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Furosemide and Mannitol Suppression of Epileptic Activity in the Human Brain

Michael M. Haglund and Daryl W. Hochman

1Departments of Surgery (Neurosurgery) and Neurobiology and 2Surgery (Experimental) and Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina

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Haglund, Michael M. and Daryl W. Hochman. Furosemide and mannitol suppression of epileptic activity in the human brain. J Neurophysiol 94: 907–918, 2005. First published February 23, 2005; doi:10.1152/jn.00944.2004. Most research on basic mechanisms of epilepsy and the design of new antiepileptic drugs has focused on synaptic transmission or action potential generation. However, a number of laboratory studies have suggested that nonsynaptic mechanisms, such as modulation of electric field interactions via the extracellular space (ECS), might also contribute to neuronal hyperexcitability. To date, a role for nonsynaptic modulation of epileptic activity in the human brain has not been investigated. Here we studied the effects of molecules that modulate the volume and water content of the ECS on epileptic activity in patients suffering from neocortical and mesial temporal lobe epilepsy. Electrophysiological and optical imaging data were acquired from the exposed cortices of anesthetized patients undergoing surgical treatment for intractable epilepsy. Patients were given a single intravenous injection containing either 20 mg furosemide (a cation-chloride cotransporter antagonist) or 50 mg mannitol (an osmolyte). Furosemide and mannitol both significantly suppressed spontaneous epileptic spikes and electrical stimulation-evoked epileptiform discharges in all subjects, completely blocking all epileptic activity in some patients without suppressing normal electroencephalographic activity. Optical imaging suggested that the spread of electrical stimulation-evoked activity over the cortex was significantly reduced by these treatments, but the magnitude of neuronal activation near the stimulating electrode was not diminished. These results suggest that nonsynaptic mechanisms play a critical role in modulating the epileptogenicity of the human brain. Furosemide and other drugs that modulate the ECS might possess clinically useful antiepileptic properties, while avoiding the side effects associated with the suppression of neuronal excitability.

INTRODUCTION

Approximately 1% of the population suffers from epilepsy, and 20–30% of epilepsy patients have seizures that are intractable to control with existing antiepileptic drugs (AEDs) (Benbadis et al. 2000; Hauser 1998). Available antiepileptic therapies are considered to be inadequate since they do not provide sufficient seizure-control in a significant proportion of epilepsy patients and are often accompanied by adverse side effects (Loscher 2002). Currently prescribed AEDs are thought to mediate their antiseizure effects by reducing neuronal excitability, either by directly affecting synaptic interactions or by reducing the likelihood of action potential generation (LaRoche and Helmers 2004). Persistent adverse effects, such as sedation and cognitive impairment, are commonly associated with AEDs because neuronal excitability is reduced indiscriminately in both epileptogenic and normal areas in the brain (Aldenkamp et al. 2003; Brodie 2001).

Previous studies on hippocampal slices suggested that antagonism of the cation-chloride cotransport system, with furosemide (Lasix) or reduced extracellular chloride, potently blocked epileptiform activity without suppressing neuronal excitability (Hochman and Schwartzkroin 2000; Hochman et al. 1995, 1999). In those studies, it was proposed that the antiepileptic action of chloride cotransport antagonism was mediated through nonsynaptic mechanisms involving the Na⁺,K⁺,2Cl⁻ cotransporter and hence potentially represented a novel approach to seizure control. The furosemide-sensitive Na⁺,K⁺,2Cl⁻ cotransporter is thought to mediate activity-evoked cell volume changes in glial cells and to play a significant role in the redistribution of potassium from the extracellular space (ECS) after increases in neuronal activity (Walz 1992, 1995; Walz and Hertz 1984; Walz and Hinks 1985).

It has long been hypothesized that alterations of the ECS could modulate neuronal synchrony by affecting nonsynaptic mechanisms such as the electrical resistance of brain tissue, extracellular ionic concentrations, local ephaptic interactions, and long-range electric field effects among neuronal populations (Dudek et al. 1986; Faber and Korn 1989; Jefferys 1995). Numerous clinical and experimental studies have shown that changes in the osmolarity of the ECS, which presumably modulates the volume fraction of the ECS by directly affecting cell volume, can significantly affect epileptogenicity (Andrew 1991). In vivo studies in rats demonstrated that systemically injected hyperosmotic solutions significantly increase electroshock seizure thresholds (Reed and Woodbury 1964) and prevent the development of kainic acid-induced seizures (Barran et al. 1987). In vitro studies on the role of nonsynaptic mechanisms in epilepsy began with the observation that synchronized discharges could occur in hippocampal slices in which chemical synaptic transmission had been eliminated by the reduction of calcium in the bathing medium (Jefferys and Hass 1982; Taylor and Dudek 1982, 1984a,b). Increasing osmolarity in this preparation with mannitol or sucrose reduced
or blocked synchronized discharges, whereas decreasing osmolarity had the opposite effect (Dudek et al. 1990; Roper et al. 1992). A study on hippocampal slices bathed in high-potassium medium suggested that alteration of the size of the ECS is a critical component in the generation of epileptiform activity (Traynelis and Dingledine 1989).

