Penetration of the Blood–Brain Barrier: Enhancement of Drug Delivery and Imaging by Bacterial Glycopeptides

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Summary

The blood–brain barrier restricts the passage of many pharmacological agents into the brain parenchyma. Bacterial glycopeptides induce enhanced blood–brain barrier permeability when they are present in the subarachnoid space during meningitis. By presenting such glycopeptides intravenously, blood–brain barrier permeability in rabbits was enhanced in a reversible time- and dose-dependent manner to agents ≤20 kD in size. Therapeutic application of this bioactivity was evident as enhanced penetration of the antibiotic penicillin and the magnetic resonance imaging contrast agent gadolinium-diethylene-triamine-pentaacetic acid into the brain parenchyma.

The brain is sequestered from components circulating in blood by the blood–brain barrier. Tight junctions between juxtaposed endothelial cells of cerebral capillaries severely restrict the passage of molecules from the blood into the brain, a property distinct from the fenestrated endothelium of peripheral vessels. The ability to reversibly permeabilize the blood–brain barrier would greatly facilitate the delivery of intravenously applied agents of potential therapeutic value for diseases of the central nervous system. It is recognized that blood–brain barrier permeability develops during the course of bacterial meningitis (1). We sought to determine if current advances in the understanding of the pathophysiology of this abnormality in meningitis could suggest novel mechanisms to enhance blood–brain barrier permeability in a controlled manner.

The bacterial components that induce blood–brain barrier permeability during meningitis have been extensively characterized during the past 10 years. One such component is endotoxin, which induces a profound inflammatory response in the subarachnoid space, including leukocytosis, brain edema, and blood–brain barrier permeability (1). In contrast, other components of the bacterial surface, particularly the glycopeptides derived from the bacterial cell wall, have been found to induce only some components of the symptom complex of meningitis, the entire spectrum of pathophysiology arising from a summation of several individual activities (2). These proinflammatory components are monomers or multimers of the basic disaccharide tetrapeptide (Tetra)[1] structure N-acetylglucosaminyl-N-acetyl-

muramyl-L-alanyl-D-glutamyl-diaminopimelyl (or lysyl)-D-alanyl-D-alanine. Variations in the peptide side chain contribute to the generation of a dozen or more components in the cell wall of a given bacterium. Intracisternal instillation of purified glycopeptides induces enhanced permeability of the blood–brain barrier, allowing influx of serum proteins. For some disaccharide tetra or tripeptides, this effect occurs without engendering other pathological elements of meningitis, such as cerebral edema, increased intracranial pressure, or recruitment of leukocytes (2). We reasoned that intravenous application of this subgroup of simple glycopeptides might induce reversible blood–brain barrier permeability of potential therapeutic value.

Materials and Methods

Induction of Extravasation of Fluorescein-labeled Dextrans into the Brain Parenchyma. New Zealand white rabbits (2 kg; Hare Marland, Nutley, NJ) were injected intravenously with either 0.1 mg purified pneumococcal cell wall or 0.9% saline. FITC dextrans of 4, 20, 40, or 70 kD (Sigma Immunochemicals, St. Louis, MO) were given intravenously 1, 3, 5, 7, or 18 h thereafter. Animals were then anaesthetized, and 30 min later, cerebrospinal fluid was collected by cisternal puncture. Animals were killed, and the lungs, liver, kidneys, spleen, heart, and brain were examined visually under UV light. For some experiments, animals were treated with 1 mg/kg of antibody to IL-1α (Roche Laboratories, Nutley, NJ).

Assessment of Blood–brain Barrier Permeability In Vivo. New Zealand white rabbits (2 kg) were placed into a stereotactic frame by a dental acrylic helmet (3) and injected intravenously with saline (negative control), 100 μg Haemophilus influenzae cell wall, or 100 μg pneumococcal cell wall (Pn). 4.5 h later, one of three radiolabeled markers was introduced intravenously: ^{14}C-dextran

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(70 kD, 100 μCi; Amersham, Arlington Heights, IL), [³H]L-glucose (150 μCi; Amersham), or [³H]penicillin (2 mCi; Merck Research Laboratories, Rahway, NJ). 30 min later, 200 μl of cerebrospinal fluid was withdrawn from the cisterna magna, 50 μl of vitreous humor was removed from the anterior chamber of the eye, 1 ml of heparinized blood was obtained by cardiac puncture, and the animals were killed by an overdose of pentobarbital. Within 5 min, 1 g of the right parietal cortex was removed onto ice. Brain samples were weighed, homogenized, in some cases depleted of capillaries by centrifugation through dextran (4), and they were solubilized with Solvable (New England Nuclear, Boston, MA). The cpm in cerebrospinal fluid, ocular fluid, serum, and brain samples were determined. The amount of penetration of the radioactive marker into the whole brain was expressed as a ratio to the amount of tracer in serum (brain index). To distinguish cpm in brain parenchyma versus that in blood in cerebral vessels, total brain cpm was corrected for the blood volume/gram brain by subtracting the cpm/gram brain of control animals receiving saline ($n = 24$). The Wilcoxon signed rank test was used for statistical analysis against saline controls.

