The Role of $GABA_B$ Receptor Activation in Absence Seizures of Lethargic (*lh/lh*) Mice

David A. Hosford,* Suzanne Clark, Zhen Cao, Wilkie A. Wilson, Jr., Fu-hsiung Lin, Richard A. Morrisett, Alexandre Huin

Lethargic (*lh/lh*) mice, which function as an animal model of absence seizures, have spontaneous seizures that have behavioral and electrographic features and anticonvulsant sensitivity similar to those of human absence seizures. Antagonists of the γ-aminobutyric $acid_B$ ($GABA_B$) receptor suppressed these seizures in lethargic mice, whereas agonists of $GABA_B$ receptors exacerbated them. Furthermore, $GABA_B$ receptor binding and synaptically evoked $GABA_B$ receptor–mediated inhibition of *N*-methyl-D-aspartate responses were selectively increased in *lh/lh* mice. Therefore, enhanced $GABA_B$ receptor–mediated synaptic responses may underlie absence seizures in *lh/lh* mice, and $GABA_B$ receptor antagonists hold promise as anticonvulsants for absence seizures.

During absence (petit mal) seizures in humans, the cerebral cortex and thalamus generate bilaterally synchronous, rhythmic 3-Hz spike-wave discharges (*1*). Studies of the mechanisms underlying these seizures implicate oscillations between interconnected neuronal structures involving both excitatory and inhibitory processes (*1*, *2*). One possible mechanism is an interplay between low threshold Ca^{2+} currents (T currents) and $GABA_B$ receptors (*3*). T currents can trigger rhythmic, oscillatory burst firing of thalamic neurons (*4*); they are reduced by therapeutically relevant concentrations of anticonvulsants that are used to treat absence seizures (*5*). Finally, T channels (inactivated at normal resting membrane potentials) are deinactivated by $GABA_B$ receptor–mediated hyperpolarizations within thalamic neurons (*6*, *7*). Taken together, these findings support the hypothesis that enhanced $GABA_B$ receptor–mediated responses play a role in absence seizures (*3*). To test this hypothesis, we used lethargic (*lh/lh*) mice, a mutant strain with spontaneous seizures (*8*) that we found was a valid model of absence seizures (*9*).

The *lh/lh* mouse, which originated at Jackson Labs (*8*), has a single-locus defect on chromosome 2 and expresses spontaneous seizures (*10*). In our first study, electroencephalographic (EEG) activity was recorded from the frontal neocortex in *lh/lh* mice and their littermates (*11*). Ninety-four percent (n = 72) of the *lh/lh* mice (all ages observed, 5 weeks to 5 months), but no *lh/+* or +/+ mice, exhibited bilaterally synchronous electrographic bursts (seizures) of 5- to 6-Hz spike-wave complexes (Fig. 1). The mean seizure duration was 1.5 s (range = 0.6 to 5.0 s), and the mean seizure frequency was 127 per hour (range = 43 to 239 per hour). The seizures were accompanied by immobility and reduced responsiveness that coincided with the duration of the electrographic burst. In contrast to earlier observations (*10*), no clonic activity or stereotypic automatisms were observed.

We tested the efficacy of anticonvulsants (*12*) against seizures in *lh/lh* mice (*13*). Compared to the vehicle control, anticonvulsants effective against absence seizures in humans produced a dose-dependent reduction in seizure frequency (ethosuximide, 400 mg per kilogram of body weight, 92% reduction, $P < 0.025$; trimethadione, 500 mg/kg, 80%, $P < 0.05$; and clonazepam, 30 μg/kg, 87%, $P < 0.025$) (Fig. 2, A and B) (*14*). The only behavioral effect of these doses was sedation produced by clonazepam (*15*).

In contrast, phenytoin and carbamazepine (anticonvulsants ineffective against absence seizures) did not reduce seizure frequency (Fig. 2C). Phenytoin's lack of effect occurred despite evidence of a central action (mild sedation) and despite mean serum levels of 21 μg/ml (*15*), within the range effective for other types of seizures (*12*). Phenytoin's lack of efficacy cannot be attributed to sedation because clonazepam was efficacious (Fig. 2, A and B) in spite of its similar sedative effect. In summary, the behavioral, electrographic, and pharmacologic features of seizures in *lh/lh* mice are similar to those of human absence seizures (*1*), validating the lethargic mouse as a genetic model of absence seizures.

