J Neurosurg 101:303–309, 2004

Blood–brain barrier opened by stimulation of the parasympathetic sphenopalatine ganglion: a new method for macromolecule delivery to the brain

David Yarnitsky, M.D., Yossi Gross, M.Sc., Adi Lorian, D.M.D., Alon Shalev, M.Sc., Itschak Lamensdorf, Ph.D., Rinat Bornstein, D.Vet., Shy Shorer, M.D., Avraham Mayevsky, Ph.D., Kaushik P. Patel, Ph.D., N. Joan Abbott, Ph.D., and William G. Mayhan, Ph.D.

Department of Neurology, Rambam Medical Center, Technion Faculty of Medicine, Haifa; BrainsGate Ltd., Raanana; Department of Maxillofacial Surgery, Poriyah Hospital, Poriyah; and Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel; Department of Physiology, University of Nebraska Medical Center, Omaha, Nebraska; and Centre for Neuroscience Research, King's College, London, United Kingdom

Object. Drug delivery across the blood–brain barrier remains a significant challenge. Based on earlier findings, the authors hypothesized that parasympathetic innervation of the brain vasculature could be used to augment drug delivery to the brain.

Methods. Using a craniotomy–cerebrospinal fluid superfusate paradigm in rats with an intravenous injection of tracer the authors demonstrated that stimulation of the postganglionic parasympathetic fibers of the sphenopalatine ganglion (SPG) increased the concentration of fluorescein isothiocyanate–dextran (4–250 kD) in the superfusate by two- to sixfold. A histological examination indicated the presence of dextran in the parenchyma. In another experiment the amount of Evans blue dye in the brain following SPG activation was similarly significantly elevated. The chemotherapeutic agents anti-HER2 monoclonal antibody and etoposide were also delivered to the brain and reached therapeutic concentrations. Brain homeostasis was not disturbed by this procedure; a measurement of nicotinamide adenine dinucleotide reduction did not show a decrease in the tissue metabolic state and brain water content did not increase significantly.

Conclusions. Sphenopalatine ganglion activation demonstrates a promising potential for clinical use in the delivery of small and large molecules to the brain.

Key Words • blood–brain barrier • parasympathetic nervous system • sphenopalatine ganglion • electrical stimulation • rat

The BBB is one of the major defense mechanisms of the brain; it regulates blood–brain molecular traffic and maintains the brain's delicate ionic and metabolic environment. Unless recognized by a specific transport system, hydrophilic molecules and lipophilic molecules larger than approximately 500 D display extremely limited penetration across the BBB. Hence the BBB has long been a major obstacle in the delivery of drugs to the brain, allowing its penetration only to small, weakly plasma-bound lipophilic molecules, and restricted entry to larger and more polar compounds and those strongly bound to plasma proteins.[2]

Many attempts had been made to enable specialized medications to penetrate the BBB by changing either the barrier or the drug or by circumventing the barrier by direct administration into brain tissue. An example of the first method is the intracarotid injection of a hyperosmolar solution, causing shrinkage of brain endothelial cells and allowing a transient increase in the permeability of the BBB.[5,15] An example of the second method is the conjugation of amino acid or other moieties to the desired molecule so that it can use a BBB transport system to cross the barrier.[14] An example of direct administration is a chemotherapy-laden gel directly placed inside a tumor resection site.[1]

In this paper we report the results of a new approach for BBB opening based on electrical stimulation of the postganglionic parasympathetic fibers of the SPG. This ganglion, classically known as the source of parasympathetic fibers to nasal and eye mucosa and the lacrimal gland, also supplies parasympathetic innervation to the brain vascular system, covering all the anterior cerebral circulation and some of the posterior circulation.[7,16,17] Electrical stimulation of parasympathetic fibers of the SPG has been shown to induce vasodilation in cerebral vessels in rats,[17] cats,[6] dogs,[19] and monkeys.[20] This effect may involve the secretion of NO and/or vasoactive intestinal peptide.[20] It has been shown,

Abbreviations used in this paper: BBB = blood–brain barrier; CSF = cerebrospinal fluid; FITC = fluorescein isothiocyanate; HPLC = high-performance liquid chromatography; LDF = laser Doppler flowmetry; mAb = monoclonal antibody; NAD = nicotinamide adenine dinucleotide; NADH = reduced form of NAD; NO = nitric oxide; SD = standard deviation; SPG = sphenopalatine ganglion.

