volume. However, this *Solemya* sp. is a relatively large animal that can burrow actively and sustain swimming by water-jet propulsion for periods of as long as 1½ minutes (*8*). Its gutless condition might have been surprising were it not for recent discoveries of several groups of gutless Pogonophora much larger than those found originally. These include large specimens of Vestimentifera (*12*) and giant tube worms collected from the volcanic vent regions of the Galápagos Rift (*13*). Jones (*14*) believes that the latter tube worms are related to the Pogonophora and confirms that they are gutless. Southward *et al.* (*15*), reporting on the value of dissolved organic molecules as food for the pogonophoran *Siboglinum fiordicum*, discussed the importance of association with microorganisms and reviewed the spectrum of physiological relationships with microorganisms that might be useful to benthic aerobes. In such symbioses, a burrow or a tube seems to be necessary to contain and confine the related organisms and prevent the dissipation of useful solutes. In comparing the biology of the *Solemya* sp. with *Siboglinum* and the Galápagos Rift tube worms, we point out a common feature: high densities of these gutless animals are found in the vicinity of extraordinary energy sources. Dense populations of *Siboglinum* are found near a commercial fish farm (*15*); the Galápagos Rift tube worms are found around volcanic vents in association with chemosynthetic bacteria (*13*); and *Solemya* sp. forms dense populations near pulp mills (*8*). This may provide a clue to the evolutionary ecology of such organisms.

ROBERT G. B. REID
Department of Biology, University of Victoria, Victoria, British Columbia, Canada V8W 2Y2
FRANK R. BERNARD
Department of the Environment, Pacific Biological Station, Nanaimo, British Columbia V9R 5K6

References

1. C. M. Yonge, *Philos. Trans. R. Soc. London Ser. B.* **230**, 79 (1939).
2. G. Owen, *Q. J. Microsc. Sci.* **102**, 15 (1961).
3. F. R. Bernard, *Venus Jpn. J. Malacol. (Kairuigaku Zasshi)*, in press.
4. E. S. Morse, *Biol. Bull. (Woods Hole, Mass.)* **25**, 261 (1913).
5. R. G. B. Reid and K. Rauchert, *Comp. Biochem. Physiol. A* **41**, 887 (1972).
6. R. G. B. Reid, *Veliger* **20**, 260 (1978).
7. ———, *Comp. Biochem. Physiol.* **17**, 417 (1966).
8. ———, *Can. J. Zool.*, in press.
9. P. W. Hochachka, in *Biochemical and Biophysical Perspectives in Marine Biology*, D. C. Malins and J. R. Sargent, Eds. (Academic Press, London, 1975), pp. 107–137.
10. S. M. Stanley, *Geol. Soc. Am. Mem. 125* (1970), p. 120.
11. A. V. Ivanov, *Pogonophora*. D. B. Carlisle, Transl. and Ed. (Consultants Bureau, New York, 1963), p. 79.
12. J. Van der Land and A. Norrevang, *K. Dan. Vidensk. Selsk. Biol. Skr.* **21**, 1 (1977).
13. J. B. Corliss *et al.*, *Science* **203**, 1073 (1979).
14. M. Jones, personal communication.
15. A. J. Southward, E. C. Southward, T. Brattegard, T. Bakke, *J. Mar. Biol. Assoc. U.K.* **59**, 133 (1979).

29 October 1979; revised 21 February 1980

Neurotransmitter Receptor Binding in Bovine Cerebral Microvessels

Abstract. *Purified preparations of microvessels from bovine cerebral cortex contain substantial levels of alpha-adrenergic, beta-adrenergic, and histamine 1 receptor binding sites but only negligible serotonin, muscarinic cholinergic, opiate, and benzodiazepine receptor binding. Norepinephrine and histamine may be endogenous regulators of the cerebral microcirculation at the observed receptors.*

Cerebral blood vessels are dynamically regulated to respond to variations in cerebral metabolic activity. Chemical factors such as carbon dioxide, potassium, calcium ions, and adenosine nucleotides are thought to accumulate within the perivascular space to couple brain blood flow and energy metabolism (*1*). Neuronal projections to cerebral blood vessels may also provide a mechanism by which the brain regulates its own blood flow, blood volume, and capillary area (*2*). The superior cervical ganglia and locus ceruleus are thought to send axonal projections that innervate small blood vessels in the forebrain (*3*). These vessels contain norepinephrine and related enzymes (*4*) as well as a beta-adrenergic-sensitive adenylate cyclase (*5*). Serotonin-containing neurons with cell bodies in the raphe nuclei also appear to project to cerebral microvessels (*6*), as may neurons containing vasoactive intestinal peptide and substance P (*7*). To clarify how transmitters might regulate vessel caliber, we measured receptor binding for a variety of neurotransmitters and drugs in purified preparations of small cerebral blood vessels. We report that microvessels from bovine cerebral cortex contain specific alpha-adrenergic, beta-adrenergic, and histamine 1 receptor binding sites.