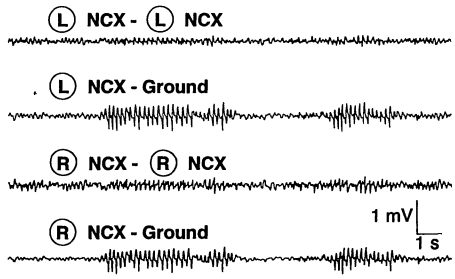

Fig. 1. EEG recording from the frontal neocortex (NCX) of spontaneous seizures in an awake, freely moving *lh/lh* mouse. Electrographic bursts (seizures) arose from and returned to a normal (waking) background. Monopolar recordings were derived from one pole of each NCX electrode and a skull-screw reference (low- and high-frequency filters, 1 and 30 Hz). L, left; R, right.

On the basis of the proposed role of $GABA_B$ receptors in deinactivating T-type Ca^{2+} channels (*3*), we hypothesized that $GABA_B$ receptor agonists would enhance the frequency of absence seizures in *lh/lh* mice, whereas $GABA_B$ receptor antagonists would inhibit them. Administration of the $GABA_B$ agonist (−) baclofen (active enantiomer in nonsalt form) produced a significant ($P < 0.001$) dose-dependent increase in seizure frequency in *lh/lh* mice (Fig. 3). The same doses of baclofen produced sedation but no seizures in matched, nonepileptic +/+ mice (n = 4), providing evidence against a nonspecific, proconvulsant effect. Administration of the systemically active $GABA_B$ receptor antagonist CGP 35348 (*16*) produced a dose-dependent decrease in seizure frequency in *lh/lh* mice. This decrease was significant after moderate doses [50 mg/kg given intraperitoneally (i.p.)] ($P < 0.05$); higher doses (100 mg/kg) virtually abolished ($P < 0.01$) absence seizures in *lh/lh* mice for up to 2 hours (Fig. 3). The only behavioral effect produced by CGP 35348 was a transient (10 min) period of torpor that began 5 min after injection. 2-Hydroxysaclofen (2-HS), a structurally different $GABA_B$ receptor antagonist (*17*), produced a dose-dependent reduction of seizure frequency that was significant ($P < 0.05$) at a total dose of 10 μg given intraventricularly (*18*) (Fig. 3). These data sup-

Table 1. $GABA_B$ receptor binding in *lh/lh* and +/+ mice. These values are the means of 12 paired samples; equivalent results were obtained in three different assays with different samples.

Receptor	K_d (nM)		B_{max} (pmol per milligram of protein)	
	lh/lh	+/+	*lh/lh*	+/+
$GABA_B$	59	58	4.3 ± 0.2*	3.4 ± 0.2*
NMDA	56	53	4.5 ± 11	3.6 ± 8
$GABA_A$	20	26	7.7 ± 0.2	8.1 ± 0.4

*$P < 0.025$, with values from *lh/lh* and +/+ membranes compared; independent t test for samples with unequal variances (*14*).

D. A. Hosford, S. Clark, Z. Cao, W. A. Wilson, Jr., F.-h. Lin, A. Huin, Departments of Medicine (Neurology) and Pharmacology, Duke University, and Veterans Administration Medical Centers, 508 Fulton Street, Building 16, Room 20, Durham, NC 27705.
R. A. Morrisett, Department of Pharmacology, University of Nebraska Medical Center, 600 South 42 Street, Omaha, NE 68198.
*To whom correspondence should be addressed.

port the hypothesis that activation of $GABA_B$ receptors is necessary for absence seizures in *lh/lh* mice.