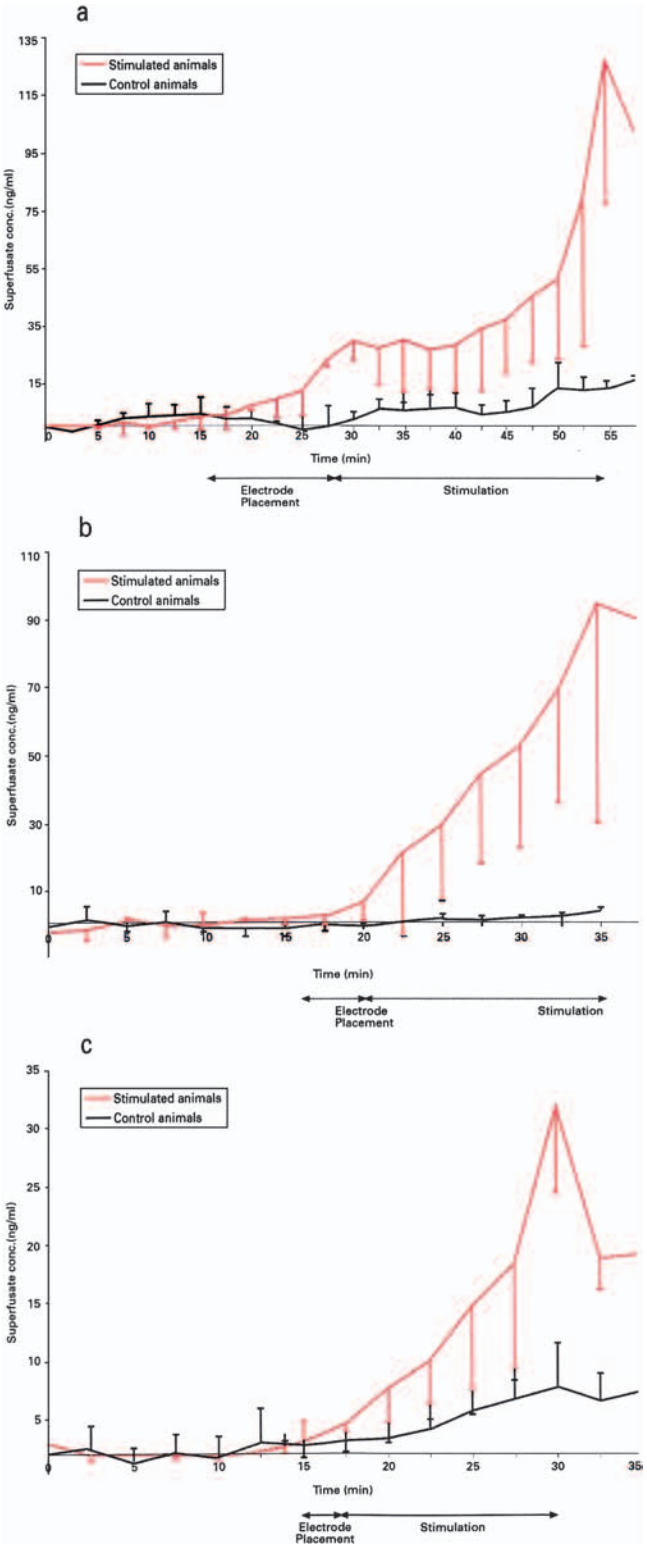

FIG. 1. Craniotomy–CSF superfusate experiments. Graphs showing dextran concentrations (conc.) in the superfusate of stimulated and control animals for different molecular weights: 4 kD (a), 70 kD (b), and 250 kD (c). Individual animal curves were horizontally shifted to align peaks. Scales for the concentration axis differ.

independently, that an increase in NO concentration in cerebral vessels due to administration of NO donors[10] or histamine[11] increases the permeability of the BBB. It was, therefore, our hypothesis that stimulation of the SPG would cause an increase in BBB permeability, in addition to vasodilation, and thus enable delivery of molecules to the brain.

Materials and Methods

All procedures were performed after approval of our protocol had been obtained from the local ethics boards.

Stimulation of the SPG

A uniform method of stimulation was used in all experiments reported here. In brief, a combined mucoperiosteal incision of the superior and inferior eyelid was performed, a flap was raised, and the medial canthus of the orbit was detached. The periosteum of the orbit together with its contents was gently retracted posteriorly and laterally, and the anterior ethmoidal nerve was exposed. The periosteum of the ethmoidal foramen that surrounds the ethmoidal nerve was carefully separated from the bone, while keeping the nerve within its periosteal envelope. Custom-made bipolar nickel-coated hook electrodes, the poles of which were 1 mm apart and the 2-mm tips exposed, were hooked onto the postganglionic parasympathetic fibers immediately lateral to the orbital opening of the ethmoidal foramen. The cathode was attached to the nasal (medial) side. During stimulation, care was taken to maintain steady positioning of the electrode to prevent tearing of the nerve and maintain electrode–nerve contact. Control animals underwent sham operations without hooking the electrodes.

Craniotomy–CSF Superfusate Experiments

Surgical Procedure. Twenty-two Wistar–Furth rats each weighing approximately 300 g were used. The rats were given an anesthetic agent (Inactin; thiobutabarbital 100 mg/kg administered intraperitoneally), a tracheotomy, and mechanical ventilation with room air and supplemental oxygen. A catheter was placed in the left femoral artery for the measurement of systemic blood pressure and for blood sampling. Another catheter was placed in the left femoral vein for tracer administration. The left ethmoidal foramen was exposed and a craniotomy was performed as previously described.[10,11] In brief, the craniotomy was performed, the dura mater incised, and the cerebral microcirculation exposed. Inlet and outlet ports were placed in the craniotomy well to allow a constant flow of superfusate over the cerebral (pial) microcirculation and cortical surface. The superfusion fluid (artificial CSF) was heated to 37°C and bubbled continuously with 95% nitrogen and 5% carbon dioxide to maintain gases within normal limits. Blood pressure and gases were continuously monitored and maintained within normal limits.

