Erectile Dysfunction in a Murine Model of Sleep Apnea

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Rationale: **Erectile dysfunction (ED) is frequent in obstructive sleep apnea syndrome (OSAS). Chronic intermittent hypoxia (CIH), one of the hallmarks of OSAS, could mediate ED.**

Objectives: **To determine whether intermittent hypoxia during sleep affects erectile dysfunction in mice.**

Methods: **Three groups of C57BL/6 mice were exposed to CIH for 5 or 24 weeks. Sexual function was evaluated by *in vivo* telemetry of corpus spongiosum pressure. Spontaneous erections, sexual activity during mating, and noncontact tests were assessed after 5 weeks of CIH and after treatment with tadalafil. Plasma testosterone was measured after 8 and 24 weeks of CIH, and the expression of nitric oxide synthase (NOS) isoforms was examined in penile tissue.**

Measurements and Main Results: **Noncontact, spontaneous, and contact sexual activity in the mice was suppressed after CIH. Spontaneous erection counts decreased after the first week of CIH by 55% ($P < 0.001$) and remained unchanged thereafter. Recovery of erectile activity during normoxia for 6 weeks was incomplete. Compared with control mice, latencies for mounts and intromissions increased by 60- and 40-fold, respectively ($P < 0.001$), and the sexual activity index decreased sixfold. Tadalafil treatment significantly attenuated these effects. Immunoblot analyses of NOS proteins in the erectile tissue showed decreased expression of endothelial NOS after CIH ($P < 0.01$), with no changes in plasma testosterone levels after 8 and 24 weeks of CIH.**

Conclusions: **CIH during sleep is associated with decreased libido in mice. The decreased expression of endothelial NOS protein in erectile tissue and the favorable response to tadalafil suggest that altered nitric oxide mechanisms underlie CIH-mediated ED. No changes in testosterone emerge after intermittent hypoxia.**

Keywords: nitric oxide synthase; erectile dysfunction; sleep apnea; intermittent hypoxia

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Erectile dysfunction is a frequent occurrence in male patients with obstructive sleep apnea; however, potential mechanisms mediating this morbidity are currently unknown.

What This Study Adds to the Field

A novel murine model that allows for sexual function assessment in freely behaving males was exposed to a model of sleep apnea and showed the emergence of marked alterations in sexual drive and erectile dysfunction that was responsive to tadalafil.

Obstructive sleep apnea (OSA), which consists of the repetitive occurrence of upper airway obstruction during sleep, is characterized by chronic intermittent hypoxia (CIH), and affects 2 to 4% of the adult population (1). Erectile dysfunction (ED) is a highly prevalent condition in patients with OSA syndrome (OSAS), and its frequency and severity appear to correlate with the severity of OSAS (2, 3). The beneficial effects of continuous positive airway pressure (CPAP) on ED among patients with OSAS suggest a potential mechanistic role for the episodic hypoxemic events during sleep.

Penile erection is a neurovascular event caused by a variety of stimuli and maintained by the interaction of central nervous system components (medial preoptic area, paraventricular nucleus of the hypothalamus, and spinal cord at T12-L3) (4–6), the peripheral nervous system, and the regulation of the smooth muscle activity within the penile vasculature and surrounding erectile tissues. In the absence of sexual stimuli, the penis is maintained in a flaccid state through predominantly sympathetic outflow and smooth muscle contraction. The principal neurotransmitter for penile erection is nitric oxide (NO), which is released from nonadrenergic, noncholinergic neurotransmission of the cavernous nerves and the endothelium (7, 8). Both constitutively expressed neuronal and endothelial NO synthase (nNOS and eNOS, respectively) isoforms mediate penile erection by producing NO from L-arginine. nNOS initiates cavernosal tissue and vascular relaxation, whereas eNOS may further facilitate blood flow into erectile tissue and maintain erection (9). NO in the smooth muscle cells binds to guanylate cyclase and catalyses cyclic guanosine monophosphate (cGMP) formation from guanosine triphosphate (GTP), which is responsible for the effect (10). Phosphodiesterase enzyme type 5 (PDE5) metabolizes cGMP into 5'GMP, which inhibits the erectile process. Tadalafil (similar to other PDE5 inhibitors such as sildenafil and vardenafil) potentiates the physiologic erectile response to sexual stimulation by decreasing the catalytic activity of PDE5, thus increasing intracellular cGMP concentrations (11). However, the expression of the nNOS isoform is required for sildenafil-induced facilitation of erectile responses *in vivo* in mice (12).