Brain microvessel preparations were made from calf brains (*8*). To separate the microvessels from surrounding parenchyma, pia and arachnoid membranes were removed with forceps under 3-diopter magnification (*6*). For each preparation, 50 g of cortical gray matter was used. Approximately 10-g lots of gray matter were removed and homogenized with two to three volumes of cold (4°C) 0.9 percent NaCl in a loosely fitting (0.1-mm clearance) Potter-Elvehjem homogenizer. The homogenates were centrifuged at 1500*g* for 15 minutes, the supernatant fractions discarded, and the pellets washed one more time. The pellets were resuspended in 60 to 80 ml of 0.25*M* sucrose, layered over gradients of 1.5 and 1.0*M* sucrose, and centrifuged in an SW 27 rotor at 58,000*g* for 45 minutes. The resulting microvessel fraction was resuspended in 100 ml of cold saline, adsorbed onto small glass beads, and washed extensively with cold buffer. The vessels were then trapped in a nylon filter (80-μm mesh), again washed extensively, and frozen until assay. Thus approximately 500 mg of microvessels were obtained from 50 g of bovine cortical gray matter. Both the microvessel preparation and frozen calf cortex were homogenized in 80 volumes of tris-HCl (*p*H 7.7 at 25°C). The tissue suspensions were sieved through 500-μm nylon mesh and immediately assayed for receptor binding.

Substantial levels of alpha-adrenergic, beta-adrenergic, and histamine 1 receptor binding were detected in the preparations (Table 1). Binding of *p*-[^{3}H]aminoclonidine and [^{3}H]WB-4101 to alpha-adrenergic receptors in the microvessels was almost half that found in bovine cortical homogenates. [^{3}H]Dihydroalprenolol binding to beta-adrenergic receptors and [^{3}H]mepyramine labeling of histamine 1 receptors were about 30 and 65 percent, respectively, of binding levels in neuronal membrane preparations. By contrast, only very low levels of binding were detected for [^{3}H]serotonin, [^{3}H]spiroperidol, and D-[^{3}H]lysergic acid diethylamide (labeling of serotonin receptors); for ^{3}H-labeled D-Ala-Leu-enkephalin and [^{3}H]naloxone (labeling of opiate receptors); for [^{3}H]flunitrazepam (labeling of benzodiazepine receptors); and for [^{3}H]quinuclidinyl benzilate (labeling of muscarinic cholinergic receptors). Interestingly, the microvessel percentage of brain benzodiazepine binding was low despite the fact that the absolute levels of binding were higher in microvessels than for most other ^{3}H-labeled ligands. In preliminary saturation experiments, the various levels of binding of ^{3}H-labeled ligands in microvessels and brain membranes reflected differences in the maximum number of binding sites,

0036-8075/80/0509-0610$00.50/0

not differences in the dissociation constant.

To ensure that the specific binding of ^{3}H-labeled ligands to alpha-adrenergic, beta-adrenergic, and histamine 1 receptors involves biologically relevant receptor sites, we evaluated drug competition at each binding site (Table 2). Both in neuronal membrane and microvessel fractions, the binding of *p*-[^{3}H]aminoclonidine and [^{3}H]WB-4101 was stereospecific with respect to the isomers of norepinephrine, with the physiologically more potent (−)isomer substantially more active at both ^{3}H-labeled ligand binding sites. The alpha-adrenergic antagonists WB-4101 and prazosin were similarly potent at brain membrane and microvessel binding sites. The binding of [^{3}H]dihydroalprenolol had properties expected of beta-adrenergic receptors in both the neuronal and microvessel fractions, with stereospecificity of epinephrine isomers and with isoproterenol being substantially more potent than norepinephrine and epinephrine. In both tissue preparations, binding of [^{3}H]mepyramine was inhibited stereospecifically by the isomers of the histamine 1 antihistamine chlorpheniramine, whereas mepyramine was markedly more potent than histamine. The limited levels of binding displayed by ligands for serotonin, opiate, benzodiazepine, and muscarinic cholinergic receptors appear to have the same drug specificity in the microvessel as in the neuronal membrane preparations. Whether this limited binding reflects a very small number of vascular receptors or merely contamination of the neuronal membranes is unclear.

