

Original Research

Epidural Pentobarbital Delivery Can Prevent Locally Induced Neocortical Seizures in Rats: The Prospect of Transmeningeal Pharmacotherapy for Intractable Focal Epilepsy

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Summary: *Purpose:* To determine whether epidural pentobarbital (PB) delivery can prevent and/or terminate neocortical seizures induced by locally administered acetylcholine (ACh) in freely moving rats.

Methods: Rats were implanted permanently with an epidural cup placed over the right parietal cortex with intact dura mater. Epidural screw-electrodes, secured to the cup, recorded local neocortical EEG activity. In the seizure-termination study, ACh was delivered into the epidural cup, and after the development of electrographic and behavioral seizures, the ACh solution was replaced with either PB or artificial cerebrospinal fluid (aCSF; control solution). In the seizure-prevention study, the epidural ACh delivery was preceded by a 10-min exposure of the delivery site to PB or aCSF. Raw EEG recordings, EEG power spectra, and behavioral events were analyzed.

Results: ACh-induced EEG seizures associated with convulsions, which were unaffected by epidural aCSF applications, were terminated by epidurally delivered PB within 2–2.5 min. Epidural deliveries of PB before ACh applications completely prevented the development of electrographic and behavioral seizures, whereas similar deliveries of aCSF exerted no influence on the seizure-generating potential of ACh.

Conclusions: This study showed for the first time that epidural AED delivery can prevent, as well as terminate, locally induced neocortical seizures. The findings support the viability of transmeningeal pharmacotherapy for the treatment of intractable neocortical epilepsy. **Key Words:** Dura mater—Neocortical seizures—Acetylcholine—Pentobarbital—Neuroprosthesis.

Pathophysiologic processes in the cerebral meninges—the dura, arachnoid, and pia maters—are involved in neurologic conditions such as meningitis, epi- and subdural hematomas, meningiomas, and metastases. Yet these membranes also have the largely unexplored potential of serving as conduits for nontraditional pharmacotherapy for intractable focal epilepsy. This assumption is based on two facts. First, the meninges are permeable for many extracellular molecules, as well as a variety of pharmaceuticals. This property of the meninges has been exploited by basic scientists in collecting neocortically released neurotransmitters [e.g., γ -aminobutyric acid (GABA)] via an “epidural cup” (Beani et al., 1968; Siniscalchi et al., 2003)

or harvesting cerebral cortical neuroactive peptides by using a plastic cylinder placed on the pial surface (Wang et al., 1983, 1986). Second, the meninges are in close proximity to the neocortical tissue that generate or propagate frontal, parietal, temporal, and occipital lobe seizures, whereas the CSF-filled intermeningeal compartment is suitable for carrying water-soluble therapeutic agents directly to the underlying epileptogenic zone(s).

It must be noted that drug deliveries through the spinal meninges have already been proven to produce clinically beneficial effects. Thus epidural and subdural (intrathecal) administrations of local anesthetics alone or in combination with opioid analgesics are now widely used in obstetric anesthesia for short-term pain relief during difficult labor and cesarean section (Liu et al., 1995; Leighton and Halpern, 2002). Similar drug administrations are also frequently used for lower abdominal, urogenital, rectal, and lower extremity surgery (Morgan et al., 2006). These treatments are based on the in vitro-verified passage of

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local anesthetics and narcotics through the spinal meninges (McEllistrem and Benington, 1993; Grouls et al., 2000). Spinal intrathecal baclofen therapy is also acknowledged to be effective in the relief of spasticity and pain associated with cerebral palsy, sclerosis multiplex, and other neurologic disorders (Guillaume et al., 2005; Ben Smail et al., 2006). These results, along with the observation that the cranial dura is as permeable to chemical compounds as the spinal dura (Moore et al., 1982), further support the idea that analogous methods might be useful for the treatment of intractable focal seizures.

In their pioneering study, Eder and his colleagues (1997) created acute seizure foci in the rat neocortex with localized bicuculline administrations, and after the establishment of EEG spiking, examined whether diazepam (DZP) injected into the epidural space could stop the epileptiform electrographic activity. They found that DZP markedly reduced the intensity of EEG spiking and concluded, "focal applications of antiepileptic drugs (AEDs) in brain may be a useful new avenue for therapy of intractable partial seizures." This conclusion was consistent with prior studies showing that intracortically infused GABA reduces motor seizures in amygdala-kindled rats (Fukuda et al., 1987) and that GABAergic agonists injected into deep brain structures exert antiepileptic effects (Browning et al., 1989; Abdul-Ghani et al., 1996; Veliskova et al., 1996).

To translate the antiepileptic potential of intracranially applied drugs into a clinically useful seizure therapy, we proposed in 2000 the development of hybrid neuroprostheses (HNP) and initiated the testing of the subdural version of these devices (Ludvig, 2000; Ludvig and Kovacs, 2002; Ludvig et al., 2005). The term "hybrid neuroprosthesis" was originally used to refer to "a microcomputer-controlled intracerebrally implanted drug delivery device, in which the timing and duration of the drug deliveries are determined by the implanted brain tissue's own electrical activity" (Ludvig, 2000). Apparently, this effort has been part of an almost simultaneously occurring intellectual current that suggested the introduction of therapeutic drug delivery implants for the treatment of focal seizures (Fischell et al., 2000; Stein et al., 2000; Litt et al., 2001). We must emphasize that unlike seizure-controlling deep-brain stimulatory devices and the recently developed responsive neurostimulator (Polkey, 2000; Turner et al., 2005; Worrell et al., 2005), which apply electrical stimulations as a means to achieve therapeutic effects, HNP-like systems, including the subdural HNP, aim to prevent and/or terminate seizures by delivering antiepileptic/antiepileptogenic drugs into the seizure-generating brain regions(s).