One mechanism that could underlie the role of $GABA_B$ receptors in absence seizures is an alteration in the intrinsic binding properties of $GABA_B$ receptors. To test this hypothesis, we examined radioligand binding to the $GABA_B$ receptor in neocortical membranes (*19*). GABA-displaceable [^{3}H]baclofen binding in the presence of isoguvacine, a $GABA_A$ agonist, was used to measure $GABA_B$ receptors (*20*). Scatchard analyses revealed a small (26%) but significant ($P < 0.006$; $n = 11$ pairs; paired t test) increase in $GABA_B$ receptor number (B_{max}) from *lh/lh* mice (4.3 ± 0.2 pmol per milligram of protein) compared to age-matched +/+ mice (3.4 ± 0.2 pmol per milligram of protein). There was no significant difference in the equilibrium dissociation constant (K_d) of $GABA_B$ receptors (Table 1). We also measured *N*-methyl-D-aspartate (NMDA) receptor binding (NMDA-displaceable [^{3}H]glutamate binding) (*21*) and $GABA_A$ receptor binding (GABA-displaceable [^{3}H]muscimol binding) (*22*), which are altered in other models. There was no significant difference in the K_d or B_{max} of NMDA or $GABA_A$ receptors in *lh/lh* and +/+ mice (Table 1), which demonstrates that the increased number of $GABA_B$ receptors in *lh/lh* mice is selective.

To test for functional alterations in $GABA_B$ receptors in *lh/lh* mice, we chose an in vitro system with previously characterized $GABA_B$ receptor–mediated function (*23*). Thus, we measured $GABA_B$ receptor–mediated inhibition of NMDA excitatory postsynaptic potentials (EPSPs) in the stratum radiatum of area CA1 of hippocampal slices (*23*). After dissection, hippocampal slices were exposed to the non-NMDA antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX) and the $GABA_A$ channel blocker picrotoxin (PTX) (*24*). When paired stimuli were delivered with an interstimulus interval (ISI) of 200 ms to the Schaffer collateral afferents of area CA1, the amplitude of the second NMDA EPSP was depressed relative to that of the first (*24*). This paired-pulse depression of the second NMDA EPSP was mediated by $GABA_B$ receptors because it was blocked by the $GABA_B$ antagonist 2-HS in seven of seven slices (Fig. 4A) and was maximally suppressed at an ISI between 100 and 300 ms (*24*), consistent with the duration of $GABA_B$ receptor–mediated inhibitory postsynaptic potentials (*4*). We compared this paired-pulse depression in *lh/lh* and +/+ mice in a blinded manner (*24*). The paired-pulse depression was significantly greater ($P < 0.05$) in hippocampal slices from *lh/lh* mice (33%) ($n = 9$ pairs) than from age-matched +/+ mice (10%) (Fig. 4B). These data demonstrate that synaptically activated $GABA_B$ receptors in area CA1 of hippocampal slices inhibit NMDA EPSPs to a greater degree in *lh/lh* mice than in their nonepileptic counterparts.

In summary, the lethargic mouse: (i) is a genetic model of absence seizures that allows study of underlying mechanisms; (ii) has seizures enhanced by $GABA_B$ receptor agonists and inhibited by $GABA_B$ receptor antagonists; (iii) has a selective increase in $GABA_B$ receptors; and (iv) has enhanced synaptic responses mediated by $GABA_B$ receptors. These findings suggest a requirement for $GABA_B$ receptors in absence seizures in *lh/lh* mice and may indicate that enhanced synaptic activation of $GABA_B$ receptors underlies this effect. $GABA_B$ receptor antagonists inhibit absence seizures in other models of absence seizures (*25*). In contrast, baclofen and CGP 35348 do not significantly modify seizures in any models of partial or secondary generalized seizures (*26*). $GABA_B$ receptor antagonists hold promise as a new class of anticonvulsants for absence seizures.