Recent animal studies have further emphasized the importance of testosterone for timely initiation and cessation of erectile activity through regulation of NOS and PDE5 gene expression (13, 14). Testosterone is necessary for normal libido and for quantitatively and qualitatively normal erections by facilitating (either directly or via its metabolites) normal penile vasodilation and tumescence in response to sexual stimuli (15–17). A conversion of testosterone to estradiol by action of aromatase is critical for sexual behavior patterns, including mount, intromission, and ejaculation (18, 19).

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The consequences of CIH during sleep on sexual behavior, including insights into mechanisms by which CIH could affect sexual behavior and erections in OSA, are not known. In particular, it is not clear whether CIH induces ED, whether NOS expression is altered, whether administration of a PDE5 inhibitor yields improved erectile function, and finally whether CIH-induced changes in erectile function, if any, are testosterone dependent.

In the present study, we used a well-validated rodent model of OSA that involves exposures to intermittent hypoxia (IH) during daylight hours (20–23) to evaluate the impact of CIH on sexual function and erectile activity. To this effect, we took advantage of a murine model developed in our laboratory that enables study of erections in unrestrained, freely behaving mice during spontaneous, mating, and noncontact activity by telemetric recording of corpus spongiosum pressure (CSP) changes in the penile bulb (24, 25). Here, we report on sexual activity changes in mice after exposures to CIH and the effects of treatment with the long-acting PDE5 inhibitor tadalafil. Plasma testosterone levels and expression NOS isoforms in the erectile tissue were also evaluated.

METHODS

All experimental procedures were in compliance with the Institutional Animal Use and Care Committee of the University of Louisville, and were in accordance with National Institutes of Health requirements for care and use of laboratory animals.

Experimental Animal Groups and Protocols

C57BL/6 male (23–27 g) and female (20–22 g) mice (Charles River Laboratories, Wilmington, MA) were housed in facilities operating at a 12:12-hour light:dark cycle (light hours, 7:00 A.M.–7:00 P.M.). Mice had free access to the standard chow and water. *See* Figure 1 for timeline.

Behavioral studies. Erectile activity was studied *in vivo* in three sexual behavioral contexts (spontaneous erections, noncontact stimulation, and mating tests with a female mouse). Behavioral tests were conducted after 5 weeks of exposure to CIH (n = 8).

Plasma sex hormones. Total plasma testosterone and estradiol were measured in normoxic (room air [RA]) mice (n = 8) and in CIH mice (after exposures lasting either 8 or 24 wk, n = 8/group).

Structural evaluation of testes. Seminiferous tubules and Leidig and Sertoli cells were examined using light microscopy in RA mice (n = 8) and in CIH mice (after exposures lasting 8 or 24 wk, n = 8/group).

Assessment of nNOS, inducible NOS, and eNOS in penile tissue. Tissue evaluation was performed in RA mice (n = 8) and in CIH mice after exposure for 8 weeks (n = 12).

Tadalafil treatment. The long-acting PDE5 inhibitor tadalafil (Eli Lilly and Co., Indianapolis, IN) was initially given orally in a mixture with peanut butter and standardized at 0.014 mg/25 g body weight (i.e., equivalent to a dose of 40 mg for humans), and given to mice after 5 weeks of CIH exposures.

Behavioral Studies

Penile erections, measured as increased bursts of CSP, were recorded in freely moving mice using telemetry, as recently described (24).

