hybrid neuroprosthesis catheters (Ludvig et al., 2005, 2006). The present experiments also revealed that a given compound with the ability to stop focal seizures upon transmeningeal delivery may not necessarily prevent the pathophysiological process. This is probably related to both the timing and other parameters of drug delivery and the capacity of the delivered compound to intervene into the distinct molecular mechanisms of seizure generation, propagation and termination. Therefore, further experiments to elaborate the seizure-controlling effects of transmeningeally delivered GABA and GABA receptor subtype agonists, as well as systematic tests of other neurotransmitters and drugs in a variety of animal models, including chronic seizure models, are needed to successfully translate the idea of controlled transmeningeal drug administration into therapy.

Acknowledgments

This study was supported by NYU/FACES. We thank Dr. Hai M. Tang for constructing the electrodes, Mr. Geza Medveczky for preparing the recording cable, and Ms. Lorraine Braithwaite-Harte for her assistance in the histological study.

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