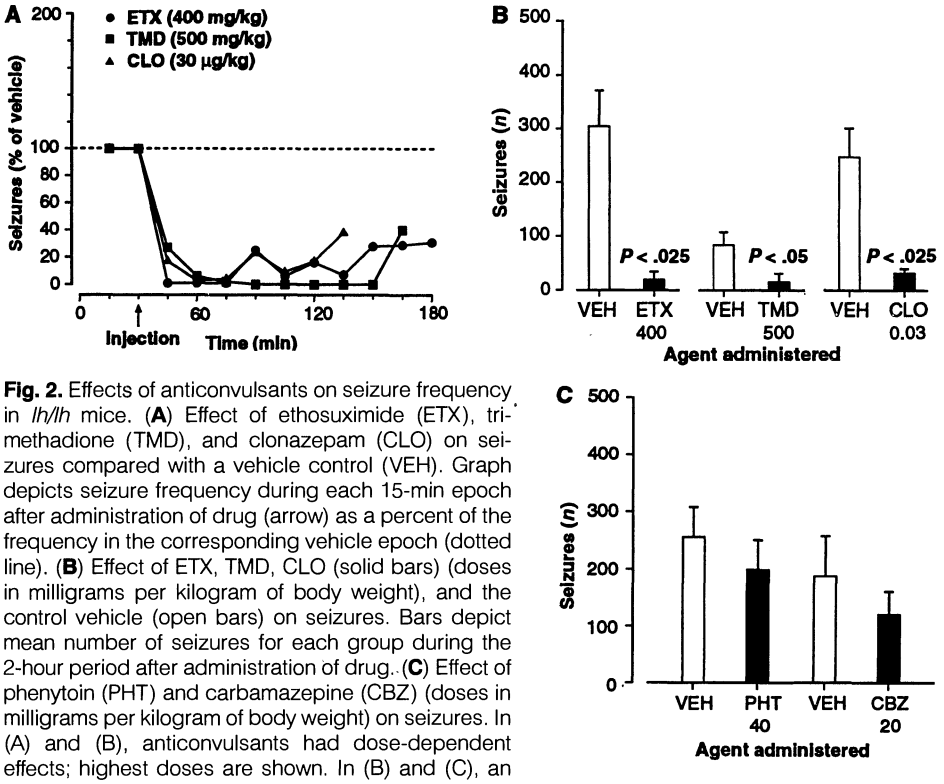

Fig. 2. Effects of anticonvulsants on seizure frequency in *lh/lh* mice. (**A**) Effect of ethosuximide (ETX), trimethadione (TMD), and clonazepam (CLO) on seizures compared with a vehicle control (VEH). Graph depicts seizure frequency during each 15-min epoch after administration of drug (arrow) as a percent of the frequency in the corresponding vehicle epoch (dotted line). (**B**) Effect of ETX, TMD, CLO (solid bars) (doses in milligrams per kilogram of body weight), and the control vehicle (open bars) on seizures. Bars depict mean number of seizures for each group during the 2-hour period after administration of drug. (**C**) Effect of phenytoin (PHT) and carbamazepine (CBZ) (doses in milligrams per kilogram of body weight) on seizures. In (A) and (B), anticonvulsants had dose-dependent effects; highest doses are shown. In (B) and (C), an independent t test for unequal variances was used to statistically compare the seizure frequency after vehicle or drug administration (*14*).