Comparison of Purified Glycopeptide Activity. Glycopeptides of *H. influenzae* were purified by HPLC as described in detail elsewhere (2). The identity of each peak was confirmed by fast atom bombardment tandem mass spectrometry. All glycopeptides from the cell wall of *H. influenzae* were tested by dividing the HPLC eluate into groups 1–6 according to the elution time. The main components of each fraction are listed in Table 2. Each component was injected intravenously at a dose corresponding to its proportion in the highly active, whole–cell wall preparation. At 4.5 h, 1 mCi of [³H]penicillin or 30 μCi of [¹⁴C]sucrose (Amersham) was injected intravenously. 30 min later, the penetration of radiolabel into the brain parenchyma was assessed as indicated above. For the most active components, the Tetra and its variants, experiments were repeated with purified material. Variations in dose and the relative time between challenge with Tetra (10 μg) and injection of the permeability marker was performed to establish the magnitude and time course of enhanced permeability.

Histopathology. Rabbits received saline (control) or pneumococcal cell wall (1 mg) intravenously. 4.5 h later, horseradish peroxidase (100,000 U; 500 mg; Sigma) was injected intravenously. 15 min thereafter, the animal was euthanized and perfused with 300 ml of 3% glutaraldehyde in 0.1 M sodium cacodylate buffer,

pH 7.3. The brain, lung, and kidney were fixed in glutaraldehyde for 48 h, sections were cut and treated with diaminobenzidine, and electron microscopy was performed by Dr. J. Elroy (Tufts University, Boston, MA).

Magnetic Resonance Imaging. Three rabbits were challenged with 1 mg of pneumococcal cell wall intravenously. 2 h later, the animals were anesthetized and the contrast agent gadolinium-diethylene-triamine-pentaacetic acid (Gd-DTPA; 1.5 cc/kg) was injected intravenously. Imaging was performed at the Small Animal Magnetic Resonance Imaging Unit, Department of Radiology, New York Hospital-Cornell Medical Center for 15 min every hour 2–6 h after cell wall challenge. T1-weighted images were compared over time for the appearance of enhancement as an indication of Gd-DTPA penetration across the blood–brain barrier.

Results

Induction of Blood–Brain Barrier Permeability to Intravenous Markers. To assess if intravenous administration of cell walls could induce blood–brain barrier permeability, rabbits were challenged intravenously with purified cell wall from one of two meningeal pathogens, Gram-positive *Streptococcus pneumoniae* or Gram-negative *H. influenzae* (2, 3). Subsequent passage out of the vascular compartment of intravenously applied fluoresceinated dextrans of various sizes was assessed visually as fluorescent staining of the brain parenchyma and cerebrospinal fluid (Table 1). Strong staining of brain by dextrans <40 kD in size was visible between 3 and 7 h after the cell wall challenge. No staining was present 1 or 18 h after challenge. Readministration of a second dose of cell wall at 24 h resulted in the same staining 5 h later as seen after a single dose, indicating no tachyphylaxis. Staining at 5 h was nearly completely eliminated by coadministration of antibody to IL-1α with cell wall, suggesting that the delay in onset of the permeability may result from a dependence on cytokine activity. Enzymatic cleavage of the Pn so as to dissociate the glycopeptide backbone from the stem peptides rendered the preparation inactive. Cerebrospinal fluid was stained sparingly; vitreous

Table 1. *Induction of Extravasation of Fluorescein-labeled Dextrans from Blood*

	UV fluorescence				
	Intravenous wall			Intracisternal wall	
Fluorescein dextran marker	Cerebral cortex	Cerebrospinal fluid	Ocular fluid	Cerebral cortex	Cerebrospinal fluid
4 kD	+ +	+/−	+/−	+ +	+ +
20 kD	+ +	+/−	−	+ +	+ +
40 kD	+/−	+/−	−	+	+ +
70 kD	−	−	−	−	+