Experimental Procedure. The electrodes were hooked onto the ethmoidal nerve fibers; thereafter, FITC-labeled dextran molecules (4–250 kD) were intravenously infused and stimulation of the SPG was begun at 10 Hz and 5 V for approximately 2 minutes. Dextrans were given in a concentration of 40 mg/ml and infused at a constant rate of 0.06 ml/minute. A superfusate sample was collected every 2.5 minutes and tracer concentrations in successive tubes were detected using quantitative fluorescence spectroscopy. The animals were killed at the end of this procedure with an injection of saturated potassium chloride. In several cases the cephalic vasculature was perfused using heparinized 0.9% saline solution (20 U heparin/ml) for 5 minutes followed by an additional perfusion with 250 ml of 4% paraformaldehyde. The brain was than removed and sectioned for fluorescence microscopy imaging.

Permeability of the BBB. The permeability of the BBB was evaluated by calculating the clearance of FITC–dextran by the area of parietal cortex that had been exposed by the craniotomy, as described previously.[12] The ratio between superfusate and blood concentration was calculated for each animal and averaged for each experimental setup.

TABLE 1

*Craniotomy–CSF superfusate experiments: peak concentrations and ratios of FITC–dextran in the superfusate and blood of experimental and control animals**

Experiment	Stimulation Group			Control Group			Overall Stimulation/Control Ratio
	Superfusate	Blood	Superfusate/Blood Ratio	Superfusate	Blood	Superfusate/Blood Ratio	
4-kD dextran (ng/ml) (3 stimulated animals, 3 control)	126.5 ± 49.7	1.60 ± 0.3	87.20 ± 35.2	17.9 ± 9.8 (p = 0.044)†	2.55 ± 1.0	20.70 ± 2.4 (p = 0.02)‡	4.2
70-kD dextran (ng/ml) (5 stimulated animals, 4 control)	93.9 ± 64.7	14.75 ± 1.1	6.56 ± 5	7.9 ± 5.5 (p = 0.013)†	7.40 ± 1.7	1.29 ± 1.1 (p = 0.04)‡	5.1
250-kD dextran (ng/ml) (3 stimulated animals, 4 control)	29.8 ± 7.3	7.36 ± 0.8	2.37 ± 1.3	5.7 ± 3.6 (p = 0.001)†	5.44 ± 0.7	1.06 ± 0.5 (p = 0.05)‡	2.2

* Values are expressed as means ± SD unless otherwise indicated.
† Probability value for the comparison between peak superfusate concentrations in the stimulation and control groups.
‡ Probability value for the comparison between stimulation and control ratios.

Closed Cranium Experiments

Evans Blue Dye. Six Wistar rats each weighing approximately 300 g received stimulation and six served as sham-operated controls. The rats were anesthetized using pentobarbital (60 mg/kg) and the right SPG was exposed and stimulated as described earlier in this paper. Stimulation was administered for 1 hour at 3.5 V by using cycles of 90 seconds on/60 seconds off. At the beginning of the experiment the rats received intravenous doses of 1.5 ml 2% Evans blue dye, which binds to plasma albumin and acts as a tracer for albumin extravasation. At the end of the stimulation, the animals were perfused with cold 0.9% sodium chloride and killed with a lethal dose of pentobarbital sodium. Following perfusion with normal saline, their brains were harvested and weighed. Tissue was homogenized in twice the volume of N,N-dimethylformamide (Bio-Lab Laboratories, Ltd., Jerusalem, Israel) and centrifuged for 10 minutes at 13,000 rpm. The amount of Evans blue–stained tissue in both hemispheres was determined spectrophotometrically (630 nm).

Infusion of FITC–Anti-HER2 mAb. Six experimental and five control Wistar–Furth rats were used in this experiment. The FITC–anti-HER2 mAb was infused. The procedure was similar to the one used to administer the FITC–dextrans. The animals' brains were removed to prepare histological fluorescence slides and for homogenization and fluorescence measurements.

Etoposide. This test group included six experimental rats and one control animal (another one died during the experiment). Ten milligrams of etoposide (Sigma Chemical Co., St. Louis, MO) was dissolved in 1 ml dimethyl sulfoxide and added to 9 ml saline. The total 10 ml of solution was infused intravenously into the animals over a 20-minute period at a constant rate of 500 μl/minute. At the end of the etoposide administration, the electrodes were hooked and the ethmoidal nerve was stimulated. Ten minutes following stimulation the animals were perfused via the left ventricle and their brains were removed. The brains were sectioned, homogenized, and analyzed for etoposide using HPLC. The cortex, cerebellum, and brainstem were weighed and homogenized in ice-cold 70% mobile phase buffer plus 30% methanol. The homogenates were centrifuged at 3000 rpm for 20 minutes at 4°C. The supernatant was transferred into a fresh microcentrifuge tube and subjected to further centrifugation at 12,000 rpm for 10 minutes at 4°C. Fifty milliliters of the supernatant was injected into an HPLC system (BAS-PM-80 delivery system with phase II-C18 column; Bioanalytical Systems, Inc., Lafayette, IN). The mobile phase consists of 76.6% 25 mM citric acid and 50 mM sodium phosphate (pH 5), and 23.4% acetonitrile. The flow rate was 1 ml/minute. Etoposide in the samples was measured using an LC-40 electrochemical detector system (Bioanalytical Systems, Inc.). The electrochemical detection signal was recorded by the PowerChrom system (ADInstruments, Colorado Springs, CO). The etoposide standard was prepared in methanol. Various concentrations of etoposide standard ranging from 0.1 to 10 mM were used to prepare a standard curve. The standard curve was made and the concentrations of etoposide in the samples were calculated using the PowerChrom system.