A fundamental question regarding the therapeutic viability of subdural HNP is whether AEDs delivered through the meninges have the capacity to prevent neocortical seizures. This is because an ideal neuroprosthetic device should be able both to prevent a seizure and promptly terminate a developing ictal event. Accordingly, the present

study was designed to assess whether drug deliveries through the meninges can prevent neocortical seizures in rats.

We used Ach as a seizure-generating agent, because its ability to induce acute neocortical EEG seizures on topical, epidural, or subdural administration is well documented (Fleming, 1963; Fleming et al., 1971; Ferguson and Cornblath, 1975). Furthermore, utilizing this particular seizure-inducing method can also provide information on whether Ach-induced neocortical epileptic events are as responsive to epidural AED delivery as are bicuculline-induced seizures (Eder et al., 1997). Moreover, because the experimenter can exert good control over the timing of acute Ach-induced neocortical seizures, the efficacy of a selected AED to prevent and/or terminate these seizures can be determined with reasonable accuracy.

PB was used as the AED. Systemically administered PB has been shown to stop experimental neocortical seizures (Mares et al., 1977; Yokoi et al., 1987; Getova and Moyanova, 1988). However, the ability of epidurally or subdurally delivered PB to prevent or terminate these seizures has not been investigated. The present study focused on the seizure-preventing/terminating potential of epidurally applied PB. We chose epidural rather than subdural drug delivery for two reasons. First, the closely attached and thin meningeal layers of the rat make it difficult to achieve reliable subdural drug deliveries without the confounding effects of piercing the pia mater or damaging the underlying cortical surface. This problem is eliminated by delivering the examined drugs epidurally. Second, recording the effects of epidurally applied drugs yields information on drug diffusion through all, and not just one, layers and compartments of the cerebral meninges.

MATERIALS AND METHODS

Animals

Eight male Long-Evans rats, weighing 350–400 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 and SUNY Downstate Medical Center.

Surgical procedures

Each rat was anesthetized with 50 mg/kg pentobarbital (PB), i.p. Additional injections of 0.1 mg/kg atropine, s.c., and 30,000 units/100 g penicillin G benzathine/penicillin G, i.m., served to reduce secretion in the respiratory system and prevent infection, respectively. The rat was placed in a stereotaxic apparatus, the skull exposed, and a 4.5-mm-diameter craniotomy was drilled in the right parietal bone with its center 2.5 mm posterior to the bregma and 2.5 mm lateral to the midline, according to the rat brain atlas of Paxinos and Watson (1988). An epidural cup constructed from a 200- μ l capped reaction tube (Analytic Lab Accessories, Rockville Center, NY, U.S.A.) was placed in the

craniotomy, touching, but not pressing, the underlying intact dura mater. Sterile bone wax was used to seal the gap between the cup and the bone. After we completed this procedure, two epidural screw-electrodes prepared from TX2-4-C stainless steel screws (Small Parts, Inc., Miami Lakes, FL, U.S.A.) were placed in the skull 2 mm from each other and 1 mm from the posterior edge of the epidural cup. An additional screw was placed in the left occipital bone to serve as grounding electrode. A Mill-Max (Oyster Bay, NY, U.S.A.) socket, with three pins connected to the electrodes via insulated wires, was also secured to the skull. The surgical procedures were completed by anchoring the entire assembly to the skull with dental acrylic and approximating the skin, treated with Animax antibiotic ointment, with a suture.

Experimental sessions

Experiments started 4–6 days after surgery. Each rat was subjected to two to four sessions, and each session consisted of a 40- to 70-min one-channel EEG recording. In each session, the rat was moving freely in a 30-cm-wide, 30-cm-long, and 35-cm-high wooden test chamber with access to food and water.

As in our previous EEG/seizure studies (Ludvig and Tang, 2000), movement artifacts were eliminated from the recordings by using a recording cable with a built-in operational amplifier (G-tech, Cortlandt Manor, NY, U.S.A.). The parietal cortical electrophysiologic signals detected with the bipolar epidural electrode were amplified ($\times 10,000$) and filtered (by using a band-pass of 1.0–100.0 Hz). The analog data were digitized at 1000 Hz with a National Instruments (Austin, TX, U.S.A.) PCI-

MIO-16E-4 (12-bit) A/D board and stored in a binary data structure by using proprietary software on a PC. In addition to acquiring the EEG data with this software, the raw EEG waves were displayed on two digital oscilloscopes. One oscilloscope (Agilent 54621A instrument, Palo Alto, CA, U.S.A.) also generated printouts of the waves during the course of the recording sessions. The screen of the other oscilloscope (a HP 54603B instrument, HP, Palo Alto, CA, U.S.A.) was continuously viewed with a Canon ZR 60 digital camcorder (Canon USA, Jamesburg, NJ, U.S.A.).

The behavior of the animal in the test chamber was continuously monitored with a JVC Compact VHS camcorder (GR-AX940, JVC, Fairfield, NJ, U.S.A.). The video signals from this camcorder and those from the one that viewed the EEG-displaying oscilloscope were mixed with the use of a Videonics MXProDV device (Videonics, Campbell, CA, U.S.A.). This allowed us to view and videotape the animal's behavior and the cortical EEG activity simultaneously. Notes were made on the rat's behavior before, during, and after drug applications.