Rabbits were injected intravenously with either 0.1 mg purified Pn or 0.9% saline. Intracisternally applied cell wall was used as a positive control. FITC dextran was given intravenously 5 h thereafter to correspond to the time of peak activity of the cell walls. 30 min later, cerebrospinal fluid and ocular fluid samples were collected, animals were killed, and the lungs, liver, and kidneys, spleen, heart, and brain were examined visually under UV light. ($n = 6$–8 animals per group; $n = 8$ eyes per group)
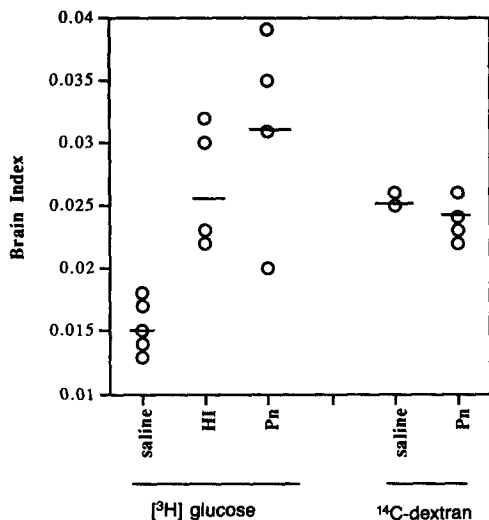
Figure 1. Penetration of radiolabeled markers into the brain parenchyma induced by cell walls. Rabbits were injected intravenously with saline (negative control), 100 μg *H. influenzae* cell wall (*HI*), or 100 μg Pn. ^{14}C-dextran or [^{3}H]glucose was introduced intravenously. 30 min after marker injection, 1 *g* of parietal cortex was excised, homogenized, depleted of capillaries, and the radioactivity per gram of the brain parenchyma was counted. Each symbol represents an individual rabbit. Horizontal lines indicate the mean penetration of each marker. Brain index: cpm per gram of brain/cpm per milliliter of serum. $P < 0.01$ for [^{3}H]glucose marker with HI wall and Pn wall versus saline.

horseradish peroxidase into the brain parenchyma (Fig. 2). Examination of the tight junctions at the cerebral capillary level did not indicate passage of the marker between cells. Rather, multiple pinocytotic vesicles were seen in cell wall-treated animals (Fig. 2, *arrows*); vesicles were very rare in control animals. Enhanced pinocytotic vesicle formation has been documented to contribute to enhanced blood–brain barrier permeability in animals with experimental meningitis (6).

Structure Activity Relationship for the Ability to Increase Blood–brain Barrier Permeability. Of particular therapeutic interest was the potential for a disaccharide peptide component of the cell wall to induce blood–brain barrier permeability similar to macromolecular cell wall. These components can not as yet be purified from pneumococcal wall. However, diverse cell wall subcomponents were separated and purified by HPLC from the peptidoglycan of *H. influenzae* (2) and were tested for the ability to induce [^{14}C]sucrose accumulation in the brain parenchyma (Table 2). Several disaccharides linked to tetrapeptides or tripeptides (groups 1 and 2) were active, increasing the penetration of the marker into brain by ~20% (2.7% of serum levels in saline group versus 3.3–3.5% in glycopeptide-treated groups). Modification of the amino acid composition of the peptide side chain was permissible for induction of blood–brain barrier permeability (Table 2), but multimerization or modification of the disaccharide reduced activity consistent with a restricted structure–activity relationship.

The induction of permeability by the Tetra was dose dependent (Fig. 3), achieving a maximum of 4.5% of serum values, i.e., approximately double that of saline controls. This accumulation occurred between 4 and 5 h after cell wall challenge, after which time the blood–brain barrier permeability returned to baseline, confirming the transient nature of the enhanced permeability (Fig. 4).

Enhancement of Penetration of Therapeutic and Imaging Agents into the Brain. To examine a therapeutic application of the bioactivity of the cell wall components, the ability to enhance penetration of the antibiotic penicillin into the normal brain was tested. In the absence of inflammation, 3% of the peak serum concentration of penicillin can be detected in brain (serum peak occurs at 30 min after intravenous injection) (Fig. 5). Cell walls and active glycopeptides resulted in between ~20 and 250% enhancement in the accumulation of penicillin in brain with a maximum brain uptake of 8% of serum levels (Table 2 and Fig. 5). Simultaneous measurement of cerebrospinal fluid penicillin penetration indicated an enhancement from 6 ± 1.3% of serum levels in control animals to 38 ± 4% in cell wall–treated animals.