Assessment of Brain Physiology During SPG Stimulation

Experiments were performed on Wistar rats in which anesthesia had been induced with an intravenous injection of 60 mg of pentobarbital. Stimuli were given at 10 Hz and 2.5 V, at cycles of 90 seconds on and 60 seconds off, for a total of 1 hour.

Mitochondrial Function. Six animals received stimulation and six served as controls. A small frontal craniotomy was performed in all animals and a monitor probe was attached and glued to the brain surface epidurally. For monitoring the NADH redox state at the brain surface, a 366-nm excitation light was passed from a fluorometer to the brain via a bundle of quartz optical fibers. The emitted light (450 nm), together with the reflected light at the excitation wavelength, was transferred to the fluorometer via another bundle of fibers. Changes in reflected light are inversely correlated to changes in tissue blood volume and also serve to correct for hemodynamic artifacts appearing in the NADH measurement. A detailed description of this method has been published.[8,9] On-line measurement of CBF from the same cortical area as the NADH measurement was done using the LDF technique. The probe attached to the brain combined optical fibers for measuring both NADH fluorescence and LDF values.

Brain Water. Brain water content was measured in several setups: 1) immediately after a 1-hour stimulation; 2) 1 hour after the end of the 1-hour stimulation; 3) 24 hours after the 1-hour stimulation; and 4) immediately after a 3-hour stimulation. After the animals had been killed, their brains were dissected into four regions: right and left hemispheres, cerebellum, and brainstem. Thereafter, brain tissue was dried in a desiccating oven at 105°C for 24 hours.

Statistical Analysis

We used t-tests for most comparisons. Data provided by the Evans blue dye experiment were analyzed using the Tukey–Kramer test. All data are presented as means ± SDs.

Results

Permeability of the BBB Measured in the CSF Superfusate

The craniotomy–CSF superfusate paradigm is a well-known technology for the determination of BBB permeability.[12] Activation of the SPG significantly increased the concentration of the dextran for all molecular weights in the superfusate, indicating an increase in BBB permeability (Fig. 1 and Table 1). The increase in permeability appeared

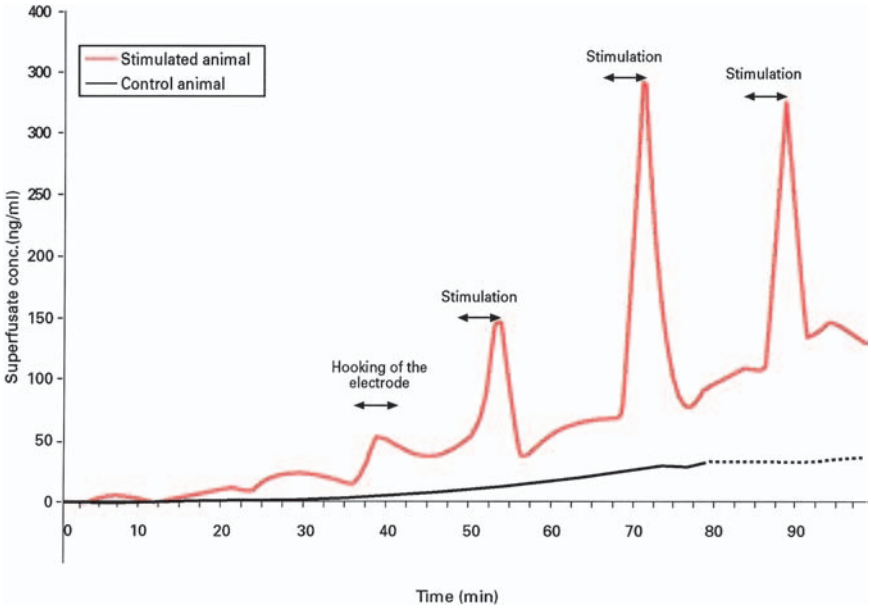

FIG. 2. Craniotomy–CSF superfusate experiment. A graph showing representative single experimental and single control values when 10 kD was administered. The FITC–dextran reversibility of the effect is clearly seen, with a rapid decrease in the superfusate concentration on cessation of stimulation. As control experiments were slightly shorter, the terminal period has been extrapolated *(dashed line)*.

to be dependent on molecular weight, with the larger 250-kD dextran penetrating less than the smaller molecules.

The dextran concentration in the superfusate quickly decreased on cessation of stimulation (Fig. 2), an indication of the reversibility of the SPG stimulation effect on BBB permeability. The continuous increase, compared with the basal (nonstimulated intervals) superfusate dextran concentration, indicated a cumulative effect of repeated SPG stimulations. Presence of FITC–dextran in brain tissue following stimulation is demonstrated in Fig. 3a.