During the experimental sessions, three solutions: Ach, PB, and aCSF were delivered into the epidural cup successively, in various orders according to four different experimental paradigms (Table 1). The delivered volume was 100 μ l for each solution in every session. The concentration for Ach was 274 mM, and that for PB was 226 mM, whenever used. The ionic composition of the aCSF was the same as in our prior microdialysis experiments (Ludvig et al., 1996; Ludvig and Tang, 2000). The recording/drug-delivery sessions started immediately after placing the rat in the test chamber. As a consequence, the animal was

TABLE 1. Design of the EEG recording/epidural drug-delivery experiments

Experimental paradigm	Rat no.	Date of session (mm/dd)	First delivered solution	Second delivered solution	Third delivered solution
1a (test)	1	10/14	Ach	PB	-
	3	10/11	Ach	PB	-
	4	10/19	Ach	PB	-
	6	10/01	Ach	PB	-
	8	10/17	Ach	PB	-
1b (control)	1	10/13	Ach	aCSF	PB
	3	10/13	Ach	aCSF	PB
	4	10/21	Ach	aCSF	PB
	6	10/04	Ach	aCSF	PB
	8	10/15	Ach	aCSF	PB
2a (test)	1	10/20 ^a	PB	Ach	-
	4	10/24 ^b	PB	Ach	-
	5	11/02	PB	Ach	-
	6	11/02	PB	Ach	-
	7	11/10	PB	Ach	-
2b (control)	1	09/30	aCSF	Ach	PB
	3	10/12	aCSF	Ach	PB
	5	11/04	aCSF	Ach	PB
	6	11/07	aCSF	Ach	PB
	7	11/12	aCSF	Ach	PB

In each experimental session, the first, second, and third indicated solutions were delivered successively into the epidural cup. Each paradigm was examined in five rats. The order of successive experimental sessions was randomized. The ID number of each rat and the date of each session are indicated; all sessions were performed in 2005. Ach-induced seizures were absent (because of PB pretreatment) only in paradigm 2a: these sessions were followed by at least one session in which the retained ability of the neocortical tissue to generate Ach-induced seizures was verified.

^aFollowed by an additional session on 10/21 with recording of Ach-induced seizure.

^bFollowed by an additional session on 10/27 with recording of Ach-induced seizure.

always awake during the application of the first drug solution. Initially, a 5- to 10-min background EEG activity was collected, while the rat was moving, grooming, eating, drinking, or resting. Next, in paradigm 1a, Ach was delivered first, kept in the cup for 10–15 min, and then either immediately or after a 15-min seizure-monitoring period replaced with PB. This latter drug was kept in the cup for 10 min before its removal. We used this arrangement to make sure that a fully developed, intense seizure activity was present at the moment of PB administration.

In paradigm 1b, Ach was again delivered first and kept in the cup for 10–15 min, but then this compound was replaced either immediately or after a 15-min seizure-monitoring period with aCSF. This latter solution was again kept in the cup for 10 min before its removal. Because in these sessions, the Ach-induced seizures were not terminated by aCSF delivery, the removal of aCSF was followed by PB delivery into the cup in an attempt to stop the ongoing seizure activity. These sessions were designed to assess whether epidurally administered PB can terminate Ach-induced seizures.

In paradigm 2a, the collection of baseline EEG data was followed by delivering PB into the epidural cup. After a 10-min exposure period, the PB solution was removed and replaced with Ach that was kept in the cup for either 10 or 30 min. We used this arrangement to make sure that Ach was present in the PB-pretreated neocortical area for even longer periods than is necessary for the induction of an Ach seizure. In each rat, paradigm 2a experiments were always followed by at least one experiment that demonstrated the retained seizure-inducing capability of the epidural Ach treatment (Table 1).

In paradigm 2b, the first epidurally delivered drug solution was aCSF, which was again kept there for 10 min and then replaced with Ach for either 10 or 30 min. Because in these sessions, the Ach-induced seizures were not prevented by the prior aCSF pretreatment, the removal of Ach was followed by PB delivery into the cup in an attempt to stop the developing seizure activity. These sessions were designed to assess whether epidurally delivered PB can prevent the Ach-induced seizures. All solutions were delivered manually, by gently holding the rat in the chamber, opening the epidural cup, and injecting the solution onto the surface of the dura mater with a 2.5-ml BAS airtight syringe. Removal of the drug solutions was carried out by handling the rat in the same way and clearing the dural surface with a fitting piece of sterile sponge. Washout of the epidurally delivered drugs from the underlying neocortical tissue was not monitored, as this procedure would have required the combination of the epidural cup technique with microdialysis or other analogous invasive sampling methods, a complex technique that has yet to be developed.

After completion of the recording/drug-delivery session, the rat was returned to the home cage. Successive sessions were separated by a minimum of a 1-day inter-

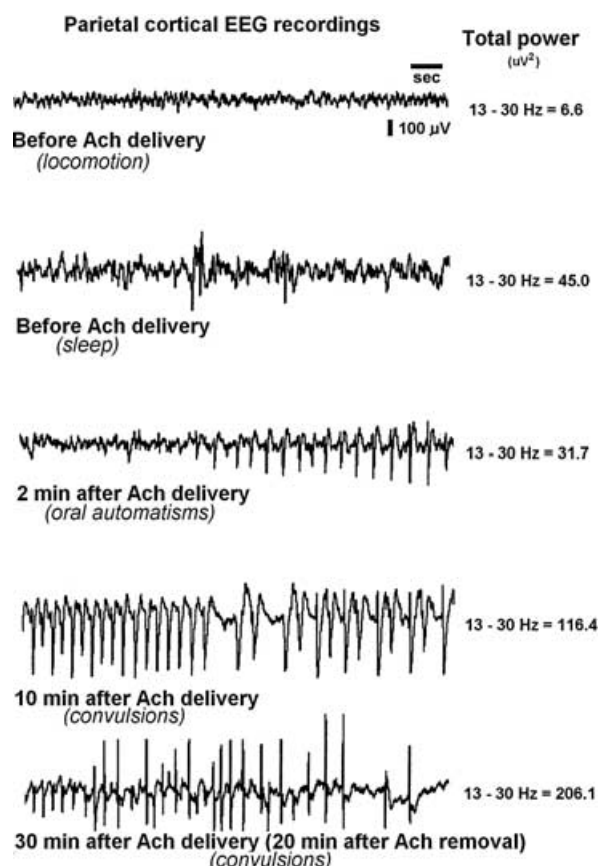


FIG. 1. Neocortical EEG recordings demonstrating the baseline electrographic activity and the spontaneous course of an acetylcholine (Ach)-induced seizure, in a freely moving rat. Calibrations for the EEG segments, the associated behavioral states, and the power of 13- to 20-Hz ("low-beta") waves within the presented segments are as indicated. Note the lack of artifacts in the recordings, the increasing severity of the seizures, the characteristic changes in low-beta power, and the sustained ictal activity 30 min after Ach delivery.