A further possible application of the bioactivity of cell wall on blood–brain barrier permeability is contrast enhancement in magnetic resonance imaging of the central nervous system. The magnetic resonance imaging agent Gd-DTPA (7) is normally excluded from the brain parenchyma. Imaging the brain hourly after intravenous cell wall challenge demonstrated Gd-DTPA enhancement over the cerebral cortex. Enhancement began at 4 h and became
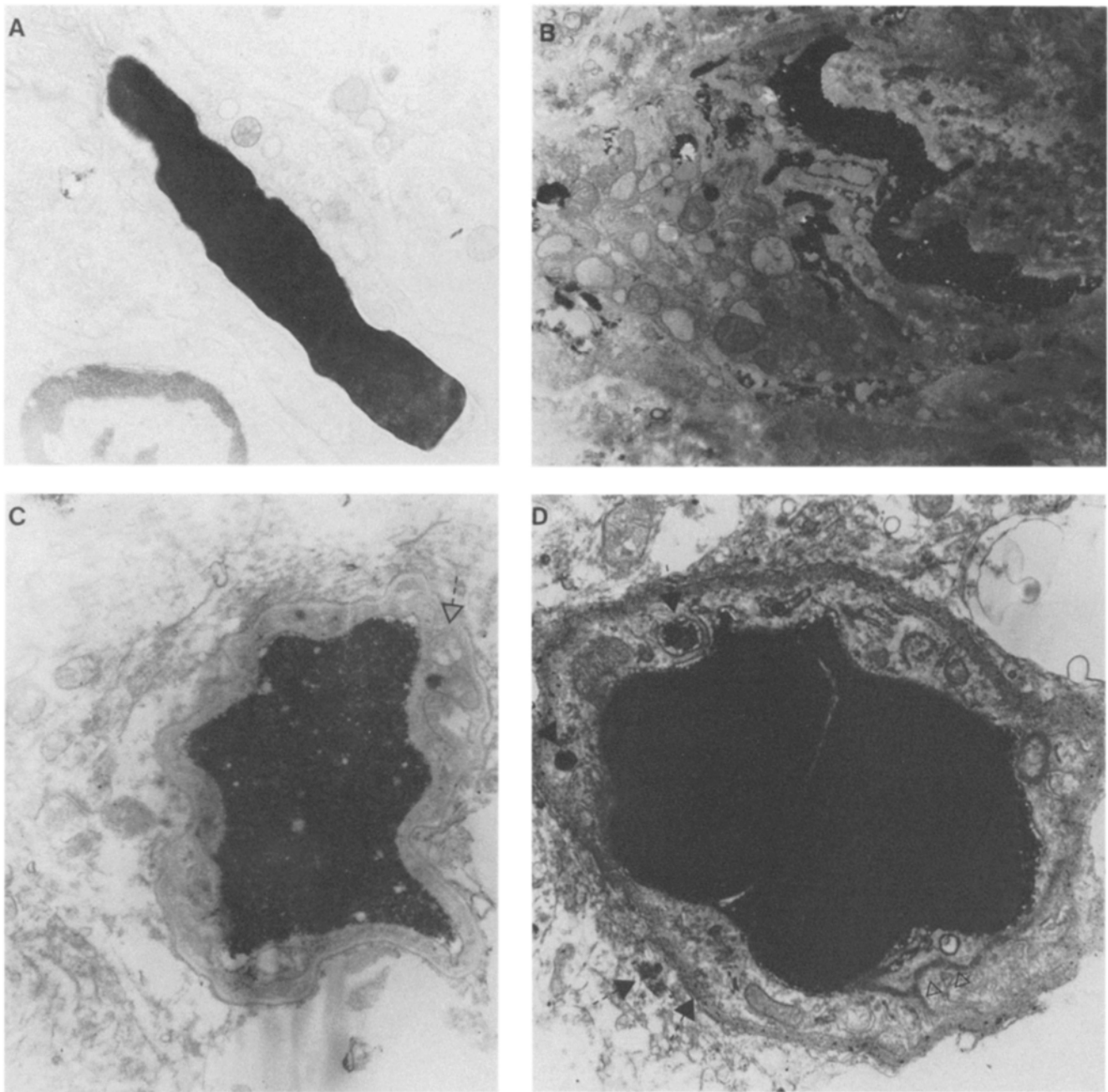
humor obtained from the eye revealed no staining; kidneys demonstrated occasional patches of staining, while the heart, lungs, liver, and spleen remained negative. Cerebrospinal fluid remained acellular with normal protein, glucose, and lactate concentrations. Brain weights and light microscopic histopathology remained normal, indicating lack of cerebral edema (data not shown). This pattern contrasted with that after intracisternal presentation of cell wall, which induced strong staining of brain and cerebrospinal fluid together with profoundly abnormal cerebrospinal fluid cytochemistry. This suggested that intravenous cell wall could induce a transient enhancement of blood–brain barrier permeability, particularly to markers 20 kD or smaller in size, without induction of undesirable parameters of inflammation. Furthermore, the effects of the cell wall appeared to be topographically restricted to the cerebral microvasculature.