Closed Cranium Tissue Homogenate Experiments

In line with the FITC–dextran superfusate experiments, the amount of Evans blue–stained brain tissue also increased after SPG stimulation to a similar extent (Fig. 4). Interestingly, a clear difference was seen between the stimulated hemisphere and the contralateral one. This is in agreement with previous histochemical reports on the mostly unilateral distribution of postganglionic parasympathetic fibers emanating from the SPG.

For evaluation of the therapeutic potential of SPG stimulation, two chemotherapeutic agents that normally do not cross the BBB, anti-HER2 mAb (an immune-based molecule primarily used against breast carcinoma) and etoposide, were administered. Stimulation of the SPG markedly increased the brain content of these agents, reaching a tissue therapeutic range (Table 2 and Fig. 3b).

Brain Physiology During SPG Stimulation

Mitochondrial Function. In stimulated animals NADH fluorescence decreased by 10.16 ± 19.42% after 20 minutes and by 29.04 ± 49.48% after 40 minutes of stimulation. Fifteen minutes after the cessation of stimulation the decrease was 43 ± 65.94% compared with baseline; these changes were not significant. For the control animals, the measurement revealed minute changes with decreases of 0.58 ± 3.59% at 20 minutes and 2.67 ± 4.96% at 40 minutes; the poststimulation measurement showed an increase of 0.93 ± 9.75% (not significant). Thus, the NADH redox state did not show a significant change subsequent to stimulation. If anything, there was a tendency toward improved oxygenation of brain tissue, as indicated by a decrease in NADH level typical of vasodilation, as evidenced by the increase in LDF values.

Brain Water Content. No significant change in brain water content was measured following SPG stimulation in any of the experimental paradigms (data not shown). A slight nonsignificant increase in brain water content was found after 3 hours of stimulation, from 78.54 to 79.63%.

Discussion

Stimulation of the SPG increased the permeability of the BBB, markedly enhancing entry of macromolecules into the brain, as demonstrated for FITC–dextran, Evans blue dye–albumin, and the chemotherapy agents anti-HER2 mAb and etoposide. Furthermore, our findings indicate that this effect is reversible, as demonstrated by the rapid decline of FITC–dextran concentrations in the superfusate at the end of the stimulation. We interpret our findings as a major advance in overcoming the obstacle of the BBB in the delivery of therapeutic agents to the brain tissue, with a substantial promise for rapid application in many currently lethal and disabling central nervous system disorders.

The SPG belongs to the parasympathetic subdivision of the autonomic nervous system. Its main ramification is traditionally known to be the mucosal membranes of the nose, the lacrimal gland, and facial skin vessels. Its effects are dilatory and secretory, causing nasal secretions, lacrimation,