session interval, during which EEG recordings were not conducted. Additional sessions were devoted in four of the examined rats to characterizing the baseline EEG during both wakefulness and sleep, the animal's behavior in the test chamber, and the spontaneous course of the Ach-induced seizures (Fig. 1). The use of localizable epidural electrodes made histologic electrode-site verification unnecessary. Histologic analysis of the drug-exposed neocortex was not an objective of the present experiments, as such analysis can be properly done only in a dedicated study with a different design, in which Ach-exposed, aCSF-exposed, and PB-exposed rats are examined separately and compared. Thus animals were not sacrificed at the end of the data collection but were kept alive to use in other epidural drug studies.

Data analysis

The EEG data were analyzed in two ways. First, the oscilloscope-generated EEG printouts, like those shown in Figs. 2 and 3, were visually examined. Second, the

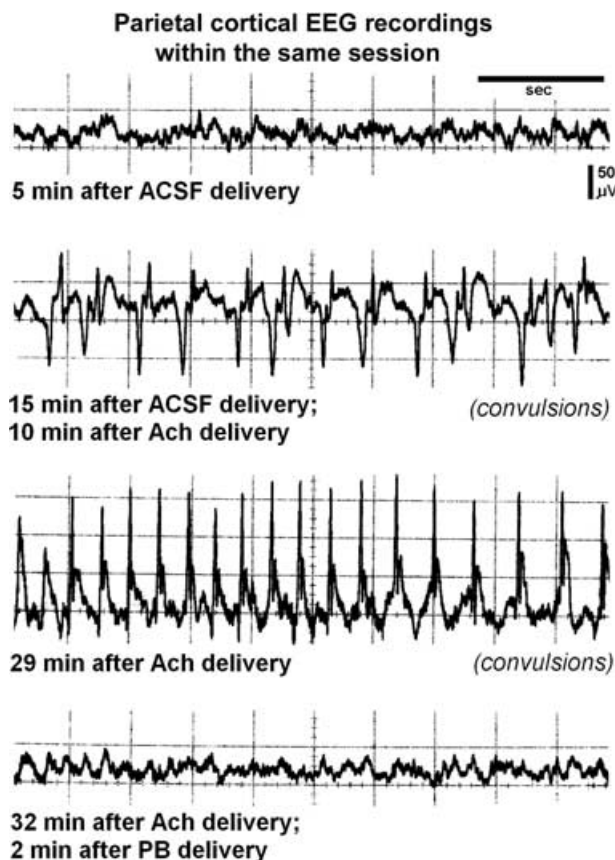


FIG. 2. Termination of an acetylcholine (Ach)-induced neocortical EEG seizure by epidurally delivered pentobarbital (PB). Calibrations, associated behaviors, and drug-delivery schedules are as indicated. Note that aCSF, delivered before Ach administration, was unable to prevent the development of the seizure. Also note that despite the presence of intense ictal EEG activity at the time of PB delivery, this drug restored the normal background EEG waves within 2 min.

stored EEG data were analyzed with proprietary software performing data playback and frequency decomposition, with a user interface written in Visual Basic and the actual EEG analysis performed in MATLAB. Fast Fourier transform (FFT) was used to assess the EEG power of the recorded waves. The power spectral density was computed with a 256-point FFT. Data playback was used to identify the onset times and termination times of the Ach-induced seizures. Representative 5- to 10-s segments from these played back EEG data (like the ones shown in Fig. 1) were processed for FFT analysis, and the total power of delta, theta, alpha, beta and gamma frequency bands was calculated. Finally, in a more comprehensive power-spectrum analysis, the total power of 1- to 4-, 4- to 8-, 8- to 13-, 13- to 20-, 20- to 30-, 30- to 50-, 50- to 80-, and 80- to 100-Hz frequency bands was computed for each consecutive 20-s period within the data file of each 40- to 70-min recording/drug-delivery session. Preliminary examination of these datasets showed that power changes in the 13- to 20-Hz (“low-beta”) frequency band were par-