The binding of *p*-[^{3}H]aminoclonidine, [^{3}H]WB-4101, [^{3}H]dihydroalprenolol, and [^{3}H]mepyramine to cerebral microvessels could involve receptors on smooth muscle, presynaptic nerve terminals, or both. Destruction of norepinephrine-containing neurons with 6-hydroxydopamine, however, fails to significantly alter the binding of ^{3}H-labeled ligands to

Table 1. Comparison of ^{3}H-labeled ligand binding in bovine cortical homogenates with binding in cortical microvessel fractions. Binding studies were performed as described by Bennett (*14*). Briefly, 100 μl of tris-HCl (*p*H, 7.7 at 25°C) or of a displacing drug, 100 μl of ^{3}H-labeled ligand, and 800 μl of tissue suspension were studied. Specific binding was defined as the excess over blanks taken in the presence of 0.1 m*M* (−)norepinephrine for alpha-adrenergic receptors, 1 μ*M* (±)propranolol for beta-adrenergic receptors, 10 μ*M* triprolidine for histamine receptors, 1 μ*M* D-lysergic acid diethylamide for serotonin receptors, 1 μ*M* levallorphan for opiate receptors, 1 μ*M* diazepam for benzodiazepine receptors, and 0.1 m*M* oxotremorine for muscarinic cholinergic receptors. In each case, approximately 0.5 mg of protein per milliliter was incubated with the ^{3}H-labeled ligands until equilibrium was reached. All experiments were performed in triplicate and repeated four to six times with less than 15 percent variation.

Binding site	Ligand	Concentration (n*M*)	Cortical neuronal membranes (femtomoles per milligram of protein)	Cortical microvessels (femtomoles per milligram of protein)	Neuronal membranes (percent)
Alpha-adrenergic	*p*-[^{3}H]Aminoclonidine	0.6	31.8	15.2	48
	[^{3}H]WB-4101	0.9	41.8	18.3	44
Beta-adrenergic	[^{3}H]Dihydroalprenolol	0.8	36.4	11.3	31
Histamine 1	[^{3}H]Mepyramine	2.0	67.8	44.0	65
Serotonin	5-[^{3}H]Hydroxytryptamine	2.0	26.4	1.36	5
	[^{3}H]Spiroperidol	0.36	18.1	1.08	6
	D-[^{3}H]Lysergic acid diethylamide	3.7	51.2	3.74	7
Opiate	D-[^{3}H]Ala-Leu-enkephalin	1.4	11.4	0.56	5
	[^{3}H]Naloxone	1.9	36.2	3.20	9
Benzodiazepine	[^{3}H]Flunitrazepam	0.7	430	37.6	9
Cholinergic	[^{3}H]Quinuclidinyl benzilate	0.2	115	8.5	7

Table 2. Comparison of drug affinities for ^{3}H-labeled ligand binding sites in bovine cerebral homogenates with affinities for binding sites in bovine cerebral microvessel fractions. For each drug, inhibition of binding was measured at three to four concentrations, and median inhibitory concentrations were determined by log-probit analysis. Each value is the mean ± standard error of the data from three to four experiments, each performed in triplicate.