This observation was extended by documenting the enhanced accumulation of intravenously applied [^{3}H]glucose but not 70-kD ^{14}C-dextran in the brain parenchyma in animals receiving intravenous cell walls of both pathogens (Fig. 1). Accumulation of [^{3}H]glucose was enhanced twofold in the cell wall–treated groups (2.5–3.1% of serum concentration was found in the brain), as compared to saline-treated control animals (1.5% of serum value). The range of accumulation was similar to that seen in animals treated with RMP-7, a bradykinin agonist known to enhance uptake across the blood–brain barrier (5).

Examination of the histopathology associated with the permeability induced by the pneumococcal cell wall indicated a significant extravasation of intravenously applied

Figure 2. Induction of penetration of horseradish peroxidase into the brain parenchyma by intravenous cell wall. 4.5 h after intravenous challenge with saline or Pn, horseradish peroxidase was injected intravenously. 15 min thereafter, the animal was killed, and the brain was fixed, cut, and treated with di-aminobenzidine. (*A* and *C*) Saline control animal at ×10,000 and ×15,000, respectively. The open arrow indicates a pinocytotic vesicle not containing label. (*B* and *D*) Cell wall–treated animal. Filled arrows indicate pinocytotic vesicles containing label (*top left*) and accumulation of label on the tissue side of the vascular cell barrier (*bottom left*). Two red cells partially occupy the intravascular space. A long segment of intercellular junction (*pair of open arrowheads*) is shown to exclude label.

prominent particularly in the region of the occipital cortex at 5 h after cell wall challenge (Fig. 6).

Discussion

Relatively few strategies enhance the penetration of therapeutic agents into the brain. Successful approaches have included covalent attachment of the agent to an anti–transferrin receptor antibody (4, 8), coadministration of the

agent with antibacterial antibodies that cross-react with brain (10), or coadministration of the agent with intraca-rotid administration of a β2-bradykinin analogue, RMP-7 (5). The intravenous application of a glycopeptide provides a potential simpler and readily reversible alternative to these more complex procedures.

Strong staining of brain by fluoresceinated dextrans ≤20 kD in size was visible between 3 and 7 h after the cell wall challenge. Permeability was confirmed by a doubling of the

Table 2. *Assessment of Blood–Brain Barrier Permeability*

Muropeptide	Structure	Brain index [^{3}H]penicillin	Brain index [^{14}C]sucrose
Saline		11.2 ± 0.2	2.75 ± 0.5
1. Tetra	GM-Ala-Glu- Dap-Ala	13.0 ± 0.8* (0.002%)	3.28 ± 0.8‡ (0.005%)
2. Tetra variants pooled	(Tetra A/S, Tetra G, Tri)	13.3 ± 2.3§ (0.002%)	ND
A. Tetra (A/S)	GM-Ala-Glu-Dap-Ser/Asp	ND	3.43 ± 0.7§ (0.005%)
B. Tetra (G)	GM-Ala-Glu-Dap-Gly	ND	3.26 ± 0.7§ (0.004%)
C. Tri	GM-Ala-Glu-Dap	ND	3.53 ± 1.3§ (0.006%)
3. Tetra^{nr}	GM^{nr}-Ala-Glu-Dap-Ala		
Tetra-Tri	GM-Ala-Glu-Dap | Ala-Da p-Glu-Ala-GM	10.8 ± 1.8	ND
Tetra-Tetra(G)	GM-Ala-Glu-Dap-Gly | Ala-Dap-Glu- Ala-GM		
4. Tetra dimer	GM-Ala-Glu- Dap-Ala | Ala-Dap-Glu-Ala GM	11.1 ± 1.2	ND
5. Tetra dimer (Anh)	GM-Ala-Glu-Dap-Ala | Ala-Dap- Glu-Ala-GM (Anh)	11.6 ± 1.8	ND
6. Tetra trimer (Anh)	GM-Ala-Glu- Dap-Ala | Ala-Dap-Glu-Ala- GM | GM(Anh)-Ala -Glu -Dap-Ala	10.0 ± 0.4	ND