Both furosemide and mannitol are known to affect the size of the ECS but through different mechanisms. Furosemide blocks activity-evoked cell swelling through antagonism of cation-chloride cotransporters, whereas mannitol removes water from intracellular compartments through osmotic forces (Geck et al. 1980; Hochman et al. 1995; Kimelberg and Frangakis 1985; Paczynski 1997; Traynelis and Dingledine 1989). Two considerations of particular clinical interest motivated the studies reported here. First, furosemide more potently blocks epileptiform activity in in vitro studies than many of the commonly prescribed AEDs (Hochman et al. 1995). Second, both furosemide and mannitol have been observed to suppress epileptiform activity in laboratory models of seizure without reducing excitatory synaptic transmission or the ability of neurons to fire action potentials (Dudek et al. 1990; Hochman and Schwartzkroin 2000; Hochman et al. 1995; Traynelis and Dingledine 1989), suggesting that the neurological side effects associated with currently prescribed AEDs might be avoided. Because the role of ECS modulation in human epileptogenicity has not yet been experimentally investigated, we studied the effects of furosemide and mannitol on patients suffering from medically intractable epilepsy.

**Methods**

**Subjects**

Intraoperative studies were performed on 27 patients, 11 of whom received no experimental treatments (control) and 16 of whom received either furosemide or mannitol during the recording sessions (experimental group). All patients were suffering from medically intractable epilepsy and had given informed consent; studies adhered to a protocol that was approved by the Duke Human Subject Committee (Institutional Review Board Protocol 2082). The control group, whose ages varied from 14 to 57 yr, consisted of 5 males and 6 females. The ages of the patients in the experimental group varied from 12 to 56 yr, consisting of 4 males and 12 females. All patients were suffering from seizure conditions that could not be adequately controlled with existing AEDs. Patients were classified as having either mesial temporal epilepsy (MTE, control group, n = 7; experimental group, n = 13) or neocortical epilepsy (NE, control group, n = 4; experimental group, n = 3), depending on where the sites of seizure onset had been identified in the mesial structures with video electroencephalographic (EEG) monitoring, concordant hypometabolism on PET in the mesial temporal lobe, and hippocampal atrophy on high-resolution MRI or implanted subdural electrode array monitoring for neocortical seizures. Some patients were involved in more than one experiment (i.e., both spontaneous spiking and stimulation-evoked afterdischarge activity were studied on the same patient).

**Intraoperative maintenance**

Patients remained on their preoperative AEDs and were anesthetized with the inhalational agent isoflurane (0.2 MAC) and intravenous remifentanil and propofol. Propofol was administered up to 10 min prior to the EEG recording session at which point propofol administration was stopped and not re-administered until the recording session had ended. Additionally, a local field block was administered consisting of 1.0% lidocaine with 1:200,000 epinephrine and 0.25% marcaine with 1:200,000 epinephrine mixed 1:1. The lidocaine/marcaine/epinephrine solution (9 ml) was mixed with a NaHCO₃ solution (1 ml) for the field block. Vital signs were monitored so that variables, such as blood pressure, blood oxygen saturation, and arterial CO₂ (paCO₂ = 35–39) and PO₂ were as close as possible between experiments. No changes in vital signs were observed during experimental treatments; importantly, neither the furosemide nor mannitol treatments caused changes in blood pressure.

**Experimental treatments**

The furosemide (Lasix) solution was composed of 4-chloro-N-(2-furfuryl)-5-sulfamoylanthranilic acid, sodium chloride for isotonicity and sodium hydroxide to adjust pH. The furosemide injection solution was a sterile, nonpyogenic solution with a concentration of 10 mg/ml and 2 ml were injected as a single bolus (20 mg). Mannitol I.V. (Mannitol injection, USP) was a sterile, nonpyogenic solution of mannitol dissolved in water for injection at a concentration of 20%. A 50-g intravenous bolus of mannitol was administered over 5–10 min.

**Electrophysiological monitoring**

After the cortex was exposed, electrophysiological recordings were acquired from an array of subdural EEG electrodes placed directly on the cortical surface. The electrode array (Ad-Tech Medical Instrument, WI) was recorded by an analog EEG machine equipped with signal amplifiers and noise filters (Grass, RI), and passed to an A/D converter and recorded as a digital signal on VCR tape cassettes (VR-100-B-8 A/D, Instrutech). Each surface EEG electrode was 5 mm in diameter with the inter-electrode distances of 10 mm. Recordings were acquired from at least seven electrodes in an array covering ~5 × 5 cm of the cortical surface. An eighth input to the amplifier received impulses from the camera used for optical imaging experiments so that images could be accurately correlated to the electrophysiological activity in time. A silver-ball reference electrode was placed on the contralateral mastoid process. All eight channels were digitized at 14-bit resolution, 11.8 k samples/s per channel. The mean voltage of the EEG recording from the interictal focus was calculated over a 20-min duration just prior to administration of either the furosemide or mannitol treatment. Only those EEG events that were at least ±3 SD from this mean were counted as spikes. This “3-sigma” criterion was chosen because it had been empirically determined to avoid overcounting ambiguous events based on waveform morphology, while still counting enough events for statistical analysis.

**Electrical stimulation of cortex**

For electrical stimulation during afterdischarge studies, a bipolar stimulating electrode (5 mm interelectrode distance), powered by a constant-current source (Ojemann Cortical Stimulator, Integra LifeSciences), was placed on the neocortex at sites distant from the interictal focus. The minimal stimulation current (4 s at 60 Hz, 1-ms biphasic pulse) required to elicit ±5 s of afterdischarge activity was determined. A recording electrode was placed between the stimulating electrodes for recording afterdischarge activity (see gray-scale image in Fig. 2, bottom middle, for the electrode configuration). The duration of afterdischarge activity was defined to be the time during which a train of spikes followed the cessation of the 4-s stimulus.