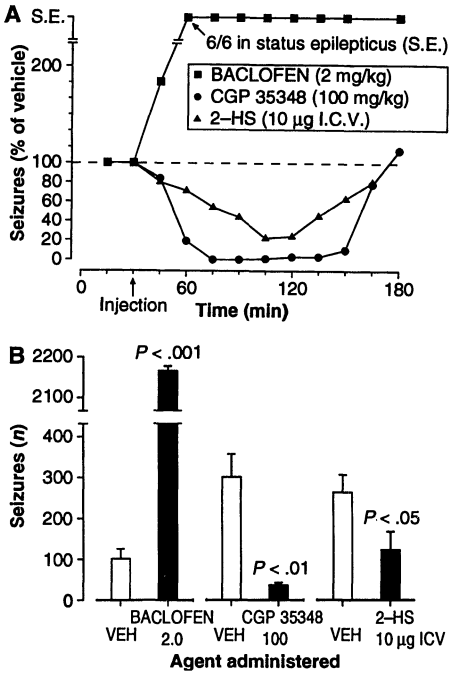

Fig. 3. Effects of $GABA_B$ receptor ligands on seizure frequency in *lh/lh* mice. (**A**) Effect of (−) baclofen, CGP 35348, and 2-HS on seizures. The effects were dose-dependent; highest doses are shown. (−) Baclofen (0.5, 2.0, and 10 mg/kg in 40% propylene glycol) and CGP 35348 (25, 50, and 100 mg/kg in 0.9% NaCl) were administered i.p.; 2-HS (total dose 0.5, 2.5, or 10 µg in 0.9% NaCl) was administered intraventricularly (ICV) (*18*). (**B**) The number of seizures during each 2-hour period after administration of the vehicle (open bars) (VEH) or drug (solid bars). Doses are as in (A). The seizure frequency during status epilepticus was arbitrarily assigned a score of 10 (a frequency ten times that after the vehicle); often, the actual score was higher than 10.

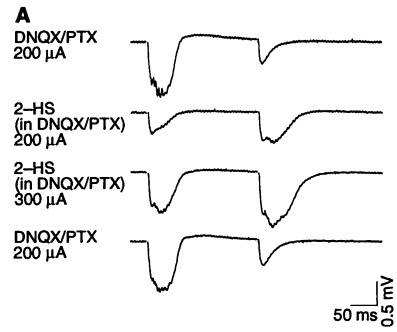

Fig. 4. Paired-pulse inhibition of NMDA EPSPs in the CA1 stratum radiatum of hippocampal slices from *lh/lh* and +/+ mice. (**A**) Extracellular recordings of paired EPSPs from a hippocampal slice of *lh/lh* (ISI = 200 ms). Top tracing: paired-pulse inhibition (PPI) of NMDA EPSPs (stimulus intensity = 200 μA); second tracing: abolition of PPI 10 min after adding 200 μM 2-HS; third tracing: persistent suppression of PPI by 2-HS (stimulus intensity = 300 μA to obtain NMDA EPSPs of amplitude similar to first tracing); and fourth tracing: return of PPI 15 min after washout of 2-HS. (**B**) Mean PPI in *lh/lh* slices (solid bar) compared to age-matched +/+ slices (open bar) (n = 9).

$GABA_B$ receptors could enhance absence seizures through diverse pre- and postsynaptic mechanisms. Presynaptic $GABA_B$ receptors may modulate absence seizures in the γ-hydroxybutyrate model (*25*), consistent with other disinhibitory actions of $GABA_B$ receptor agonists (*27*). Conversely, in the lethargic mouse, a selective increase in $GABA_B$ receptors and their enhanced postsynaptic responses could produce a more effective hyperpolarization. This could deinactivate T-type Ca^{2+} channels and trigger the synchronous neuronal burst firing of absence seizures (*3*, *6*). Although there was only a modest (26%) increase in the number of $GABA_B$ receptors in *lh/lh* mice, our measurements were made in membranes pooled from all neocortical regions. The $GABA_B$ receptor density in a particular fraction of these membranes may be increased to a greater degree. Identification of particular structures with increased $GABA_B$ receptors may be crucial to the understanding of the function of these receptors in absence seizures.