Spontaneous erections. CSP was continuously recorded 24 hours per day in freely moving mice (one mouse per cage). After recording baseline CSP activity in normoxia (RA; 72 h), mice were exposed to CIH for 8 weeks, and then returned to RA for an additional 6 weeks. Count per day of spontaneous erections was evaluated in the baseline, after CIH, and after treatment with tadalafil in both RA and CIH mice.

Contact (mating) tests. Contact (mating) tests with a female mouse were conducted in the male home cage in RA and after 5 weeks of CIH. The baseline CSP was recorded for 10 minutes and after a receptive female was placed into the cage for 24 hours. Latencies to mounts (M), intromissions (I), ejaculations, and sexual activity index (I/[I+M]) were evaluated. Flaccid and tumescence pressures and maximal pressures during full erections (P > tumescence pressures +100 mm Hg) were analyzed at baseline, after CIH, and after treatment with tadalafil for CIH-exposed mice. Estrous phase in females was induced by estradiol (0.02 ml, 2 mg/ml; Sigma, St. Louis, MO) and progesterone (0.01 ml, 50 mg/ml; Sigma) dissolved in sesame oil and injected subcutaneously 24 hours and 4–5 hours before the test, respectively.

Noncontact test ("psychogenic" response). Tests were conducted in the male mouse home cage in RA, after 5 weeks of CIH, and after treatment with tadalafil for CIH-exposed mice. After CSP baseline recordings for 5 minutes, a female mouse was placed into the cage for the 20-minute test. Mice were separated from each other by a two-layer (distanced at 1 cm) wire mesh and thus were prevented from physical contact, while olfactory, visual, and auditory sensing continued. Short, 0.4–0.5-second CSP peaks with amplitudes of 80–120 mm Hg appeared in the records of CSP after placement of a female mouse. Peaks were counted for each minute interval during the 20-minute test in RA-exposed mice, in CIH-exposed mice, and in CIH-exposed mice after treatment with tadalafil.

Telemetry Transducer Implantation

Procedures were performed as described earlier (24, 25). Briefly, male mice (23–27 g) were anesthetized with pentobarbital (50 mg/kg, intraperitoneally). Incisions were made along the abdominal and perineal midline. Sterile telemetry transmitters (PhysioTel, model TA11PA-C20; DSI, St. Paul, MN) were coated with biologically inert material, placed intraperitoneally, and secured to the abdominal wall by three stitches during incision wound closure. The bulbospongiosus muscle overlaying the bulb of the corpus spongiosum was exposed, and the distal portion was gently retracted so that a small area over the tunica albuginea of the distal bulb could be visualized. The wall of the bulb was punctured with a 23-gauge needle, and the catheter tip was carefully advanced for 2 to 3 mm inside the corpus spongiosum, and secured with 1 to 2 drops of tissue adhesive (Vetbond; DSI). After the completion of surgical procedures, mice recovered for 7 days before recordings were initiated.

CIH

Animals were exposed to IH in chambers operating under a 12-h light:dark cycle (Oxycycler model A44XO; Biospherix, Redfield, NY). Oxygen concentrations in the chambers were automatically adjusted according to the desired profile by a computerized system controlling the gas valve outlets. IH consisted of alternating 21% O_2 and 7.8% O_2 every 360 seconds for 12 hours per day during daylight (22, 23). Total air-exchange intervals during these experiments were approximately 240 seconds (for 7.8% O_2) and 120 seconds (for room air), resulting in 10 hypoxic events per hour of exposure. Deviations from the desired O_2 concentration were adjusted by addition of N_2 or room air through the solenoid valves. For the remaining 12 hours of the dark period, O_2 concentrations were kept at 21%. Ambient CO_2 in the chamber was maintained at less than 0.03%. The cycling oxygenation profiles aimed to reproduce repetitive oxyhemoglobin desaturations to mimic moderate to severe apneic episodes during sleep. Indeed, in a separate group of four mice, oxyhemoglobin saturation (Sp_{O_2}) was measured in restrained waking conditions while exposing the animals to the IH protocol. Mean nadir Sp_{O_2} values revolved around 76 to 78% for all mice.