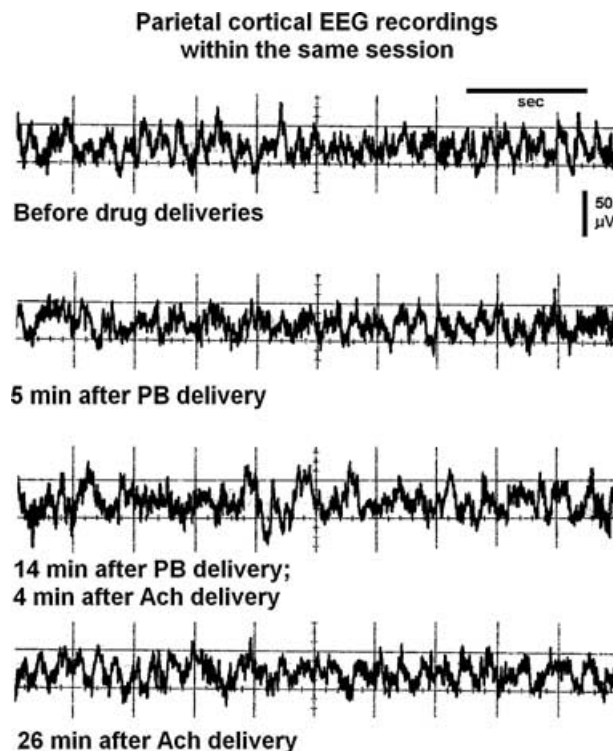


FIG. 3. Prevention of an acetylcholine (Ach)-induced neocortical EEG seizure by epidurally delivered pentobarbital (PB). Calibrations and drug-delivery schedules are as indicated. Note the lack of epileptiform EEG activity during Ach exposure. Behavioral seizure signs were also absent throughout this experiment. The similarity of the EEG waves is because they were all collected during locomotion.

ticularly useful indicators of seizure activity. Therefore we selected this frequency band for further analysis and determined the peak power of this band before drug applications, as well as during the exposures to Ach, aCSF, and PB (Fig. 4). Specifically, peak power was defined as the highest power value (μV^2) within the EEG recording segment that was collected (a) before drug deliveries, (b) during the presence of Ach in the cup, (c) during aCSF exposure, and (d) during PB exposure. The dataset with peak power values was introduced into statistical analysis (see later).

The behavioral data were analyzed with the notes made during the experimental sessions, as well as by viewing the generated videotapes. Detailed behavioral analysis was not an aim of the study.

Statistical analysis, by using the SPSS program package, was performed using the peak power data as dependent variables. Three analyses were conducted. The first analysis, a paired *t* test, examined the power characteristics of the pre-seizure and subsequent Ach-induced seizure phases. The second analysis, a factorial repeated measures analysis of variance (ANOVA) with a between-subject factor and a within-subject factor, determined whether the effect of PB on the ongoing seizure activity was

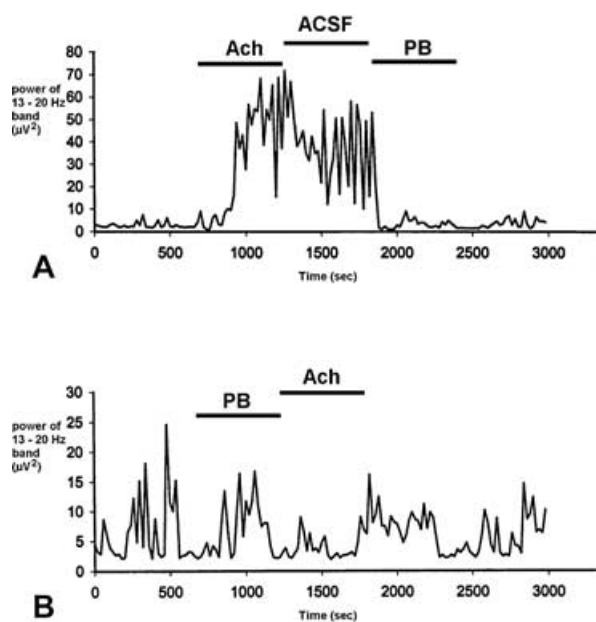


FIG. 4. Fast Fourier transform (FFT) analyses of the power of 13- to 20-Hz waves during the course of a seizure-termination experiment (**A**) and a seizure-prevention experiment (**B**). Horizontal axis, recording session time; vertical axis, power of the selected frequency band. Drug-exposure periods are as indicated. Note in (**A**) the clear power increase during acetylcholine (Ach) exposure and that this power increase is maintained, albeit at a slightly lesser degree, after the replacement of the epileptogenic compound with aCSF. The power of 13- to 20-Hz waves promptly returns to normal after pentobarbital (PB) delivery. As (**B**) illustrates, the Ach-related marked power increase is completely prevented by prior PB administration.

significant. The between-subject factor had two levels: the rat group in which Ach-induced seizures were followed by aCSF (control solution) delivery, and the rat group in which Ach-induced seizures were followed by PB delivery. The within-subject factor had also two levels: the condition of Ach-induced seizure and the condition of subsequent test-drug (aCSF or PB) delivery. The analysis was followed by post hoc (pairwise) comparisons. The third analysis, also a factorial repeated measures ANOVA with a between-subject factor and a within-subject factor, determined whether PB could prevent neocortical seizures. The between-subject factor had two levels: the rat group in which the Ach delivery was preceded with aCSF (control solution) exposure, and the rat group in which the Ach delivery was preceded by PB exposure. The within-subject factor had three levels representing the predrug phase, the drug (PB or aCSF)-treatment phase and the Ach-exposure phase. This analysis, illustrated in Fig. 5, also used post hoc (pairwise) comparisons.

RESULTS

Baseline data

Before drug applications, the rats displayed normal behavior in the test chamber. In this period, spontaneous

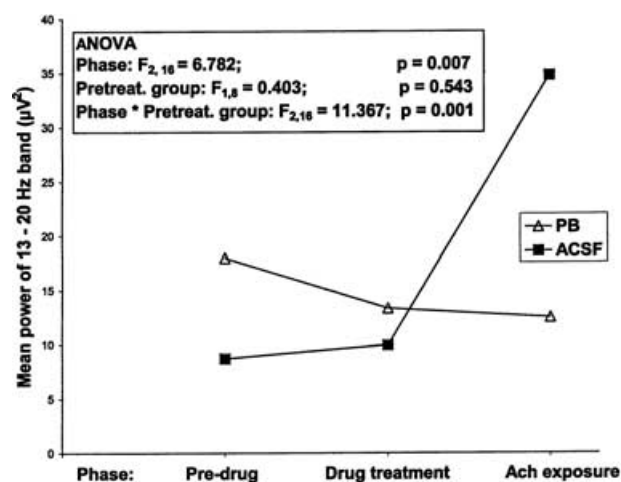


FIG. 5. Statistical analysis of the seizure-prevention study. The means of peak 13- to 20-Hz power values in three experimental phases: before drug deliveries, during test-drug pretreatments, and subsequent acetylcholine (Ach) exposures, are plotted for pentobarbital (PB)-pretreated (open triangles) and aCSF-pretreated (solid squares) rat groups. The results of data analysis with factorial repeated measures analysis of variance are indicated. The highly significant interaction between the experimental phase factor and the rat group factor indicates that PB pretreatment exerted a pharmacologically specific effect on the seizure-related power-increasing action of Ach.