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10. As shown in early reports, *lh/lh* mice had seizures exhibiting motionless staring, clonic activity, or both [R. L. Sidman, M. C. Green, S. H. Appel, *Catalog of the Neurological Mutants of the Mouse* (Harvard Univ. Press, Cambridge, MA, 1965), p. 34] and bursts of 6-Hz spike-wave complexes [J. L. Noebels, *Adv. Neurol.* **44**, 97 (1986)].
11. Bipolar recording electrodes were implanted bilaterally into the frontal neocortex (1.0 mm posterior to the bregma, 1.0 mm lateral to the midline, and 1.0 mm below the dura) of 8-week-old male mice anesthetized with sodium pentobarbital (50 mg/kg). Recording sessions began 1 week later.
12. We chose three doses of each anticonvulsant to encompass median effective dose values against seizures in other models [S. M. Taylor, G. D. Bennett, L. C. Abbott, R. H. Finnell, *Eur. J. Pharmacol.* **118**, 163 (1985); A. H. Heller, M. A. Dichter, R. L. Sidman, *Epilepsia* **25**, 25 (1983); R. L. Krall, J. K. Penry, H. J. Kupferberg, E. A. Swinyard, *ibid.* **19**, 409 (1978)]. We administered ethosuximide (100, 200, and 400 mg/kg in 0.9% NaCl), trimethadione (100, 300, and 500 mg/kg in 0.9% NaCl), clonazepam (3, 10, and 30 μg/kg in 40% polyethylene glycol 400 mixed with 0.9% NaCl), phenytoin (10, 20, and 40 mg/kg in 0.9% NaCl), and carbamazepine (5, 10, and 20 mg/kg in 12.5% propylene glycol).
13. Groups of 8-week-old male mice (n = 6 per group; one group per drug) underwent six 3-hour EEG recording sessions. Successive doses of an anticonvulsant were administered [5 ml per kilogram of body weight (ml/kg) i.p.] to each group 30 min after the beginning of recordings on days 2, 4, and 6. Vehicle (5 ml/kg i.p.) was administered on days 1, 3, and 5. Recordings were divided into twelve 15-min epochs, and a blinded observer counted bursts as seizures if they: (i) were comprised of epileptiform spikes (<70 ms per spike), (ii) had a typical frequency (5 to 6 Hz), (iii) had a duration >0.6 s, and (iv) were recorded in at least two channels simultaneously. Interobserver reliability was >90%.
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15. During sessions, animals were observed for sedation, increased ataxia, immobility, and stereotypic or clonic activity. One hour into each session, 30-μl blood samples were obtained from the retro-orbital sinuses. Serum-acetonitrile mixtures were separated by high-pressure liquid chromatography (HPLC) to measure anticonvulsant levels, as modified from P. M. Kabra, B. E. Stafford, and L. J. Marton [*Clin. Chem.* **23**, 1284 (1977)].
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18. Twenty-two–gauge guide cannulae were cemented bilaterally over the lateral ventricles (0.5 mm posterior to the bregma; 1.0 mm lateral to the midline; cannula tip 1.0 mm below the dura and 0.8 mm above the ventricles) of anesthetized (sodium pentobarbital; 50 mg/kg i.p.) 8-week-old male *lh/lh* mice. A bipolar EEG electrode was implanted into the right frontal neocortex (*11*). One week later, injection cannulae were inserted into the ventricles of awake animals under gentle, hand-held restraint; Hamilton syringes were used to inject the drug or vehicle bilaterally (0.5 μl each side; 0.5 μl per minute). Histologic placement was verified by intraventricular injections of Evan's blue dye after the last experiment.
19. Anesthetized (chloroform) 5-week-old male *lh/lh* and +/+ mice were decapitated; neocortical tissue was dissected and prepared as per D. R. Hill and N. G. Bowery [*Nature* **290**, 149 (1981)]. The protein concentration of the final resuspension was 500 to 1500 μg/ml [O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951)].
20. Methods were validated by a demonstration of appropriate equilibrium curves, pH, and ion requirements for $GABA_B$ receptors (*19*). Specific binding (65% of total) displayed appropriate displacement by ligands for $GABA_B$ receptors but not by ligands for $GABA_A$, NMDA, or quisqualate receptors. For binding isotherms, incubations (20 min at 25°C) included membranes, [^{3}H]baclofen (2 to 500 nM), with (nonspecific binding) or without (total binding) 500 μM GABA (*19*). After centrifugation (15,000*g* for 10 min at 4°C) and washing, bound ligand was quantitated (liquid scintillation spectrometry).
21. NMDA receptor binding was measured as described [G.-C. Yeh, D. W. Bonhaus, J. V. Nadler, J. O. McNamara, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 8157 (1989)].
22. $GABA_A$ receptor binding was measured with modifications from C. Shin and co-workers [*J. Neurosci.* **5**, 2696 (1985)].
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24. Paired 5-week-old male *lh/lh* and +/+ mice were anesthetized (chloroform) and decapitated; hippocampal slices were prepared as described (*23*). The hippocampal slices in a superfusion chamber were perfused at 29°C (*23*). A tungsten electrode in the stratum radiatum of CA1 delivered paired pulses (0.1-ms biphasic square waves) to Schaffer collaterals. EPSPs were recorded extracellularly in the stratum radiatum of CA1 with the use of glass microelectrodes (impedance 2 to 10 megohms) filled with 150 mM NaCl. After obtaining stable responses, we added 10 μM DNQX and 30 μM PTX to the perfusions. Subsequent EPSPs were mediated primarily by NMDA receptors, because 100 μM D-aminophosphonovalerate reduced the amplitude of the EPSPs by 77% in seven of seven slices. There was no significant difference in the amplitude of single (nonpaired) NMDA EPSPs in *lh/lh* and +/+ slices. We varied the currents (50 to 1500 μA) to obtain maximal, stable responses (ISI = 200 ms); the ISI was then varied from 50 to 1000 ms. Waveforms obtained with 200-ms ISIs were used to measure paired-pulse depression (amplitude of the second NMDA EPSP divided by that of the first). Paired-pulse depression was compared between paired *lh/lh* and +/+ slices for statistical significance (Mann-Whitney U test) (*14*).
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SWD in: (i) a rat genetic model of absence seizures (intrathalamic administration) [Z. Liu, M. Vergnes, A. Depaulis, C. Marescaux, *Neuroscience* **48**, 87 (1992)] and (ii) two pharmacologic models of absence seizures (i.p. administration) [O. C. Snead, *Eur. J. Pharmacol.* **213**, 343 (1992)].
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28. B63HF1 is the F_1 from C57Bl/6JEi females crossed with C3H/HESnJ males. We refer to the B63HF1 strain, with which *lh/lh* is congenic, as wild or +/+. We maintain *lh/lh* and +/+ mouse colonies at the Duke University Vivarium, using stocks from Jackson Laboratory (Bar Harbor, ME).
29. Supported by grants from the Veterans Administration (D.A.H. and W.A.W.) and NIH (D.A.H., W.A.W., and R.A.M.). We thank H. Bittiger for the gift of CGP 35348 [European patent 463560-A1 (2 January 1992); priority CH-002092 (22 June 1990)]; V. H. MacMillan for HPLC measurements; N. Lambert, D. V. Lewis, J. O. McNamara, D. D. Mott, C. Shin, and H. S. Swartzwelder for critical reading and discussion; and B. Worrell and S. Sneed for administrative assistance.