Testosterone and Estradiol Assessment

Mice were anesthetized with pentobarbital (50 mg/kg). Blood obtained by intracardiac puncture was allowed to clot, and then centrifuged at 4,000 rpm for 15 minutes to separate serum. Serum total testosterone was measured using a commercial solid-phase RIA (Coat-A-Count Total Testosterone, product code TKTT1; Diagnostic Products Corp., Los Angeles, CA) with a sensitivity of 0.4 ng/ml, and intra- and interassay coefficients of variation of less than 5% and less than 9%, respectively. Serum estradiol was measured using a commercial kit 3RD Generation Estradiol RIA (catalog no. DSL-39100; Diagnostic Systems Laboratories, Inc., Webster, TX) with a sensitivity of 0.6 pg/ml intra- and interassay variability of less than 6% and less than 10%, respectively.

Hematoxylin-and-Eosin Staining of Testes

Testicular specimens were fixed overnight in Bouin's solution and, after a serial dehydration in alcohol (80–100%) and xylene, were embedded in paraffin. Five-micrometer sections were cut and stained with ordinary hematoxylin and eosin (HE). The structure of seminif-

erous tubules and the morphology, the number, the distribution, and the proportion of different-stage germ cells (Sertoli cells) were viewed on the HE-stained sections.

Western Immunoblotting

Penile tissues from CIH mice (8-wk exposure) and controls (RA) were homogenized at 0°C with a tissue blender in radioimmunoprecipitation (RIPA) buffer (1% nonylphenoxylpolyethoxylethanol [NP]-40, 0.55 deoxycholate, 1% sodium dodecyl sulfate [SDS], 1 mM sodium orthovanedate, 0.5 ml phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 20 μg/ml leupeptin, and volume made in 10 ml of phosphate-buffered saline) in a ratio of 1:4 wt/vol. Homogenates were centrifuged at 14,000 rpm for 10 minutes and supernatants or soluble fractions were collected, and subjected to protein content estimation using the Bradford method (DC-Bio-Rad protein assay, BioRad Laboratories, Hercules, CA). Proteins (50 μg/sample) were then separated on SDS–polyacrylamide gel electrophoresis, 8–16% Tris-glycine gels (Invitrogen, Carlsbad, CA), and transferred on a 0.2-μM nitrocellulose membrane. Membranes were blocked for 1 hour in a 5% nonfat dry milk solution in TBS-Tween (Boston BioProducts, Worcester, MA). After overnight incubations with rabbit polyclonal antibodies of nNOS (1:500), inducible NOS (iNOS) (1:500), or eNOS (1:500) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), membranes were washed and incubated for 1 hour with a horseradish peroxidase–labeled goat anti-rabbit antibody (1:5,000; Santa Cruz Biotechnology). In initial experiments, a positive control was included. Proteins were visualized by enhanced chemiluminescence (Amersham, Piscataway, NJ), and semiquantitative analysis of each of the NOS isoform bands was performed by scanning densitometry. In addition, blots were reprobed with a monoclonal antibody against β-actin for further standardization of potential loading inequalities. At least six different experiments were conducted for NOS isoform.

Data Analysis

Data were tabulated, and responses were compared using analysis of variance or Student's *t* tests followed by Newman-Keuls *post hoc* tests. A P value of less than 0.05 was considered to achieve statistical significance. The results are expressed as mean ± SEM.

RESULTS

NOS Isoform Expression

There was a significant decrease in eNOS expression in rats exposed to CIH for 8 weeks (Figure 2; $P < 0.01$) but not at 4 weeks (data not shown), as compared with the control group (RA). No differences in nNOS and iNOS immunoreactivity emerged in CIH-exposed mice.

Sex Hormone Concentration

No changes were found in the level of total serum testosterone (both 8- and 24-wk exposures) and estradiol after 8 weeks of CIH exposure compared with normoxic mice (Table 1). However, there was a significant increase in estradiol concentrations in male mice exposed to CIH for 24 weeks (Table 1).