behavioral seizures were not observed. During movement, the parietal cortical EEG activity was characterized by the dominant occurrence of theta waves, whereas during quiet wakefulness and sleep, the EEG traces were dominated by irregular, large-amplitude delta waves (Fig. 1). When the rat was awake but immobile, 1- to 4-s spindles of rhythmic, high-amplitude, 7- to 11-Hz waves often occurred; this activity could be promptly interrupted with a sound stimulus, like clapping. The mean power of the 13- to 20-Hz frequency band before epidural drug deliveries in all experiments was 11.3 ± 1.7 (mean \pm SEM); this value was 9.2 ± 1.7 in the subset of experiments in which the pre-seizure phase was followed by Ach delivery. Epidural applications of Ach resulted in EEG seizures in all rats, with an onset time of 224.1 ± 41.9 s in seven rats. In the eighth rat, these seizures developed after unusually long (> 10 -min) latencies. Further experiments on this rat were not conducted. Unless terminated with PB, the ictal EEG activity was maintained for 40–60 min (Fig. 1) before resolving spontaneously. Removal of the Ach solution from the epidural cup did not stop the ongoing EEG seizure activity (Fig. 1).

As the EEG seizure evolved, the total power of 13- to 20-Hz frequency waves increased markedly (Fig. 1), peaking at 466.2 ± 51.2 s after the start of Ach exposure, to a mean value of 66.5 ± 16.4 . This seizure-related value was significantly higher than the baseline power of 13- to 20-Hz frequencies before epidural drug deliveries (paired t test; $p = 0.005$; $n = 10$). Fig. 4A

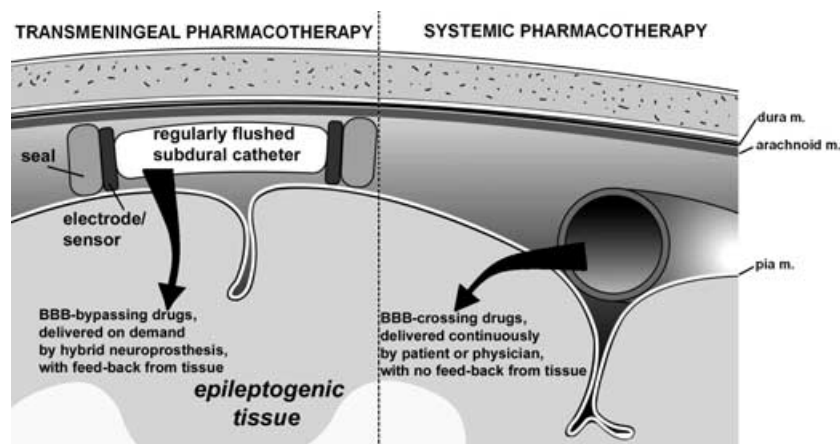


FIG. 6. Schematic diagram illustrating the principal characteristics of systemic and transmeningeal pharmacotherapies. In contrast to the traditional systemic (e.g., oral) pharmacotherapeutic approach, transmeningeal pharmacotherapy offers drug deliveries directly and exclusively to the epileptogenic brain tissue, via sealed, single or multiple, regularly flushed, subdural/epidural catheter units equipped with recording electrodes/sensors that provide feedback from the exposed neural tissue.

demonstrates the typical effect of an Ach-induced seizure on the power of this frequency band. The power of other frequency bands also increased, in varying degrees and at various times, during the EEG seizures. Sound stimuli, like clapping, did not influence ictal EEG events. The Ach-induced EEG seizures were accompanied in all rats with myoclonic jerks and convulsions characterized by contralateral forelimb and/or hindlimb clonus. Oral automatisms were also displayed, and the convulsions often became bilateral. Tonic seizures (Ludvig and Moshe, 1989) never appeared, but symmetrical forelimb clonus with rearing, resembling stage 4 seizures according to Racine's classification (Racine, 1972; Ludvig and Moshe, 1988) did develop in three rats. Signs of a generalized activation of the parasympathetic system (e.g., increased defecation) were not observed.

Seizure-termination study

Epidural delivery of PB during an ongoing Ach-induced seizure fully terminated both the electrographic and the behavioral seizure activity within 138 ± 10 s (Fig. 2), in all examined rats ($n = 7$), regardless of the length of the preceding Ach exposure and well before such seizures would spontaneously resolve (40–60 min after Ach application). The seizure terminated within 60 s of epidural PB delivery in three rats. Handling of the rats during the procedure of PB delivery never altered the ongoing EEG seizure activity. In contrast, aCSF delivered in the same fashion did not stop the seizures. The ANOVA dedicated for analyzing the effects of PB and aCSF on ongoing seizure activity, with data from paradigms 1a and 1b, showed that the within-subject effect (indicating change from seizure condition to test-drug condition; $F_{1,18} = 7.76$; $p = 0.012$) was significant, whereas the between-subject effect (indicating difference between the PB-subjected and aCSF-subjected groups; $F_{1,18 \text{ was}} = 1.89$; $p = 0.185$) was, understandably,