**Optical imaging**

The intraoperative optical imaging technique used in these studies was similar to what has previously been reported, where it was demonstrated that changes in the activity-evoked optical spectroscopic properties of the cortex can be used to provide high-resolution maps of epileptiform activity in humans (Haglund et al. 1992). A 4 ×
4-cm glass plate was gently placed on the cortical surface to prevent movement artifacts from respiration and heartbeat. The cortex was illuminated with 535-nm light, which has been shown to provide accurate localization of neuronal activity in human neocortex (Haglund and Hochman 2004). The cortical surface was uniformly illuminated with four fiberoptic lights regulated by a stable DC power supply (SCHOTT Fostec) and filtered with 535 ± 5-nm band-pass optical filters. Images were acquired with a cooled 12-bit digital CCD camera (Roper Scientific, NJ). Sequences of images were integrated over 200-ms intervals and stored on hard disk for off-line analysis. During each stimulation trial, 400–600 images were acquired. To visually demonstrate the spread of stimulation-evoked optical changes over the cortex, “difference images” were generated by subtracting a randomly chosen prestimulation image (i.e., control image), acquired during a 10-s interval prior to stimulation, from all of the images in its associated series. Each difference image thus represented the absolute change in the optical signal from the chosen control image. The difference images were then divided by the control image to provide a map of percentage change. To make small changes more apparent, the images were pseudo-colored with a “spectrum” lookup table, shown in the color bar of Fig. 4. High-frequency noise was removed by applying a Gaussian low-pass filter to the images to improve appearance; such processing was not observed to significantly affect the spatial features of the optical maps as compared with the raw, unprocessed images. Each image was processed in the identical manner.

Estimation of electrical resistance changes

Although the configuration of the stimulation and recording electrodes did not allow for a quantitative estimation of absolute tissue resistivity, it was possible to estimate percentage changes in tissue resistance from baseline conditions (Plonsey and Barr 2000). In several experiments, prior to the administration of furosemide or mannitol, the cortex was stimulated with at least three different current amplitudes within the range of 6–18 mA (4 s of stimulation at each current). For these experiments, the “stimulation artifacts” recorded by each of the surface EEG electrodes (i.e., the voltage responses recorded by each electrode during the time the stimulation current was applied) was re-digitized at a high sampling rate (10 kHz) from the stored data. Because the stimulation-current had a sinusoidal waveform of 60 Hz, the voltage response from the stimulation current could be faithfully dissociated from the underlying physiological EEG signal. A computer program written by Hochman automatically determined the peak amplitudes for each cycle of the voltage-response during the 2-3-s interval from the onset of the stimulation current. The average of these peak voltages was determined for each stimulation trial and was used to represent the voltage response in further calculations. The average values recorded at each electrode in the array during a given stimulation trial were used to calculate the SD of the resistance change for that trial. By Ohm’s law, assuming that the tissue resistance was not changed during baseline conditions, the magnitudes of the voltage responses divided by the stimulation currents could be used to give an estimate of the electrical resistance of the tissue. Prior to administration of furosemide or mannitol treatments, this estimate of tissue resistance should remain constant throughout the range of stimulation currents, as was observed to be the case (Fig. 5A, left). Only data from the electrodes that showed a nearly ideal ohmic response were used for these calculations (Fig. 5A), and data from those electrodes that failed to show such a response were excluded from the analysis. Following these criteria, data from at least four of seven surface electrodes were used in the analysis for each experiment. After the administration of furosemide or mannitol, the cortex of each subject was stimulated every 3–7 min for the remainder of the recording session at some fixed current strength. This posttreatment stimulation current strength was identical to one of those used during the pretreatment stimulation trials. In this manner, percentage changes in tissue resistance after any experimental treatments were estimated.

Calculation of power spectra

For each experiment, the power spectrum of the EEG data recorded from the array of surface electrodes was calculated over two time intervals: a 10-min interval prior to the administration of any experimental treatment and the final 10 min of the recording session after the administration of either furosemide or mannitol; during this final 10-min interval, furosemide or mannitol had been administered ≥20 min prior to the recording. Prior to any analysis, electrical stimulation artifacts were deleted from the data, and data from electrodes that showed significant spurious noise (such as the noise introduced from loose electrode contacts) were excluded. For each of the two time intervals, the separate EEG recordings from each of the remaining electrodes (4–7 electrodes for each experiment) were re-organized into single, continuous data files (1 file for the pretreatment data and 1 file for the posttreatment data), and the power spectrum was calculated for each of these files. In this way, the power spectrum included the total power contributed by each of the recording electrodes in the array. A test calculation from a single data set that was re-digitized at a high rate (10 kHz) showed that >95% of the power was localized at frequencies <50 Hz. Hence to facilitate the ease of computations, subsequent calculations were performed on data that had been re-sampled at a lower frequency of 100 Hz, and the power spectra were only calculated for those frequencies <50 Hz. The total power contributed from each frequency for a given interval was calculated from the raw power spectrum. For visualization purposes (Fig. 6), smoothed, denoised estimates of the power spectra were calculated using a wavelet shrinkage algorithm (Gao 1997). All calculations were performed using S-Plus (Insightful).