Sexual Activity

Spontaneous erections. The count of spontaneous erections at baseline was 29 ± 3/day. After 1 week of CIH, the number of daily spontaneous erections decreased by 55% ($P < 0.001$; Figure 3), remaining close to this level for the following weeks of CIH exposure (Figure 3). Six weeks of recovery in normoxia after CIH were associated with a return of the number of daily spontaneous erections to within 74.1% of baseline levels (Figure 3). Treatment with tadalafil increased the number of spontaneous erections in control mice by 19%, and in mice exposed to 5 weeks of CIH by 38.9%, with essentially normalization of spontaneous erectile activity with treatment ($P > 0.05$ compared with RA; Figure 4).

Noncontact tests. After 5 weeks of CIH exposure, noncontact sexual activity was significantly suppressed (Figure 5). Tadalafil administration was accompanied by significant improvements in activity in the CIH mice (Figure 5).

Mating tests. After 5 weeks of CIH exposures, there were significant increases in the latencies to mounts (>60-fold; $P < 0.001$) and in the latencies to intromissions (>40-fold; $P < 0.001$). This was also reflected in a marked decrease in the I/M+I index (6-fold; $P < 0.001$; Figure 6). Of note, one of the seven mice exposed to CIH retained normal sexual activity. In five out of seven CIH-exposed mice, ejaculations did not occur; in one mouse, the latency to ejaculation increased to 660 minutes, and in another mouse, the latency was unaltered by CIH. These parameters were favorably affected by treatment with tadalafil (Figure 6). Indeed, administration of tadalafil increased tumescence pressure in the CIH-exposed mice ($P <$

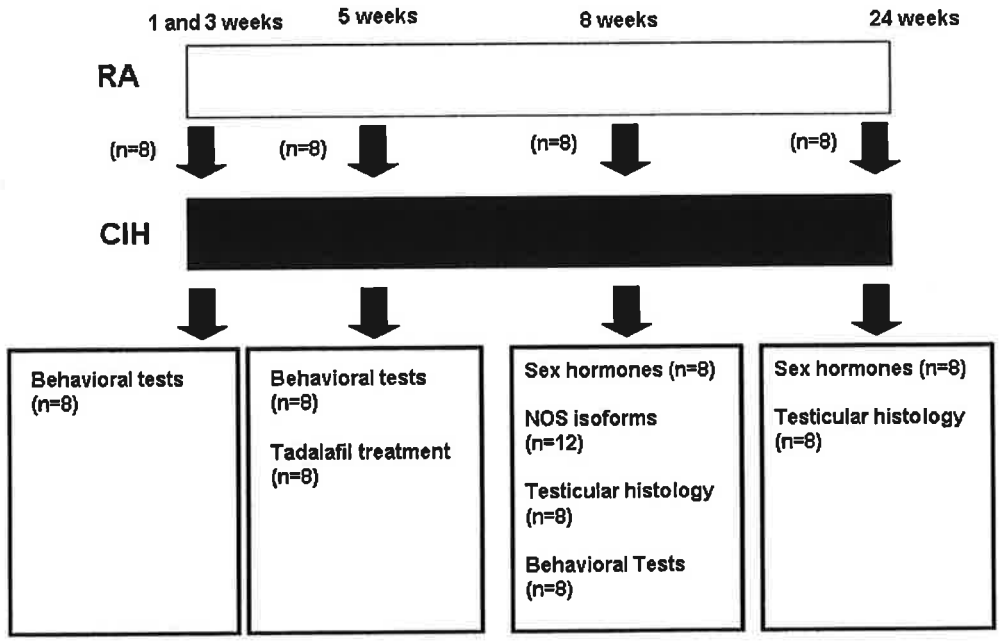

Figure 1. Schematic timeline for the various groups of mice exposed to either room air (RA) or chronic intermittent hypoxia (CIH) and the various tests administered at each time point. Number of animals per time point in indicated in parentheses. NOS = nitric oxide synthase.