not. No significant interaction was found between the two factors ($F_{1,18} = 1.37$; $p = 0.257$). Post hoc tests revealed that in this subset of experiments, the delivery of PB reduced the peak power of 13- to 20-Hz waves from the preceding, seizure-related 50.5 ± 10.9 value to 6.4 ± 2.4 , which was a robust, highly significant effect ($p = 0.001$). In contrast, when aCSF was delivered in the epidural cup, the difference between the preceding seizure-related peak power (52.8 ± 18.9) and the subsequent, corresponding value during aCSF exposure (34.9 ± 4.3) was not significantly different ($p = 0.363$). The post hoc tests also showed that the seizure-related power values before aCSF delivery and those before PB delivery (see earlier) were similar ($p = 0.903$), whereas the power values after the application of aCSF and those after PB application (see earlier) were significantly different ($p < 0.001$). Once a seizure had been stopped by PB, the ictal EEG waves did not reappear, except in one occasion of the 15 analyzed seizure terminations. In that case, the electrographic seizure returned with lower-amplitude spikes, finally to disappear 9 min later. After PB exposure, the EEG activity was comparable to the pre-seizure background activity, and the rat displayed normal behavioral patterns, without sleep or signs of anesthesia. Fig. 4A illustrates the characteristic changes in the power of 13- to 20-Hz waves during the course of an experiment, where the Ach-induced neocortical seizure was maintained during epidural aCSF administration, but stopped with the similar delivery of PB.

Seizure-prevention study

Epidural delivery of PB before Ach application completely prevented the development of electrographic and behavioral seizures. This is demonstrated in Fig. 3. In contrast, when aCSF was delivered before Ach exposure, the seizures developed in all experiments, as in the one represented in Fig. 2. The ANOVA dedicated to analyzing the

effects of PB and aCSF on the epileptogenic potential of subsequent Ach exposures, with data from paradigms 2a and 2b, showed that the within-subject effect (indicating changes across predrug, drug treatment, and Ach exposure phases; $F_{2,16} = 6.782$; $p = 0.007$) was significant. The between-subject effect (indicating difference between the aCSF-pretreated and PB-pretreated groups; $F_{1,8} = 0.403$; $p = 0.543$) was, understandably, not significant. However, the interaction between the two factors was highly significant ($F_{2,16} = 11.367$; $p = 0.001$). These key results are shown in Fig. 5. The post hoc tests revealed that in this subset of experiments, the peak power values during PB delivery (13.3 ± 3.1) and the peak power values during the subsequent Ach exposure (12.4 ± 6.5) were statistically similar ($p = 0.890$). In contrast, the peak power values during aCSF delivery (9.9 ± 3.1) and the peak power values during the subsequent Ach exposure (34.8 ± 6.5) were significantly different ($p = 0.003$), highlighting the marked seizure-related power increase that was totally absent when PB was delivered before Ach. Figure 4B demonstrates the ability of PB to prevent the power increase of 13- to 20-Hz waves during Ach exposure. The behavior of the rats during the presence of PB in the epidural cup and that during the presence of aCSF were indistinguishable and comprised moving, grooming, eating, drinking, and resting. In this pre-Ach phase, gross alterations in the background EEG activity were not detected.

Other observations

Two additional observations are noteworthy. First, PB delivery onto a right-hemisphere seizure focus could terminate severe EEG seizures associated with bilateral convulsions, as determined by visual monitoring of the animals' behavior. Unfortunately, our one-channel recording method was not suitable to monitor the propagation of Ach-induced seizures and the spread of PB-generated inhibition in the spike-sustaining circuitry. Finally, as Fig. 1 illustrates, a clear EEG seizure, especially at its beginning, could produce periods of spikes and spike-wave discharges with low-beta-frequency power similar to the power of this frequency component during sleep. The power of this frequency band increased markedly only in later seizure stages, during the sustained generation of high-amplitude, rhythmic epileptiform discharges. Because our experimental design assured that Ach was administered during wakefulness and not during sleep, and because PB was delivered in later seizure stages, the FFT analysis correctly identified the periods of seizures and drug effects.

DISCUSSION

We found that PB delivered epidurally can both prevent Ach-induced seizures and abort ongoing Ach-induced seizures. These findings support other studies that indicate that compounds delivered epidurally or subdurally

can profoundly alter the function of the underlying cerebral cortex (Fleming et al., 1971; Tanganelli et al., 1992; Eder et al., 1997; Stein et al., 2000; Kiss et al., 2004; Ludvig et al., 2005). The delivery of drugs to either of these spaces could be of potential benefit to patients with epilepsy.

Ach, because it contains a quaternary amine, does not cross the blood-brain barrier (BBB) and thus cannot enter into the bloodstream after epidural application. Therefore the Ach-induced seizures in this study were more likely due to diffusion of the molecule through the subdural space into the neocortical tissue than to systemic effects. This explains why peripheral cholinergic effects, which characteristically accompany seizures induced by systemically injected pilocarpine (Turski et al., 1983), did not occur in the rats we examined. The preservation of wakefulness after PB deliveries further indicates that drugs delivered through the cerebral meninges exert their effects primarily through local penetration into the neural tissue, not via diffusion into the vascular system. Nevertheless, the possibility cannot be ruled out that a portion of the Ach and/or PB molecules did enter into the circulation via damaged blood vessels under the epidural cup, contributing to the net electrographic and behavioral effects of these drugs. Evaluating this possibility remains for future studies that will monitor serum levels of Ach, PB, and other drugs on epidural delivery. Such neurochemical measurements will be critical for fully understanding the mechanism of action of epidurally/subdurally delivered epileptogenic and antiepileptic compounds.