All glycopeptides from the cell wall of *H. influenzae* were tested for the ability to induce accumulation of [^{3}H]penicillin in rabbit brain 5 h after challenge ($n \geq 4$ animals per substance). The main components of fractions tested as groups 1–6 are listed. Brain index: [cpm per gram of brain/cpm per milliliter of serum] ×100 ± SD. Values in parentheses are the percentage injected dose accumulating in brain above that of saline controls. Analysis of individual glycopeptides in Groups 1 and 2 was also performed with 30 μCi of [^{14}C]sucrose according to the same protocol. ND, not determined. Significant difference from saline controls: *$P = 0.04$; ‡$P = 0.05$; §combination of groups 2 A–C $P = 0.02$. GM, N-acetylglucosaminyl-N-acetylmuramic acid; Anh, 1,6 anhydro GM; nr, nonreduced; Dap, diaminopimelic acid. Amino acids are designated by the conventional three-letter code.

accumulation of [^{3}H]glucose. The exclusion of larger molecules, such as 70-kD dextran, was validated using radiolabeled material. This exclusion limit differs from that seen during bacterial meningitis, where passage of albumin (66 kD) and even larger molecular weight species >120 kD is markedly increased (10). Electron microscopic examination of the cerebral capillary endothelium in meningitis indicates that two processes occur to enhance permeability: increased pinocytotic vesicle formation and loss of tight junctions between endothelial cells (1, 6). The pathology of the effects of cell wall on cerebral capillaries differed from

meningitis in that tight junctions remained intact while pinocytotic vesicle activity was enhanced. This suggests that the effect of cell walls may involve increasing transcellular transport of intravascular components rather than induction of loosening of tight junctions between endothelial cells. Increased trafficking, as opposed to physical opening of tight junctions, would be compatable with the observed preservation of restriction of penetration of larger molecular weight species.

The effect of cell walls appeared to be strong on the cerebral microvasculature, the site of the blood–brain barrier,
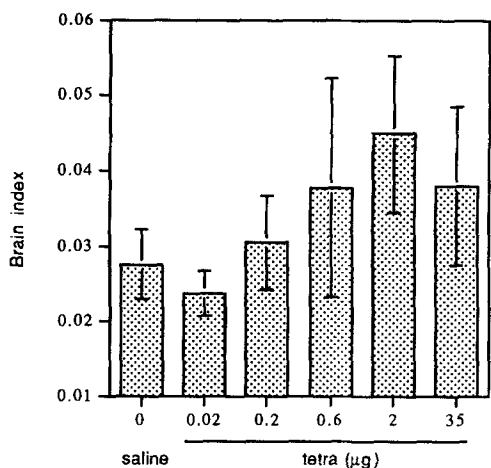
Figure 3. Dosage effect on Tetra bioactivity. Tetra was injected intravenously at the indicated dose, followed by 30 μCi of [^{14}C]sucrose after 4.5 h. Brain index = cpm per gram brain/cpm per milliliter serum ± SD; value of 4.4 for 2 μg Tetra is equivalent to 0.007% of the injected dose and differs from control at $P < 0.01$.

Figure 5. Penetration of [^{3}H]penicillin into the brain parenchyma induced by cell walls. Rabbits were challenged with the indicated cell wall as in Fig. 1. [^{3}H] penicillin was introduced intravenously at 4.5 h after cell wall. Each symbol represents an individual rabbit. Horizontal lines indicate the mean penetration of each marker. Brain index: cpm per gram of brain/cpm per milliliter of serum. $P < 0.001$ for HI wall and Pn versus saline.

as indicated by the accumulation of label in the brain parenchyma. A lesser effect was evident for penetration into cerebrospinal fluid. The blood–brain barrier arises at the cerebral capillary endothelium, while the barrier between blood and the subarachnoid space arises at the level of the specialized epithelium of the choroid plexus. The continuity of interstitial fluid of the brain with cerebrospinal fluid may allow material crossing the cerebral microvasculature to accumulate in the subarachnoid space. Although also delineated by tight junctions, examination of the blood–ocular barrier indicated no substantial enhancement of penetration of 20-kD fluoresceinated dextran into the vitreous fluid of the eye. Furthermore, no staining of other peripheral organs such as the lungs, spleen, liver, or kidneys were noted, suggesting that this effect is either limited to or most pronounced on endothelial cells of the central nervous sys-

tem. Investigation of a possible receptor mediating this effect will reveal if distribution of receptor can account for differences in the biological activities of cell walls at these different sites.