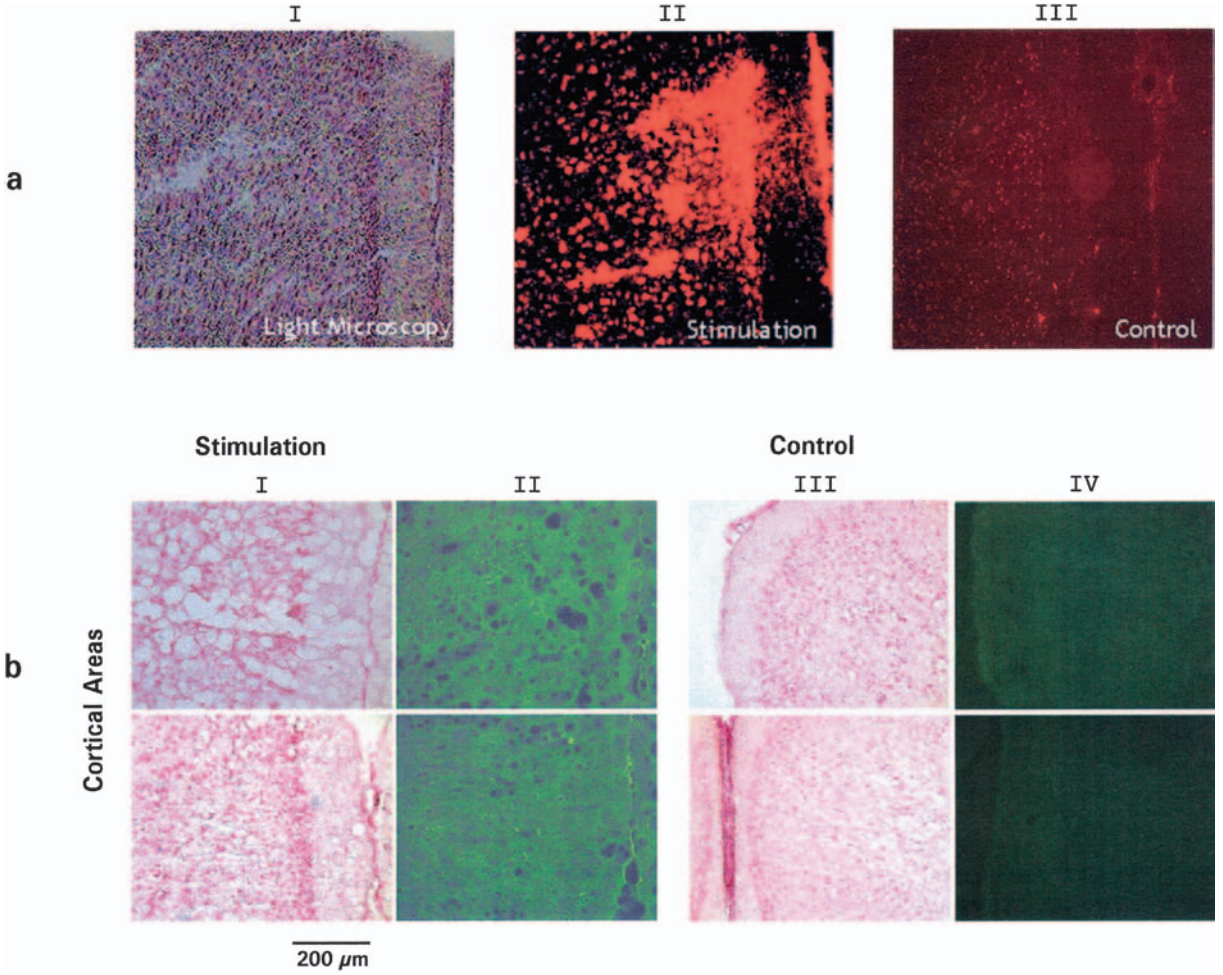

FIG. 3. Fluorescence microscopy images and photomicrographs of brain tissue. a: Frontal cortex as it appears with light microscopy (I) and with fluorescence of the same region due to FITC–dextran (10 kD) with (II), and without (III) stimulation. b: Cortical areas in animals treated with anti-HER2 mAb. Tissue shown with light microscopy (I and III) and corresponding area viewed with fluorescence microscopy (II and IV) for stimulated and nonstimulated animals, respectively. The lack of fluorescence in tissue from control animals is in clear contrast with the prominent fluorescence in tissue from animals that received stimulation. Original magnifications × 100. H & E used for light microscopy (aI, bI and III).

and flushing of the face. More recently, its relevance for intracranial vessels has been recognized. Suzuki and colleagues[17] and later Hara, et al.,[7] mapped the projections of parasympathetic fibers from the ganglion to most of the cerebrovascular bed. Later, a vasodilatory effect of SPG stimulation on these vessels was shown.[19,20] Based on these lines of evidence, we hypothesized that electrical stimulation of the SPG would induce both vasodilation and increased BBB permeability of cerebral vessels innervated by its fibers.

In a study of the dura mater in rats, Delepine and Aubineau[4] showed an increase of 200% in plasma protein extravasation in response to electrical stimulation of the SPG. This effect was abolished by an infusion of atropine and mimicked by an infusion of carbachol. This indicates that cholinergic transmission is central to the effect, most likely at the ganglionic level. Furthermore, to rule out a possible antidromic firing from nociceptors as a mediator neurogenic inflammation, some of the rats were pretreated with capsaicin, which diminished, but did not block the effect. Our histological investigations and measurements of brain homogenate concentration clearly demonstrated that the effect we describe is not limited to changes in meningeal vessels.

A major question concerning this effect regards whether it is an opening of tight junctions or a transcellular vesicular transport. The finding of greater penetration for molecules of lower molecular weight is an indication that the tight junction is the site of action because transcytotic vesicle penetration would tend to result in similar concentrations in the superfusate, independent of molecular weight.

Nitric oxide is known to be the neurotransmitter of autonomic nonadrenergic noncholinergic functions and is a potent vasodilator and inducer of plasma protein extravasation in many body systems. Recently, Mayhan[10] showed that administration of NO donors to brain vessels causes an increase in the BBB permeability of these vessels (see also the review by Thiel and Audus[18]).

The dextrans used in this study are large hydrophilic molecules. The wide range of molecular weights implies wide

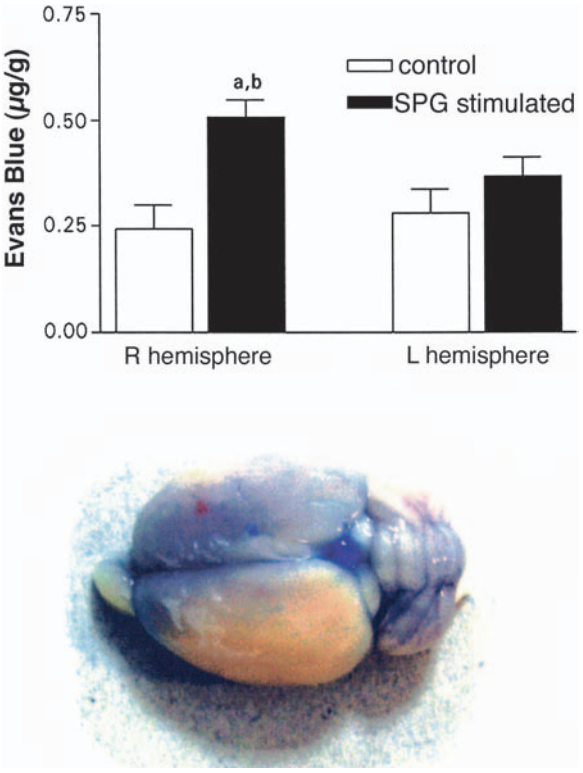

FIG. 4. Closed cranium experiments: Evans blue dye in the brain after stimulation of the right SPG. *Upper:* Bar graph of tissue homogenate concentrations showing significant differences between stimulated and control right hemispheres (a, $p < 0.01$) and stimulated and control left hemispheres (b, $p < 0.05$). *Lower:* Macroscopic view of the brain showing a clear blue stain in the stimulated hemisphere and the cerebellum and no dye in the other hemisphere.

potential applications for molecules of different sizes, including immunoglobulins, genes, chemokines, antibodies, and even viral vectors. As many larger lipophilic molecules have restricted passage across the BBB as a result of efflux by P-glycoprotein in the luminal membrane of the brain endothelium, opening of tight junctions could allow such molecules to bypass the P-glycoprotein to some extent and reach higher concentrations in the brain. A very useful clinical application of this method can therefore be foreseen for both hydrophilic and lipophilic agents.