Analysis

After digitization, EEG and optical data were analyzed off-line using a combination of commercial software and custom software written by Hochman. Interspike intervals were calculated from the digitized EEG recordings for each experiment using the three-sigma criterion described in the preceding text. Calculation of means ± SD, t-test, and confidence intervals for the interspike interval data, and the generation of graphs were performed using S-Plus V 6.2 (Insightful). Optical images were processed with MetaMorph (Universal Imaging).

RESULTS

All experiments were performed intraoperatively on patients during their surgical procedure for the treatment of intractable epilepsy. After the cortex was exposed, optical imaging was performed while electrophysiological recordings were acquired from an array of EEG electrodes placed directly on the cortical surface. In accordance with an approved IRB, experiments were limited in duration. Consequently, it was only possible to observe the effects following experimental treatments for ~40 min. Patients were classified as having either mesial temporal epilepsy (MTE) or neocortical epilepsy (NE), depending on where the sites of seizure onset had been identified prior to these experiments with a subdural electrode array or video EEG monitoring.

Effects of furosemide on spontaneous epileptic spiking

In a first set of studies, the effects of furosemide on spontaneously occurring interictal activity were studied on five patients (4 MTE and 1 NE; data from an individual patient is

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A single intravenous bolus injection of furosemide (20 mg) was administered to each patient after ≥20 min of recording control (i.e., pretreatment) EEG data; the final dose being 0.18–0.45 mg/kg depending on the weight of the patient. Within 10–30 min after administration of furosemide, the frequency of the interictal spikes was significantly reduced in all five patients (Fig. 1, 2nd trace) with all spontaneous spiking being abolished during the final 10 min of the recording session in three patients (2 MTE; 1 NE). Several minutes prior to the decrease in interictal activity, there was a noticeable change in the morphology of the interictal spikes that their peak-to-peak amplitudes decreased, with a proportion of spikes losing their biphasic structure and becoming broader (Fig. 1, right). Variability in the time to the reduction in the frequency of spontaneous interictal activity (10–30 min) was observed and is reflected in the larger error bars in the average patient response during the initial period following furosemide treatment (Fig. 1, bottom right graph). There was no correlation between the onset times or magnitudes of effects, and the milligram/kilogram dosage of furosemide. It was noted that although the frequency of the spontaneous epileptic spikes was significantly reduced in all patients, the magnitude of the baseline spontaneous activity recorded by all of the electrodes at sites showing no epileptic activity remained unchanged (data not shown).

To characterize the magnitude of the effect of furosemide on spontaneous interictal activity for individual patients, the average of the interspike intervals was tabulated over two time intervals; the 20-min duration just prior to administration of furosemide and the duration lasting from t = 20 min to t = 40 min after furosemide administration. The average reduction in spiking frequency over these five patients, when comparing the pre-furosemide data to the post-furosemide data, was ~60%. It was found that prior to furosemide administration, the average interspike interval (i.e., the time between occurrences of consecutive spontaneous interictal spikes that were ≥3 SD from the mean voltage) of the data pooled over all five patients was 9.6 ± 0.65 (SE) s with the 99% confidence interval (CI) of [7.9 s, 11.3 s]. After furosemide treatment, the mean interspike interval was increased to 21.9 ± 3.9 s (99% CI = [11.8 s, 31.9 s]). A t-test of the means of interspike intervals occurring before and after furosemide treatment rejected the null hypothesis that the treatment had no effect (P < 0.0001). Spontaneous spiking was more dramatically suppressed and appeared to be completely blocked in several patients during the last 5 min of the recording session. The graph in Fig. 1, bottom, shows the normalized average reduction in spiking frequency over time, averaged over all five patients.

**Effects of furosemide on electrical stimulation-evoked epileptic discharges**

The next set of studies tested the effects of furosemide on electrical stimulation-evoked epileptiform activity, or “after-discharge activity,” on eight patients (6 MTE, 2 NE; Fig. 2). A bipolar electrode was placed on the cortex and the minimal stimulation current (4 s at 60 Hz; biphasic; 1 ms/phase) required to elicit ≥5 s of afterdischarge activity was determined. A recording electrode was placed between the stimulating electrodes for recording afterdischarge activity (see gray-scale image in Fig. 2, bottom middle, for the electrode shown in Fig. 1). These patients were selected on the basis of having interictal EEG activity consisting of spontaneously occurring spikes that could be unambiguously identified and counted. In each experiment, the site of the maximal spontaneous interictal activity was located. This site of maximal activity, referred to as the “interictal focus,” tended to be highly localized in that recordings from electrodes 1–2 cm away showed significantly fewer or no interictal spikes. One subdural electrode was placed as close as possible to the interictal focus, which in the MTE patients was on the parahippocampal gyrus and presumably reflected activity generated by mesial structures (Fig. 1; black traces). Recordings were acquired from ≥6 other electrodes in an array covering approximately 5 cm x 5 cm of the cortical surface. The closest electrode, which showed little or no interictal activity, was used for comparison as ‘background’ EEG activity (Fig. 1; gray traces). The average value of the voltage from the interictal focus recording was calculated for the 20 min duration prior to furosemide administration. Only those EEG events that were ≥3 SD from this average value were counted as spikes (Fig. 1; solid horizontal lines on top left traces).
The duration of afterdischarge activity was defined to be the time during which a train of spikes followed the cessation of the electrical stimulus, where spikes were defined as those EEG events that were \( \pm 3 \) SD from the mean of the voltage of prefurosemide activity. For each patient, the minimal current was selected that consistently evoked afterdischarge activity in three consecutive stimulation trials. The furosemide treatment resulted in either a complete blockade (4 patients: 3 MTE, 1 NE) or in a reduction by \( \frac{1}{2} \) (4 patients: 3 MTE, 1 NE) of the duration and amplitude of the afterdischarge activity (Fig. 2A). The average reduction in the duration of stimulation-evoked afterdischarge activity over these eight patients was \( 85 \pm 14.6\% \) (mean \( \pm \) SD). In comparing the mean duration of the afterdischarge activity before and after furosemide treatment, a Wilcoxon rank-sum test rejected the null hypothesis that the furosemide treatment had no effect \( (P < 0.01) \).