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Nitric Oxide: A Physiologic Mediator of Penile Erection

Arthur L. Burnett, Charles J. Lowenstein, David S. Bredt, Thomas S. K. Chang, Solomon H. Snyder*

Nitric oxide (NO) is a cytotoxic agent of macrophages, a messenger molecule of neurons, and a vasodilator produced by endothelial cells. NO synthase, the synthetic enzyme for NO, was localized to rat penile neurons innervating the corpora cavernosa and to neuronal plexuses in the adventitial layer of penile arteries. Small doses of NO synthase inhibitors abolished electrophysiologically induced penile erections. These results establish NO as a physiologic mediator of erectile function.

Nitric oxide mediates bactericidal and tumoricidal actions of macrophages (*1*) and blood vessel relaxation of endothelial cells (*2*). NO may also be a major neuronal messenger (*3*). Immunohistochemical studies localize NO synthase (NOS) to neurons in the brain as well as to discrete populations of autonomic nerves in the periphery (*4*), where NO fulfills most characteristics of a neurotransmitter. For instance, NOS is highly localized to cell bodies and fibers of the myenteric plexus of the gastrointestinal pathway (*4*). The nonadrenergic, noncholinergic relaxation evoked by physiologic stimulation of myenteric plexus neurons is potently and selectively blocked by NOS inhibitors (*5*).