The method of measurement we used for the craniotomy–superfusate experiments was based on the model developed by Mayhan and Heistad,[12] in which the brain surface is constantly superfused with artificial CSF. Previous studies in which this model has been used have shown that dextran extravasation during perturbation of the brain (acute hypertension, infusion of hyperosmolar solutions, and application of inflammatory mediators) reflects a disruption of the BBB, primarily in cerebral venules and veins.[12] Care was taken, therefore, to maintain a constant blood pressure during our experiments. Although the craniotomy–superfusate model has the advantage of reflecting the time course of the stimulation effect, it could be criticized because it involves a surgical procedure to expose of the brain immediately before the experiment. This could cause some local tissue damage and artificially raise extravasation, which would explain the gradual increase in the baseline concentration of the dextrans in the superfusate (Fig. 2). The brain homogenate and histological experiments and tissue staining, however, are free of this potential difficulty and clearly show the BBB disruption effect. Fluorescence microscopy imaging (Fig. 3) demonstrated florescence not only in the capillaries and pia mater, but also in the brain parenchyma itself. This is evidence of the penetration of FITC–dextran across the parenchymal BBB.

A major concern for any disruption of the BBB is the possible loss of the protective function of this barrier, its presumed raison d'être. This could lead to penetration of unwanted molecules; changes in the ionic, metabolic, or osmotic balance; or invasion of immunogenic or infectious elements. These possible effects have not yet been systematically studied. Our understanding of the potential clinical use of this method is that repeated short activations will be made at the height of blood concentration of the relevant medication. This could be designed as cycles lasting several minutes up to a few hours, occurring once every several days or weeks. For such a working paradigm, one can consider migraine attacks, which represent neurogenic inflammation of cerebral vessels and probably cause a similar transient effect on the BBB;[13] despite possible opening of the BBB migraineurs do not show any sign of long-term injurious effects on brain function between attacks. Similarly, most patients who contract meningitis or encephalitis sustain some transient damage to their BBB, usually lasting several days or longer, and recover completely.[3,21] An extrapolation from these clinical examples to our model indicates no long-term damage to the brain.

As seen in Fig. 2, the present experiments demonstrate the short duration of BBB disruption; the clear decrease in dextran concentration in the superfusate immediately on cessation of stimulation indicates the reversibility of the effect. This is a major safety feature in favor of this proposed method for BBB opening. Nevertheless, we conducted two additional experiments to explore the potential injurious effects of SPG stimulation on brain physiology. We found no injurious effect on the metabolic state of the brain, as shown by the NAD/NADH balance and no significant brain edema caused by SPG stimulation. Therefore, SPG stimulation appears to be a safe procedure for future administration to humans, and a regimen of repeated short activations should not cause brain damage. The surgical procedure to expose the ethmoidal nerve in the rat was designed as an acute procedure; the size and accessibility of the human equivalent mean that implantation of an electrode could be done as a minor procedure. The greater palatine canal, which extends from the roof of the oral cavity up into the sphenopalatine fossa, can serve as a convenient route for introduction of a stimulating electrode to the ganglion.

Potential applications of this stimulation method in human disease are wide, including improvement in the delivery of the following agents: 1) drugs used in chemotherapy for primary and secondary brain tumors; 2) immune molecules tailored to attack specific targets such as the amyloid protein in cases of Alzheimer disease; 3) growth factors to stop degeneration and enhance regeneration in cases of neurodegenerative diseases such as Parkinson, Alzheimer, Huntington, amyotrophic lateral sclerosis, and others; 4) genes for modification of genetically based diseases; and 5) future molecules to be developed once it is realized that the

TABLE 2

*Brain homogenate concentrations of anti-HER2 mAb and etoposide**

Chemotherapeutic Agent	Stimulaton Group			Control Group			Stimulation/Control Ratio		
	Cortex	Brainstem	Cerebellum	Cortex	Brainstem	Cerebellum	Cortex	Brainstem	Cerebellum
anti-HER2 mAb (6 stimulated animals, 5 control)	26.0 ± 19.5	43.7 ± 27.3	8.7 ± 5.7	5.2 ± 2 ($p = 0.02$)†	2.4 ± 1.2 ($p < 0.01$)†	2.6 ± 0.7 ($p = 0.02$)†	5.0	17.9	3.4
etoposide (6 stimulated animals, 1 control)‡	309.3 ± 133.2	ND	ND	ND	ND	ND	>6.9	NA	NA

* Values are expressed as means ± SD (μg/ml for anti-HER2 and ng/ml for etoposide) unless otherwise indicated. Abbreviations: NA = not applicable; ND = not detectable.