The bioactivities of bacterial glycopeptides are numerous and depend critically on the structure of the glycopeptide and the nature of the target eukaryotic cell. For instance, modification of the disaccharide moiety to a 1,6 anhydro linkage defines the sleep peptide known to induce slow
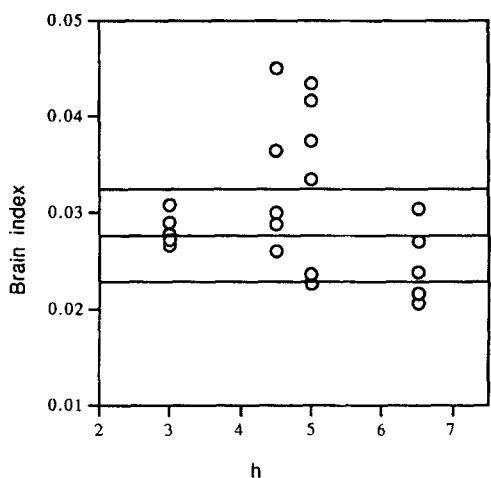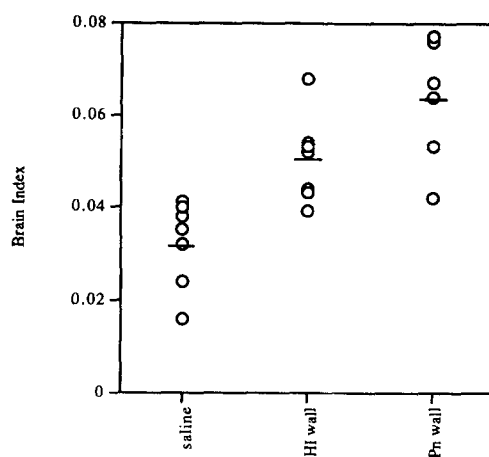

Figure 4. Time course of enhanced blood brain barrier permeability. Tetra (10 μg) was injected intravenously at 0 h in groups of five or more animals followed by an injection of 30 μCi of [^{14}C] sucrose at 4.5 h. Each symbol represents an individual animal. Brain index = cpm per gram brain/cpm per milliliter serum ± SD.
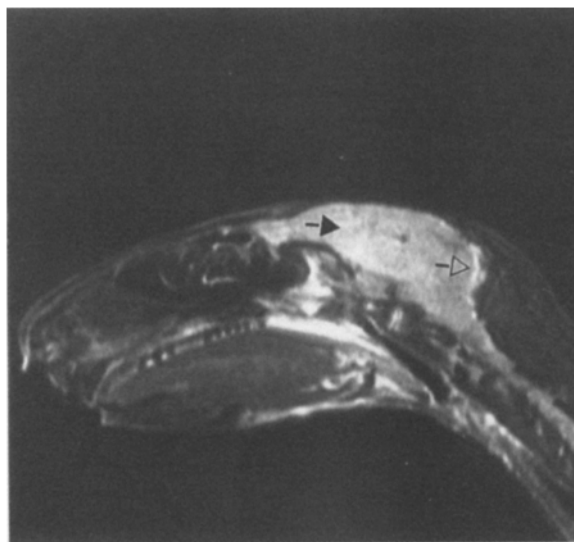

Figure 6. Sagittal magnetic resonance image of a rabbit challenged with Pn. The rabbit was anesthetized and challenged with 1 μg of pneumococcal cell wall intravenously. 4 h later, the contrast agent Gd-DTPA was injected intravenously, and imaging was performed for 15 min every hour. The T1-weighted image shown was acquired at 5 h. Normal brain parenchyma excludes Gd-DTPA and remains grey (black arrow). Gd-DTPA penetration through the blood–brain barrier results in a white enhancement that is pronounced over the occipital cortex in this image (open arrow). This image is representative of results obtained in three animals.

wave sleep in rabbits upon intravenous administration (11), while a lactyl-tetrapeptide derivative is toxic to respiratory ciliated cells (12). The most active structure in this study was a disaccharide linked to three or four peptides. The activity of the component was dose dependent, and it occurred in a strict time-dependent fashion, peaking at 4–5 h after dose. In contrast to other known activities, modification of the amino acid composition of the peptide side chain was permissible for induction of blood–brain barrier permeability, but multimerization or modification of the disaccharide reduced activity consistent with a restricted structure–activity relationship.