The neocortical seizures induced by epidural Ach application in our experiments were promptly and effectively terminated by epidural applications of PB. This was evident in the EEG recordings (Fig. 2), power-spectrum analyses (Fig. 4A), and behavioral records. During aCSF exposures, the power of 13- to 20-Hz waves was often slightly less than during the preceding Ach exposure (Fig. 4A) because the test solutions (aCSF and PB) were delivered after Ach was removed and because the seizure-related power increase often peaked before the delivery of these test solutions. As a consequence, the ANOVA used for the seizure-termination study yielded no significant within-subject/between-subject interaction. However, all post hoc analyses, including the one that showed no significant aCSF effect on Ach-related power increase, shed light on the same robust phenomenon: the ability of PB to stop neocortical seizures on epidural administration. This is consistent with the previous observations that epidurally delivered DZP can reduce the intensity of bicuculline-induced EEG spike activity (Eder et al., 1997) and can decrease the frequency of bicuculline-induced seizures (Stein et al., 2000) in the rat neocortex. Expanding these observations, our study suggests that different types of neocortical seizures can be controlled by epidural/subdural drug applications.

The most significant finding that emerged from our experiments is that epidural delivery of an AED could fully prevent the development of neocortical seizures. The concentration of PB we used was relatively high: 226 mM. This raises the possibility that the seizure-preventing effect was due to nonspecific, nonpharmacologic actions. However, the fact that another drug, Ach, in a similar concentration range, caused completely different actions makes that possibility unlikely. Importantly, whereas PB pretreatment likely caused subtle electrophysiologic alterations that have yet to be described, the overall EEG activity during the sole presence of this drug was very similar to the normal (predrug) EEG patterns. Thus neurotoxic effects did not take place during PB pretreatment. A remarkable feature of the seizure-preventing action of epidurally administered PB pretreatments was that this effect was observed after the removal of the drug from the epidural area. This could simply be due to the sustained diffusion and/or delayed clearance of PB within the exposed neocortical cell layers. However, it also is possible that penetration of PB molecules into the neocortical tissue was followed by prolonged binding to postsynaptic GABA [or other (e.g., substance P) receptors] (Okamoto et al., 2003; Wan et al., 2003), long-lasting induction of inhibitory neurotransmitter release via presynaptic receptors (Rohde and Harris, 1983; Murugiah and Hemings, 1998), sustained modulation of second-messenger systems (Gonzales and Mendez-Bobe, 1996; Humar et al., 2004), or other molecular cascades. Future studies with intracortical sampling and tissue biopsy methods may uncover these mechanisms.

These results highlight the potential of transmeningeal, epidural, or subdural drug deliveries to prevent and terminate neocortical seizures. Figure 6 outlines a model of the envisioned "transmeningeal" pharmacotherapy and its differences from the traditional, systemic pharmacologic approach. Transmeningeal pharmacotherapy would have several advantages over oral and parenteral routes. First, it offers therapeutic control over epileptogenic neocortical circuitries that are both unresponsive to systemically administered drugs and, because of their localization in motor or language areas, unsuitable for resective surgery. Second, it offers the ability to administer a range of compounds, including peptides and proteins, which may have unique antiepileptogenic effects yet are excluded from the physician's therapeutic repertoire because of their inability to cross the BBB. Third, it offers pharmacologic treatment without the numerous adverse effects of systemic drug administration. These include the rare, idiosyncratic, and common dose-related side effects. Even in the case of central nervous system side effects, systemic drug delivery affects widespread brain areas that are often unrelated to seizure generation or spread. Thus many adverse effects likely result from drug effects on brainstem structures that are not directly related to the drug's mechanism

of antiepileptic action. Transmeningeal pharmacotherapy would have disadvantages. First, it requires the implantation of subdural/epidural electrode-catheter units coupled to an HNP-like device. The goal is to control the timing and duration of drug delivery based on continuous feedback from the exposed neural tissue. Such implantations require neurosurgical procedures that carry risks such as hemorrhage and infection, albeit importantly, to a lesser extent than intraparenchymal/deep-brain implantations. Second, numerous technical and scientific problems must be solved before transmeningeal pharmacotherapy can become an effective, safe, and reliable therapeutic modality for intractable focal epilepsy. Is transmeningeal drug delivery as effective in controlling toxin- or injury-induced chronic, spontaneous seizures in rats (D'Ambrosio et al., 2005; Nilsen et al., 2005) as it is in controlling bicuculline- and Ach-induced acute seizures? Should subdural HNPs deliver conventional AEDs or rather a combination of water-soluble, natural neurotransmitters and neuromodulators? Do tolerance and/or detrimental plastic changes develop in the drug-exposed neocortex after the long-term use of a subdural drug-delivery device? These are just a few of the many questions that remain to be studied in the laboratory.

In summary, our study revealed that epidural PB delivery could terminate as well as prevent neocortical seizures induced by local Ach exposure in rats. Based on these and prior observations by other groups (Eder et al., 1997; Stein et al., 2000) and ours (Ludvig et al., 2005), we propose that drug administrations across the meninges may become effective treatments for intractable focal epilepsy. Although the present animal study did not prove that such transmeningeal drug deliveries will produce better therapeutic results than traditional, systemic drug treatments, our data perhaps justify the consideration of pilot neurosurgical studies to evaluate whether epileptiform EEG activity in the human neocortex can also be stopped or significantly reduced by locally applied drugs. Indeed, this information would help to assess and compare the seizure-controlling potential of on-demand electrical-stimulation and drug-delivery neuroprostheses (Fischell et al., 2000; Ludvig, 2000; Litt et al., 2001; Ludvig and Kovacs, 2002; Iasemidis et al., 2005; Turner et al., 2005; Worrell et al., 2005).

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