† Probability values are for the comparison between the stimulation and control groups.

‡ The lowest detectable concentration was 45 ng/ml.

BBB can be overcome. Furthermore, molecules that are in excess in the brain, such as the amyloid protein in cases of Alzheimer disease, can be expected to clear out into the plasma if the BBB obstacle is removed.

Conclusions

Using several lines of evidence, the present study shows that the BBB can be temporarily opened by electrical stimulation of the SPG in the rat. Limited safety studies attest to the lack of substantial side effects. Potential application of this technique in humans is wide, including brain tumors, and neurodegenerative and immune-based brain diseases.

Acknowledgment

These studies were sponsored by BrainsGate, Ltd. We thank Dr. Judith Sonn for her skillful help with the NADH measurement studies.

References

1. Brem H, Piantadosi S, Burger PC, et al: Placebo-controlled trial of safety and efficacy of intraoperative controlled delivery by biodegradable polymers of chemotherapy for recurrent gliomas. The Polymer-brain Tumor Treatment Group. **Lancet 345:**1008–1012, 1995
2. Cornford EM, Hyman S: Blood-brain barrier permeability to small and large molecules. **Adv Drug Deliv Rev 36:**145–163, 1999
3. Coyle PK: Chronic and recurrent meningitis, in Gilchrist JM (ed): **Prognosis in Neurology.** Boston: Butterworth-Heinemann, 1998, pp 239–243
4. Delepine L, Aubineau P: Plasma protein extravasation induced in the rat dura mater by stimulation of the parasympathetic sphenopalatine ganglion. **Exp Neurol 147:**389–400, 1997
5. Doolittle ND, Miner ME, Hall WA, et al: Safety and efficacy of a multicenter study using intraarterial chemotherapy in conjunction with osmotic opening of the blood-brain barrier for the treatment of patients with malignant brain tumors. **Cancer 88:**637–647, 2000
6. Goadsby PJ: Sphenopalatine ganglion stimulation increases regional cerebral blood flow independent of glucose utilization in the cat. **Brain Res 506:**145–148, 1990
7. Hara H, Zhang QJ, Kuroyanagi T, et al: Parasympathetic cerebrovascular innervation: an anterograde tracing from the sphenopalatine ganglion in the rat. **Neurosurgery 32:**822–827, 1993
8. Mayevsky A: Brain NADH redox state monitored in vivo by fiber optic surface fluorometry. **Brain Res 319:**49–68, 1984
9. Mayevsky A, Chance B: Intracellular oxidation-reduction state measured in-situ by a multichannel fiber-optic surface fluorometer. **Science 217:**537–540, 1982
10. Mayhan WG: Nitric oxide donor-induced increase in permeability of the blood-brain barrier. **Brain Res 866:**101–108, 2000
11. Mayhan WG: Role of nitric oxide in histamine-induced increases in permeability of the blood-brain barrier. **Brain Res 743:**70–76, 1996
12. Mayhan WG, Heistad DD: Permeability of the blood-brain barrier to various sized molecules. **Am J Physiol 248:**H712–H718, 1985
13. Moskowitz MA: Neurogenic inflammation in the pathophysiology and treatment of migraine. **Neurology 43 (Suppl 3):**S16–S20, 1993
14. Pardridge WM: Drug and gene delivery to the brain: the vascular route. **Neuron 36:**555–558, 2002
15. Rapoport SI: Advances in osmotic opening of the blood-brain barrier to enhance CNS chemotherapy. **Expert Opin Investig Drugs 10:**1809–1818, 2001
16. Suzuki N, Hardebo JE, Kahrstrom J, et al: Selective electrical stimulation of postganglionic cerebrovascular parasympathetic nerve fibers originating from the sphenopalatine ganglion enhances cortical blood flow in the rat. **J Cereb Blood Flow Metab 10:**383–391, 1990
17. Suzuki N, Hardebo JE, Skagerberg G, et al: Central origins of preganglionic fibers to the sphenopalatine ganglion in the rat. A fluorescent retrograde tracer study with special reference to its relation to central catecholaminergic systems. **J Auton Nerv Syst 30:**101–109, 1990
18. Thiel VE, Audus KL: Nitric oxide and blood-brain barrier integrity. **Antioxid Redox Sign 3:**273–278, 2001
19. Toda N, Ayajiki K, Tanaka T, et al: Preganglionic and postganglionic neurons responsible for cerebral vasodilation mediated by nitric oxide in anesthetized dogs. **J Cereb Blood Flow Metab 20:** 700–708, 2000
20. Toda N, Tanaka T, Ayajiki K, et al: Cerebral vasodilatation induced by stimulation of the pterygopalatine ganglion and greater petrosal nerve in anesthesized monkeys. **Neuroscience 96:** 393–398, 2000
21. Tyler KL: Acute viral enecephalitis, in Gilchrist JM (ed): **Prognosis in Neurology.** Boston: Butterworth-Heinemann, 1998, pp 233–238

Manuscript received October 13, 2003.

Accepted in final form April 2, 2004.

Address reprint requests to: David Yarnitsky, M.D., Department of Neurology, Rambam Medical Center, Bat Galim, Haifa 31096, Israel. email: davidy@tx.technion.ac.il.