To test whether the furosemide suppression of afterdischarge activity resulted from an increase in the afterdischarge threshold (i.e., an increase in the minimal current required to elicit afterdischarge activity), the stimulation current was incrementally increased in two patients that had experienced a complete blockade of afterdischarge activity (Fig. 2B). It was found that sufficiently large stimulation currents were able to elicit afterdischarge episodes at least as long in duration as those observed prior to furosemide treatment (B, bottom).

**Effects of mannitol on spontaneous and electrical stimulation-evoked epileptic activity**

If furosemide mediates its suppression of epileptic activity through modulation of the size of the ECS, then it would be expected that osmotic agents might also have significant effects on epileptic activity. A comparison of the reported effects of mannitol (an osmotic agent) and furosemide in in vitro studies supports this notion (Hochman et al. 1995; Traynelis and Dingledine 1988). To test this hypothesis, we repeated the preceding studies on four patients who were given a single...
Changes in the spread of electrical stimulation-evoked activity revealed by optical imaging

To examine the effects of furosemide and mannitol on the spread of activity over the cortex, optical images of the activity-evoked changes in light absorption by the cortical tissue were acquired during the electrical stimulation of the cortex at sub-threshold levels (i.e., the magnitude of the stimulation current was set just below to what was necessary to evoke afterdischarge activity; Fig. 4). Hemodynamic changes underlie the activity-evoked optical changes in vivo and are thought to be a reliable surrogate for neuronal activity in the cortex (Grinvald et al. 1988; Haglund et al. 1992; Ngai et al. 1988). These optical imaging studies were performed on three patients treated with mannitol and two patients with furosemide (all MTE patients). Images acquired at the end of 4 s of subthreshold stimulation were used for comparison. Within 30 min after treatment, both mannitol and furosemide significantly blocked the spread of activity over the cortex elicited by electrical stimulation, reducing the extent of activated cortex by >50% in all cases (Fig. 4). Although the spatial extent of the spread of the optical signal was reduced, the magnitude of the optical change in between the two poles of the bipolar stimulating electrode was not diminished. These results suggest that mannitol and furosemide suppressed the spread of synchronous stimulation-driven activity over the cortex, but they did not suppress neuronal firing in response to direct electrical stimulation.

Changes in the electrical resistance of tissue after furosemide and mannitol treatments

It is possible that the furosemide and mannitol treatments affected the response of the cortex to electrical stimulation by altering the electrical resistance of the tissue. This possibility was studied by analyzing the voltage responses recorded by the surface EEG electrodes during electrical stimulation of the cortex (furosemide, n = 3; mannitol, n = 3). For the duration of the recording session prior to the infusion of furosemide or mannitol, it was observed that the recorded voltage responses varied linearly in proportion to the magnitude of the stimulation current, confirming that Ohm’s law could be used to estimate percentage changes in tissue resistance (Fig. 5A). This constant relationship between stimulation current and voltage response was observed to hold with sufficient reliability for the purposes of this study, though this ohmic response appeared to hold more reliably at stimulation currents >8 mA (Fig. 5A, right). In the data sets analyzed, the cortex was stimulated at a fixed current for the duration of the recording session following injection of furosemide or mannitol. There appeared to be a transient decrease in tissue resistance (<2%) within the first 5 min after administration of furosemide; however, there was no significant change in tissue resistance between the time that furosemide was first administered and the end of the recording session when the afterdischarge activity and interictal spiking were maximally diminished (Fig. 5B, left). Mannitol treatment resulted in a greater initial decrease in tissue resistance (maximum observed change of 10%) lasting for a longer duration than the changes elicited by furosemide but also recovered to baseline levels by the end of the recording session at the time when diminution of afterdischarge activity and interictal spiking was maximal.