The magnitude of the enhanced accumulation of intravenous marker in the brain parenchyma ranged from ∼20 to 250%. Accumulation of [^{3}H]glucose in the brain doubled in cell wall–treated animals as opposed to controls. This effect is similar to that seen with the same marker in animals treated with RMP-7, a bradykinin agonist known to enhance uptake across the blood–brain barrier (5). In healthy individuals, ∼3% of peak serum concentrations of penicillin can be found in brain tissue. This value increases to ∼15% in gravely ill patients with bacterial meningitis, characterized by severe breakdown of the blood–brain bar-

rier and pathological brain edema, suggesting that this level of permeability may not be compatible with recovery. The glycopeptides applied here resulted in up to 8% of the serum concentration of penicillin appearing in brain tissue without apparent brain edema or residual neurological effects in the experimental animals. The enhanced permeability was transient, and it indicates that the blood–brain barrier can be permeabilized to this degree reversibly, without generation of brain edema.

This enhanced level of penetration of penicillin (0.002–0.01% of injected dose) is within the 0.001–0.1% range of penetration of an injected dose documented for some small peptides that cross well into the brain (13). Thus, small glycopeptides may provide a new avenue for development of agents useful in delivering treatments for diseases of the central nervous system across the blood–brain barrier. Increasing the penetration of an antibiotic into the brain could be of therapeutic importance when efficacy is marginal by conventional dosing, for example, such as when fungi or resistant bacteria are the cause of central nervous system disease. Greater impact of this type of therapeutic approach would most likely accrue through antitumor therapy or imaging, as demonstrated in Fig. 6.

References

1. Quagliarello, V., and W.M. Scheld. 1992. Bacterial meningitis: pathogenesis, pathophysiology and progress. N. Engl. J. Med. 327:864–872.
2. Burroughs, M., E. Rozdzinski, S. Geelen, and E. Tuomanen. 1993. A structure-activity relationship for induction of meningeal inflammation by muramyl peptides. J. Clin. Invest. 92: 297–302.
3. Tuomanen, E., H. Liu, B. Hengstler, O. Zak, and A. Tomasz. 1985. The induction of meningeal inflammation by components of the pneumococcal cell wall. J. Infect. Dis. 151: 859–868.
4. Friden, P., L. Walus, P. Watson, S. Doctrow, J. Kozarich, C. Backman, H. Bergman, B. Hoffer, F. Bloom, and A. Granholm. 1993. Blood-brain barrier penetration and in vivo activity of an NGF conjugate. Science (Wash. DC). 259:373–377.
5. Inamura, T., T. Nomura, R. Bartus, and K. Black. 1994. Intracarotid infusion of RMP-7, a bradykinin analog: a method for selective drug delivery to brain tumors. J. Neurosurg. 81: 752–758.
6. Wispelwey, B., A.J. Lesse, E.J. Hansen, and W.M. Scheld. 1988. Haemophilus influenzae lipopolysaccharide–induced blood brain barrier permeability during experimental meningitis in the rat. J. Clin. Invest. 82:1339–1346.
7. Mathews, V.P., M.A. Kuharik, M.K. Edwards, P.G. D'Amour, B. Azzarelli, and R.G. Dreesen. 1988. Gd-DTPA-enhanced

MR imaging of experimental bacterial meningitis: evaluation and comparison with CT. Am. J. Roentgenol. 9:1045–1048.
8. Friden, P., L. Walus. G. Musso, M. Taylor, B. Malfroy, and R. Starzyk. 1991. Anti-transferrin receptor antibody and antibody-drug conjugates cross the blood-brain barrier. Proc. Natl. Acad. Sci. USA. 88:4771–4775.
9. Tuomanen, E., S. Prasad, J. George, A.I.M. Hoepelman, P. Ibsen, I. Heron, and R. Starzyk. 1993. Reversible opening of the blood brain barrier by anti-bacterial antibodies. Proc. Natl. Acad. Sci. USA. 90:7824–7828.
10. Kadurugamuwa, J.L., B. Hengstler, and O. Zak. 1989. Cerebrospinal fluid protein profile in experimental pneumococcal meningitis and its alteration by ampicillin and anti-inflammatory agents. J. Infect. Dis. 159:26–33.
11. Krueger, J., D. Davenne, J. Walter, S. Shoham, S. Kubillus, R. Rosenthal, S. Martin, and K. Biemann. 1987. Bacterial peptidoglycans as modulators of sleep. Brain Res. 403:258–266.
12. Goldman, W.E., D.G. Klapper, and J.B. Baseman. 1982. Detection, isolation, and analysis of a released Bordetella pertussis product toxic to cultured tracheal cells. Infect. Immun. 36: 782–794.
13. Banks, W., K. Audus, and T. Davis. 1992. Permeability of the blood-brain barrier to peptides: an approach to the development of therapeutically useful analogs. Peptides. 13:1289–1294.