TABLE 1. SERUM CONCENTRATIONS OF TESTOSTERONE AND ESTRADIOL IN ROOM AIR– AND CHRONIC INTERMITTENT HYPOXIA–EXPOSED MICE

	Testosterone (*ng/dl*)	n	Estradiol (*pg/ml*)	n
Two months				
RA	24.6 ± 0.6	6	15.6 ± 0.5	6
CIH	22.6 ± 0.1	6	15.5 ± 1.6	6
Six months				
RA	158.2 ± 52.0	10	19.1 ± 1.0	13
CIH	159.4 ± 50.8	9	22.7 ± 0.5*	12

Definition of abbreviations: CIH = chronic intermittent hypoxia; RA = room air.
Data are presented as mean ± SE.
* $P < 0.05$ vs. RA.

0.05), and showed a trend toward effective increases in erectile maximal pressures (Table 2).

HE Staining of Testes

When blindly compared with normoxic mice, sections stained with HE showed normal testicular structure and spermatogenesis, as well as normal Sertoli and stromal cells in and around the seminiferous tubules, such that the two exposure conditions were indistinguishable.

DISCUSSION

This study reports the occurrence of ED in a murine model of OSA. Our findings implicate a multifactorial role for CIH in the complex impairments of erectile function that have been reported in patients with OSA, since altered elements of erectile function, including reduced eNOS expression, and sexual drive emerged among mice exposed to CIH during sleep.

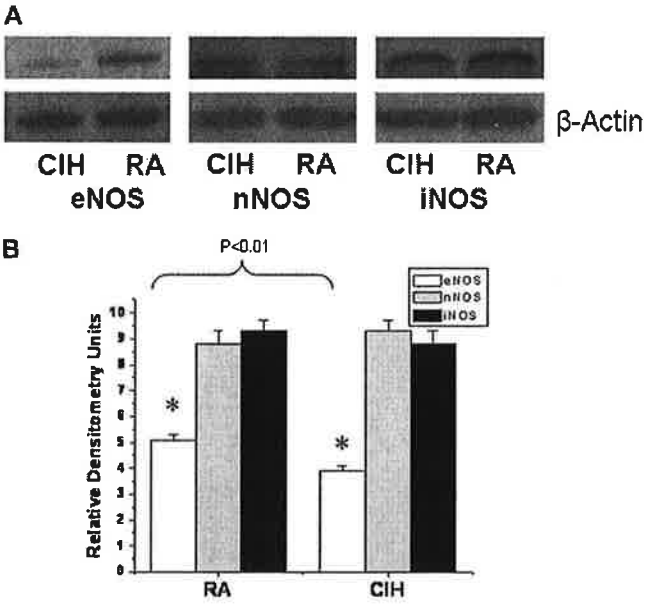

Figure 2. Effect of chronic intermittent hypoxia (CIH) on nitric oxide synthase (NOS) isoform protein levels in the penile tissue of mice. (*A*) Representative Western blots are shown for control (room air [RA]) and CIH for 8 weeks in mice. Blots were reprobed with a monoclonal antibody against β-actin for standardization of loading inequalities. (*B*) Quantification of immunoreactivity in the penile tissue from mice exposed to CIH (n = 12) and RA (n = 8). Immunoreactivity is expressed in mean densitometry units corrected for β-actin (relative units). eNOS = endothelial NOS; nNOS = neuronal NOS; iNOS = inducible NOS. *$P < 0.01$.

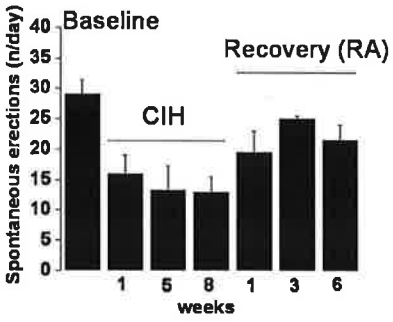

Figure 3. Spontaneous erections in mice during exposure and recovery from chronic intermittent hypoxia (CIH). Effects of CIH on spontaneous erectile activity were measured on Weeks 1, 5, and 8, and during normoxic (room air [RA]) recovery on Weeks 1, 3, and 6. n = 8/group.