FIG. 3. Suppression of spontaneous epileptic spiking activity after mannitol administration. Electrophysiological activity was recorded from EEG electrodes placed on the cortical surface (parahippocampal gyrus) before and after administration of a 50-g intravenous injection of mannitol (data from an individual patient is shown in the top 2 traces, top left). As in Fig. 1, events that differed from the mean activity by >3 SD, indicated by the horizontal lines, were counted as spikes. Mannitol suppressed the frequency of spontaneous activity within 30 min after administration in all patients. Prior to mannitol treatment, all spikes typically had a sharp biphasic waveform (1st top right trace). Several minutes prior to mannitol suppression of the spontaneous activity, many spikes appeared to become broader with diminished peak-to-peak amplitude (2nd top right trace), similar to what was observed during the furosemide study shown in Fig. 1. The starred arrows on the leftmost side of the traces mark spikes that were plotted at a faster time course (top right traces). A graph of the average number of spikes occurring per minute, averaged over the 4 patients, is shown bottom right, where a smooth curve was fitted to the data as in Fig. 1. The black bar indicates the time period over which the mannitol bolus was administered. Error bars show 90% confidence intervals and were calculated as explained in the Fig. 1 legend.

50-g intravenous injection of mannitol. First, the effects of mannitol on spontaneous interictal activity were studied (Fig. 3; 3 MTE, 1 NE). As in the previous set of furosemide experiments, data were analyzed over two time intervals: the 20-min duration prior to mannitol treatment, and the final 20 min of the recording session after mannitol treatment. After mannitol administration, the average spontaneous interictal spiking frequencies of the four patients was reduced by ~60%. Prior to administration of mannitol, the mean interspike interval was 3.5 ± 0.12 s (99% CI = [3.2 s, 4.0 s]). After mannitol treatment, the mean interspike interval was increased to 8.3 ± 0.36 s (99% CI = [6.3 s, 9.2 s]). A t-test rejected the null hypothesis that the mean interspike intervals before and after mannitol administration were the same (P < 0.0001). Immediately prior to the mannitol suppression of interictal activity, the morphology of a proportion of the spikes was changed in a manner similar to what was observed with furosemide (Fig. 3; top right traces). In a following set of studies, the effects of mannitol on the duration of electrical stimulation-evoked neocortical afterdischarge activity were studied on four patients (recordings not shown). The mean reduction of afterdischarge duration after mannitol treatments was 41.8 ± 8.0%. Similar to the furosemide observations, there was significant variability in onset time of suppression of epileptic activity after the mannitol injections.
Changes in EEG voltage activity after furosemide and mannitol treatments

Analyses were also performed on data acquired from all subjects in the Experimental group to examine the effects of furosemide and mannitol on spontaneous “normal” EEG activity recorded from surface electrodes at sites that recorded “normal” EEG activity (i.e., at sites that showed no interictal spiking). Two intervals of EEG recordings were analyzed for each of the experiments: the 10-min interval prior to treatment and the final 10-min interval at the end of the recording session after furosemide or mannitol administration. First, to test for general effects on the magnitude of the EEG activity, the absolute values of the EEG voltage recordings were summed for the pretreatment and posttreatment intervals over all recording sites that did not show interictal spiking. Variation in the responses were observed after furosemide and mannitol treatments with the data from one subject for each treatment showing a slight decrease and all other subjects showing a slight increase in the magnitudes of EEG activity. On average, there was an increase in the absolute values of EEG activity following both furosemide and mannitol treatments; furosemide resulted in an increase of 2.3 ± 4.8% (mean ± SD) and mannitol in an increase of 5.8 ± 3.7%. Next, power spectra were calculated for the pre- and posttreatment data. Again, there was some variation among the individual responses of changes in the total power (summed from 0 to 50 Hz). On average over all subjects, furosemide resulted in a change of 4.1 ± 15.8% and mannitol in a change of 10.2 ± 12.1%. Examination of plots of the power spectra for each individual subject suggested that changes after treatment in the magnitude of the power were localized to a narrow range of frequencies within 3 to 10 Hz (Fig. 6).

FIG. 4. Optical imaging shows reduced cortical spread of stimulation-evoked activity after mannitol and furosemide treatments. Optical imaging was used to map the spatial extent of activated cortex during 60-Hz electrical stimulation. Shown are 2 different patients, one who was treated with mannitol (top) and another with furosemide (bottom). The gray-scale images on the left show the appearance of the cortex illuminated with 535 nm (green) light, and the location of the bipolar stimulating electrodes (marked with “S”) and the recording electrode (marked with “R”). For these studies, the cortices of patients were stimulated for 4 s with current that was 1 mA below the stimulating threshold required for eliciting afterdischarge activity. Images acquired at the end of 4 s of sub-threshold stimulation were used for comparison. The pretreatment responses of the cortices of 2 patients are shown in the middle pseudo-colored images. Using the same stimulation current, the responses of the cortices were again mapped 30 min after treatment with mannitol (A, right) and furosemide (B, right). Both mannitol and furosemide reduced the spread activation over the cortex by >50% in all subjects. However, the magnitude of the response to electrical stimulation close to the stimulating electrodes was not reduced. Orange dotted lines on the left gray-scale images show the maximum spread of activity over the cortex before treatments; blue dotted lines show the maximum spread after treatments. Images were pseudocolored to enhance the visibility of small changes; maximum changes (8%) were set to white, and the minimum changes (0%) black.
Analysis of spontaneous epileptic spike activity in control subjects

To address the possibility that the observed effects of furosemide and mannitol treatments were simply a function of time while recording under the experimental conditions, data from patients who received no treatments over similar recording durations were examined. These patients were involved in other studies where spontaneous interictal spiking was recorded intraoperatively for 50 minutes where no pharmacological treatments other than the standard medications were administered. The interspike intervals were tabulated for 11 patients for whom neither furosemide or mannitol treatments were administered. There was never an observed instance of any significant decrease in spiking frequency during the recording session; rather, on average, spiking frequency appeared to gradually increase over time (Fig. 6).