Penile erection is thought to involve parasympathetic, neuronally mediated relaxation of the blood vessels as well as of the trabecular meshwork of smooth muscle that comprises the corpora cavernosa (*6*). The neuronal chemical mediator of erection has not been established. Vasoactive intestinal polypeptide (VIP) occurs in limited populations of nerve fibers in the penis (*7*), but direct administration of VIP does not fully mimic physiologic erection (*8*). In isolated smooth muscle from the corpus cavernosa of several species, relaxation evoked by electrical field stimulation could be blocked by NOS inhibitors, as reported in some studies (*9*) but not in others (*10*). Blockage of relaxation by NOS inhibitors can establish NO as a mediator of cavernosal muscle relaxation but does not permit conclusions as to whether it is a neuronal, transmitter-like messenger and a physiologic mediator of erection.

Several portions of the genitourinary tract of rat displayed substantial NOS activity, monitored by the conversion of [^{3}H]arginine to [^{3}H]citrulline (Table 1). High concentrations in the pelvic plexus, referred to in the rat as the major pelvic ganglion, suggest a neuronal role for NOS. Amounts of NOS in the membranous urethra exceeded amounts in the pelvic plexus and were three to four times larger than those in the penis and the bladder neck and considerably larger than those in the prostate. This regional distribution of NOS activity was confirmed by protein immunoblot (*11*).

We conducted immunohistochemical staining of rat penile tissue (Fig. 1) with an antiserum that is highly selective for NOS and stains NOS specifically in a variety of rat peripheral tissues and in brain tissue (*4*). All immunohistochemical staining of NOS in penile tissues was blocked by preabsorption with recombinant NOS protein (*11*).

The antibody to NOS stained the pelvic plexus and its axonal processes that form the cavernous nerve (Fig. 1A), located immediately adjacent to the deep cavernosal artery, the major arterial source of the corpus cavernosum. In the proximal penis, the nerve plexus in the adventitia of the deep cavernosal arteries stained prominently as did neuronal processes in the sinusoids and the periphery of the corpora cavernosa (Fig. 1B). This staining circumscribed the corpora cavernosa directly below their fibrous capsules, the tunica albuginea. Neuronal staining of the deep cavernosal arteries continued as the arteries subdivided into the intracorporal network of helicine arteries (Fig. 1D). In the most distal portion of the corpora, staining diminished as the helicine arteries were replaced with cavernous spaces (Fig. 1E). The distal part of the penis superficial to the corpora cavernosa contained dorsal penile nerve fibers that stained for NOS (Fig. 1, E and F). Dorsal penile and cavernosal arteries stained for NOS both in the adventitial and endothelial layers, although endothelial staining was faint in the cavernosal vessels. NOS staining in the urethra was associated with neuronal fibers coursing through the smooth muscle or the submucosal vasculature or both (Fig. 1E) and fits with the substantial urethral NOS catalytic activity (Table 1).

The neural specificity of NOS staining was established by bilateral cavernous nerve transection, after which we no longer observed penile neurons stained for NOS (Fig. 1C), although endothelial staining persisted (*11*). To ensure that this distribution was not species-specific, we conducted immunohistochemical localizations of NOS from the penes of dogs and demonstrated essentially identical localizations to nerve plexuses in the adventitial layers of penile arteries and the dorsal nerves of the dog penes.

The localization of NOS to neuronal fibers innervating blood vessels and the corpora cavernosa of the penis suggested a possible role for NO as a neuronal mediator of erection. We examined this possibility in a rat model of penile erection in which we electrically stimulated the cavernous nerves of intact rats by using optimal parameters that evoked physiologic erection (*12*). L-Nitroarginine, a potent and selective inhibitor of NOS, markedly diminished penile erections (Table 2). As little as 1 mg per kilogram of body weight (mg/kg) administered intravenously (i.v.) significantly reduced erection, and 2.5 mg/kg produced more than a 50% reduction. At 5 mg/kg, nitroarginine almost completely inhibited erection. Intravenous bolus injections of

A. L. Burnett and T. S. K. Chang, Department of Urology, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205.
C. J. Lowenstein, Department of Cardiology, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205.
D. S. Bredt and S. H. Snyder, Departments of Neuroscience, Pharmacology, and Molecular Sciences, and Psychiatry, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205.
*To whom correspondence should be addressed.