Binding site	^{3}H-Labeled ligand	Drug	Median inhibitory concentration	
			Cortical neuronal membranes	Cortical microvessels
Alpha-adrenergic	*p*-Aminoclonidine	(−)Norepinephrine	24 ± 0.88	35 ± 10
		(+)Norepinephrine	690 ± 120	1,200 ± 490
		WB-4101	75 ± 45	73 ± 47
		Prazosin	5,800 ± 1,700	5,700 ± 1,800
	WB-4101	(−)Norepinephrine	2,400 ± 700	2,100 ± 380
		(+)Norepinephrine	73,000 ± 16,000	73,000 ± 16,000
		WB-4101	0.64 ± 0.12	0.61 ± 0.10
		Prazosin	1.4 ± 0.65	0.86 ± 0.12
Beta-adrenergic	Dihydroalprenolol	(−)Epinephrine	1,000 ± 460	1,400 ± 790
		(+)Epinephrine	16,000 ± 6,300	17,000 ± 6,600
		Isoproterenol	87 ± 27	77 ± 18
		(−)Norepinephrine	2,000 ± 790	2,200 ± 720
Histamine 1	Mepyramine	Histamine	23,000 ± 2,000	46,000 ± 7,900
		Mepyramine	3.3 ± 0.47	3.1 ± 0.88
		D-Chlorpheniramine	4.3 ± 0.71	3.8 ± 1.1
		L-Chlorpheniramine	250 ± 62	300 ± 35

alpha-adrenergic (*9*) or beta-adrenergic (*10*) receptors. In addition, the microvessel preparations used here were more than 95 percent pure as determined by light (including phase-contrast) microscopy and transmission and scanning electron microscopy (*6*). These considerations, along with the negligible levels of serotonin, benzodiazepine, opiate, and muscarinic cholinergic receptors, strengthen the conclusion that the adrenergic and histamine 1 receptors involve cerebral microvessels. Despite the relative microvessel enrichment of histamine 1 and adrenergic receptors, microvessels, which account for < 1 percent of the brain's mass, provide only a small portion of the brain's total content of these receptors.

In general, alpha agonists constrict and beta agonists dilate cerebral vessels (*1*), whereas serotonin constricts or dilates depending on the route of administration, species, and state of vascular contractility (*11*). The negligible serotonin receptor binding detected here [despite apparent serotonin innervation of cerebral microvessels (*6*)] may reflect species or regional variation. Histamine, localized in mast cells surrounding cerebral blood vessels (*12*), may account for histamine-elicited constriction as well as dilatation via a histamine 2 receptor-linked adenylate cyclase (*13*).

The existence of norepinephrine and histamine receptors in the brain's microvessels suggests that these transmitters are endogenous regulators of the microcirculation. Drugs that affect these receptor sites might be useful in treating cerebral blood vessel abnormalities.

STEPHEN J. PEROUTKA
Departments of Pharmacology and Experimental Therapeutics and *Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205*
MICHAEL A. MOSKOWITZ
Laboratory of Neuroendocrine Regulation, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge and *Section of Neurology, Peter Bent Brigham Hospital, Harvard Medical School, Boston, Massachusetts 02115*
JOHN F. REINHARD, JR.
Laboratory of Neuroendocrine Regulation, Massachusetts Institute of Technology
SOLOMON H. SNYDER
Departments of Pharmacology and Experimental Therapeutics and *Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine*

References and Notes

1. W. Kuschinsky and M. Wahl, *Physiol. Rev.* **28**, 656 (1978).
2. M. E. Raichle, B. K. Hartman, J. O. Eichling, L. G. Sharpe, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3726 (1975).
3. T. Donath, *Acta Morphol. Acad. Sci. Hung.* **16**, 285 (1968); B. K. Hartman, D. Zide, S. Udenfriend, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2722 (1972); M. L. Rennels and E. Nelson, *Am. J. Anat.* **144**, 233 (1975).
4. F. M. Lai, S. Udenfriend, S. Spector, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 4622 (1975).
5. T. J. Herbst, M. E. Raichle, J. A. Ferrendelli, *Science* **204**, 330 (1979); J. Nathanson and G. Glaser, *Nature (London)* **278**, 567 (1979).
6. J. F. Reinhard, Jr., J. E. Liebmann, A. J. Schlosberg, M. A. Moskowitz, *Science* **206**, 85 (1979).
7. L. I. Larrson, L. Edvinsson, J. Fahrenkrug, R. Hakanson, C. Owman, O. Schattalitzky, D. Muckadell, F. Sundler, *Brain Res.* **113**, 400 (1976); V. Chan-Palay, *Wenner-Gren Cent. Int. Symp. Ser.* **30**, 39 (1977); *Brain Res.* **102**, 103 (1976).
8. B. Mrsulja, B. J. Mrsulja, T. Fujimoto, I. Klatzo, M. Spatz, *Brain Res.* **110**, 644 (1976).
9. D. C. U'Prichard, D. A. Greenberg, S. H. Snyder, *Mol. Pharmacol.* **13**, 454 (1977).
10. J. R. Sporn, B. B. Wolfe, T. K. Harden, P. B. Molinoff, *ibid.* **13**, 1170 (1977).
11. L. Edvinsson, J. Cervosna, L. I. Larrson, C. Owman, A. L. Ronnberg, *Neurology* **27**, 878 (1977); L. Edvinsson and J. E. Hardebo, *Acta Physiol. Scand.* **97**, 523 (1976).
12. B. Jarrott, J. T. Hjelle, S. Spector, *Brain Res.* **168**, 323 (1979).
13. L. Edvinsson and C. Owman, *Neurology* **25**, 271 (1975).
14. J. P. Bennett, Jr., in *Neurotransmitter Receptor Binding*, H. I. Yamamura, S. J. Enna, J. J. Kuhar, Eds. (Raven, New York, 1978), pp. 57-90.
15. S.J.P. is a recipient of medical scientist training program grant 5T 32 GM0309 from NIH. M.A.M. is an established investigator of the American Heart Association. This work was supported by PHS grants HL-22573-01, NS-15201 (M.A.M.), MH-18501, DA-00266 (S.H.S.), and by grants from the McKnight and Hartford foundations. The authors thank R. M. Lebovitz for excellent technical assistance and D. C. Hanks for manuscript preparation.