To analyze whether the apparent increase in spiking frequency over time was statistically significant, the data for all 11 patients were pooled over two time intervals: the initial 20 minutes of the recording session and the final 20 minutes of the recording session. The mean of the spiking frequency during the first 20 minutes of the recording session was 10.1 ± 0.45 (SE) spikes/min (95% CI = [9.2, 10.9]) and for the last 20 minutes was 11.6 ± 0.42 spikes/min (95% CI = [10.8, 12.4]). A t-test rejected the null hypothesis that there was no difference between the pre- and posttreatment means (P = 0.0064). These results suggest there was a significant increase in spiking frequency of ~15% over the course of the recording sessions in the control group of subjects.

DISCUSSION

The most important and surprising finding of this study is that furosemide and mannitol suppress epileptic activity in the human brain. These studies were performed on patients who were suffering from medically intractable epilepsy, and who were still taking their antiepileptic medications at the time the
experiments were performed (i.e., the epileptic activity being recorded from patients during these experiments was resistant to standard AEDs). Hence furosemide and mannitol suppressed epileptic activity, which could not be suppressed by standard medications. Further, these compounds suppressed spontaneous spiking and stimulation-evoked discharges in every patient tested, regardless of whether they were suffering from neocortical or mesial temporal epilepsy, suggesting that these drugs affect general mechanisms, necessary for the maintenance of epileptiform activity, common to all patients. The magnitude of normal EEG activity recorded by electrodes at nonepileptic sites was not diminished in amplitude by these treatments, suggesting that the suppression of epileptic activity was not mediated through a global suppression of neuronal activity. This observation parallels the results reported in in vitro studies showing that furosemide and mannitol suppress epileptiform activity without suppression of action potential generation or excitatory synaptic transmission. (Dudek et al. 1990; Hochman and Schwartzkroin 2000; Hochman et al. 1995, 1999; Traynelis and Dingledine 1989).

Changes in interictal spike frequency in untreated subjects

To address the possibility that the observed decrease in interictal spiking after furosemide and mannitol treatments was simply a function of recording time, data from 11 subjects who received no treatments were analyzed. It was found that over the course of continuous EEG recording for 50 min, rather than there being a decrease in spike frequency, there was a statistically significant increase in spike frequency of ~15%. This result suggests that the neuronal excitability increases over time under standard recording conditions in the operating room. A possible explanation for this observation is that the administration of propofol, one of anesthetic components, was stopped 10 min prior to the EEG recording session and was not re-administered until after the recording session was completed. This is a standard clinical practice during intraoperative EEG mapping procedures, with the purpose of allowing the suppressed EEG activity to increase to more active levels. Although propofol is believed to be fast-acting, it may be that there is a prolonged “wearing-off” phase that becomes apparent with quantitative analysis of the EEG activity. Importantly, this observed increase in interictal spiking needs to be considered when interpreting the data acquired in our studies. Specifically, if the increase in interictal activity observed in the control subjects is reflective of a general increase in neuronal excitability over time, it may be that this increase in excitability is the cause of the increases in the amplitudes and power spectra of the EEG recordings. This would suggest that furosemide and mannitol specifically suppress abnormal epileptiform activity without suppressing normal EEG activity.

Whatever the explanation for the observed increase in interictal spiking in the control group of subjects, this result strongly supports our interpretation that furosemide and mannitol suppress interictal activity. The experimental conditions favored an increase in interictal spike frequency over time, yet furosemide and mannitol both significantly suppressed interictal spiking on average by ~60% and completely abolished all interictal activity in several patients by the end of the recording session.

Suppression of epileptic EEG activity after furosemide and mannitol treatment

Furosemide and mannitol are both known to modulate the size of the extracellular space but through different mechanisms (Geck et al. 1980; Kimelberg and Frangakis 1985; Paczynski 1997; Walz and Hertz 1984). Further, neither compound is thought to suppress action potential generation or excitatory synaptic transmission (Dudek et al. 1980; Hochman and Schwartzkroin 2000; Hochman et al. 1995; Traynelis and Dingledine 1989). Because both compounds similarly sup-
pressed epileptic activity in this study, our results are consistent with the notion that they share a common mechanism of action involving the modulation of size and water content of the ECS. A common mechanism of action is also consistent with the observation that both furosemide and mannitol similarly changed the morphology of the spontaneous spikes immediately prior to the suppression of interictal activity. These changes in spike morphology are similar to what was observed in previous studies on hippocampal slices; chloride cotransport antagonism induced a broadening and diminution in the amplitude of population spikes recorded by CA1 and CA3 field electrodes immediately prior to the blockade of epileptiform activity. Simultaneous intracellular recordings from pairs of pyramidal cells showed that these changes in the population spike morphology were correlated to the desynchronization of action potential firing times (Hochman and Schwartzkroin 2000). Taken together, these observations suggest that furosemide and mannitol may be mediating their antiepileptic effects in the human brain by desynchronizing action potential firing times independent of effects on neuronal excitability.

Furosemide and mannitol completely abolished or significantly reduced the cortical electrographic seizure activity elicited by electrical stimulation in all patients tested. However, by increasing the stimulation currents, afterdischarge episodes could be elicited with durations and amplitudes at least as large as those that could be elicited prior to treatment. This result demonstrates that furosemide and mannitol increase the minimal current required to elicit ictal discharges, suggesting that perhaps these treatments decrease the ability of cortical tissue to maintain and propagate seizure activity.

Do changes in tissue resistance underlie the antiepileptic effects of furosemide and mannitol?

Because furosemide is believed to block activity-evoked cell volume changes through antagonism of chloride cotransport, and mannitol is thought to cause a reduction in cell volume through removal of water from intracellular compartments, both treatments might be expected to cause a global change in the electrical resistance of brain tissue. However, several results reported here indicate that a more complicated explanation may be required for the antiepileptic actions of furosemide and mannitol. First, our estimates of percent-changes in the electrical resistance of tissue after furosemide and mannitol administration (Fig. 5) show that the time courses of changes in the tissue resistance are not correlated to the suppression of interictal spike frequency or the duration of afterdischarge activity. Furosemide and mannitol induced their maximal changes in tissue resistance within 5 min after the one-time intravenous injection, yet this was prior to any significant reduction in interictal spike frequency and afterdischarge suppression. Further, changes in tissue resistance recovered to baseline levels by the end of the recording session when interictal spiking and afterdischarge durations were maximally suppressed. Second, because power ($P$) is related to resistance ($R$) and current ($I$) through Ohm’s law as $P = R*I^2$, a global change in tissue resistance would be expected to result in a constant change in the power spectrum at all frequencies, and this was not observed to be the case.

A possible explanation for the lack of correlation between the observed changes in tissue resistance and the suppression of epileptic activity is that perhaps furosemide and mannitol do not mediate their antiepileptic effects simply by altering the baseline electrical resistance of brain tissue but rather through their effects on activity-evoked volume changes. In vitro data are supportive of this notion in the case of furosemide, where quantitative measurements showed the exposure of slices to furosemide did not result in changes in the baseline volume fraction of the ECS (Sykova et al. 2003). Additionally, the size of the antidromic spike in the CA3 region of hippocampal slices was not altered by prolonged exposure to cation-chloride cotransporter antagonists, suggesting that electrical resistance of the tissue was not altered by these treatments (Hochman and Schwartzkroin 2003). However, the in vitro mannitol data are more difficult to interpret with regard to this notion; because mannitol is an osmolyte, as long as it is present in the perfusion medium it will result in a change in the electrical resistance of the tissue that will necessarily be concomitant with any effects...
on the epileptogenicity of the tissue. It is known that furosemide blocks activity-evoked volume change in brain tissue (Holthoff and Witte 1996; MacVicar and Hochman 1991; Sykova et al. 2003), and mannitol reduces activity-evoked changes in the electrical resistivity of hippocampal slices (Fox et al. 2004).

Interpretation of the optical-imaging data

Our interpretation of the optical-imaging data relies on the following assumption: changes in the in vivo intrinsic optical signal (IOS) are positively correlated to changes in neuronal activity. Even though a complete understanding of the mechanisms underlying the generation of IOS is lacking, this correlation is generally consistent throughout the literature (Grinvald et al. 1988; Haglund et al. 1992; Seth et al. 2003; Ts’o et al. 1990). There are at least three components that contribute to the changes of IOS: changes in blood volume, changes in blood oxygenation, and blood-independent changes involving ion fluxes and cell volume changes (Grinvald et al. 1988; MacVicar and Hochman 1991). It is believed that the intrinsic signal in vivo is dominated by hemodynamic components (Grinvald et al. 1988). In our study, we used 535-nm light for illumination, which has been shown to be highly selective for blood volume changes in vivo (Haglund and Hochman 2004) and minimizes the blood-independent component of the IOS (MacVicar and Hochman 1991). Because blood volume changes are thought to be tightly correlated to neuronal activity (Ngai et al. 1988), we believe the IOS recorded in our studies does indeed represent changes in neuronal activity. Given this interpretation, our data suggest that mannitol and furosemide treatment result in a decrease in the spatial extent of cortex activated by 60-Hz electrical stimulation of the cortical surface. Presumably, this type of stimulation elicits a synchronous discharging of a population of neurons. Hence, the optical-imaging data suggest that furosemide and mannitol reduce the spatial extent through which a synchronous drive can activate neurons in the neocortex. Because the IOS showed no diminution in its amplitude in the area between the two poles of the bipolar stimulating electrode, it is suggested that furosemide and mannitol treatment did not result in a reduction in action potential firing evoked by direct electrical stimulation. These interpretations would be consistent with in vitro studies on hippocampal slices showing that chloride cotransport antagonism desynchronized the timing of synaptically evoked action potentials, but did not affect the ability of neurons to generate action potentials (Hochman and Schwartzkroin 2000).

Clinical implications

Because large quantities (50-g boluses) of mannitol were used in these studies to suppress epileptiform activity, this compound would not be a practical chronic antiepileptic treatment. However, relative to laboratory studies that required >40 mg/kg of furosemide to block kainic acid-induced epileptiform activity in rats (Hochman et al. 1995), surprisingly small doses of furosemide (0.18–0.45 mg/kg, depending on the weight of the patient) were highly effective in suppressing epileptic activity in human patients. Because doses of furosemide that are 50- to 200-fold greater than what was used in this study have been given chronically to patients with man-
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