26 December 1979; revised 11 February 1980

Single-Shot Channel Activation Accounts for Duration of Inhibitory Postsynaptic Potentials in a Central Neuron

Abstract. *In the goldfish Mauthner cell, inhibitory postsynaptic currents evoked by intracellular stimulation of presynaptic neurons decay exponentially, with a mean time constant of 6.65 milliseconds. Analysis of membrane conductance fluctuations induced by iontophoresis of glycine and γ-aminobutyric acid indicates a mean inhibitory channel lifetime of 7.15 milliseconds. The results thus suggest that the relaxation kinetics of activated inhibitory channels are rate-limiting during decay of the inhibitory postsynaptic current.*

Inhibitory postsynaptic potentials (IPSP's) recorded from vertebrate central neurons, particularly those in the brain, are often long lasting (*1*), presumably because of (i) a prolonged elevation of inhibitory transmitter concentration in the synaptic cleft or (ii) a long mean lifetime of activated inhibitory channels. The first possibility implies that repetitive transmitter-receptor interactions, resulting perhaps from repetitive firing of the presynaptic terminals, restricted diffusion of the transmitter from the synaptic cleft, or relatively slow transmitter uptake, contribute to the underlying inhibitory postsynaptic current (IPSC). In contrast, the second possibility implies that transmitter concentration falls rapidly at active synapses, with IPSC duration reflecting the relaxation kinetics of inhibitory channels, most of which open only once. In the case of the frog neuromuscular junction, Magleby and Stevens (*2*) and Anderson and Stevens (*3*) concluded that channel gating properties, rather than the loss of acetylcholine from the endplate region, are rate-limiting during the normal endplate current decay. That conclusion was based in part on results obtained with the analysis of conductance fluctuations (*4*). This analytical approach, however, has been restricted so far to peripheral junctions (*3, 4*) and to neurons in vitro (*5*); it has not yet been applied to vertebrate central neurons in situ, where the experimental analysis is more difficult.

Having analyzed inhibition at the goldfish Mauthner cell (M-cell) (*6, 7*), we now present evidence, based on measurements of both unitary IPSC decay and conductance fluctuations, that suggests that most channels open and close only once during an IPSP. Our results thus extend to at least some central neurons the basic findings of Stevens and his colleagues.

Goldfish (*Carassius auratus*) 12 to 18 cm long and immobilized with curare (0.15 mg) were used. The procedures for artificial respiration, exposure of the medulla, and identification of the M-cell after its antidromic activation, were similar to those described previously (*8-10*). Intracellular recordings were obtained from the M-cell soma or proximal lateral dendrite. Injections of Cl^- ion from the intracellular recording electrodes were used to displace the IPSP reversal potential (*11*) so the recorded IPSP's were depolarizing. In the first of these experimental series, we obtained intracellular recordings simultaneously from identified interneurons (Fig. 1A), the so-called passive hyperpolarizing potential (PHP) cells (*10*), which have been shown to me-

0036-8075/80/0509-0612$00.50/0