Furthermore, CIH-related effects were not testosterone dependent, and did not appear to be associated with disruption of testicular anatomy, including the number and appearance of Leidig and Sertoli cells.

Before we discuss the potential significance of our findings, several technical issues deserve comment. First, our chronic model allows for naturalistic observation and quantification of sexual behavior in male mice. Erectile events in mice are organized in clusters, and the rhythmic nature of these events underlies the activity of one or more spinal generators (26–28) involving parasympathetic, sympathetic, and somatic pathways (29), all of which are under both inhibitory and facilitatory control (30–33). Thus, although dissection of the effects of CIH on each of the neural pathways is not possible in a freely behaving mouse model, substantial insights into the coordinated activity of the system and exploration of the potential link between OSA (or rather of its surrogate CIH) and ED were possible. Second, we have only explored the consequences of longstanding CIH, such that the impact of sleep fragmentation, IH-induced sleepiness and altered mood, and the potential interactions between CIH and sleep disruption on erectile function remain to be elucidated. We should also point out that the frequency of catheter technical malfunction markedly increases after 5 to 6 weeks after implantation, a factor that dictated pharmacologic intervention within this temporal window to optimize reliability of pressure measurements, and to minimize the number of mice used.

CIH-related impairments of erectile function could involve deficits in the following areas: (*1*) central neural mechanisms, (*2*) peripheral neural mechanisms (i.e., autonomic nervous system and noncholinergic, nonadrenergic transmission), and (*3*) peripheral erectile tissue (34).

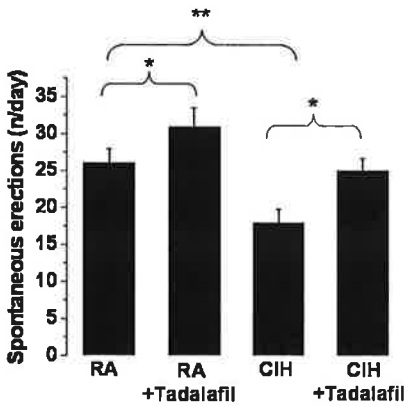

Figure 4. Effects of tadalafil on spontaneous erections in mice exposed to chronic intermittent hypoxia (CIH) for 8 weeks and in control mice. RA = room air. n = 8 mice/group. *$P < 0.01$, **$P < <0.001$.

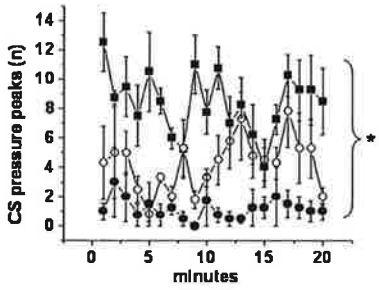

Figure 5. Effects of chronic intermittent hypoxia (CIH) and tadalafil on the number of corpus spongiosum pressure (CSP) peaks recorded in male mice during noncontact tests with an estrous female mouse. Data represent counts per minute of CSP peaks (80–120 mm Hg and 0.4–05-s duration; n = 8 mice/group). *Solid squares*, baseline (RA); *solid circles*, 5 weeks of CIH; *open circles*, 1 hour after tadalafil administration in CIH mice. *$P < 0.0001$ room air versus CIH.

Central Neural Mechanisms

Experimental mice demonstrated changes in complex sex-oriented behaviors after CIH, such as a decreased number of spontaneous erections, increased latencies for mounts and intromissions, decreased tumescence pressure during intromissions, reduced overall mating activity (I/M+I index), less frequent occurrence of ejaculation, and diminished sexual activity during noncontact behavior. These changes could reflect CIH-induced brain injury to neural regions involved in sexual behavioral control. The various olfactory, visual, auditory, somatosensory, and sexual recognition stimuli that affect sexual behavior are integrated within the medial preoptic area (MPOA) (4–6) and the paraventricular nucleus of the hypothalamus (4, 6, 35). In rats, exposures to CIH have been associated with significantly lower levels of both nNOS mRNA and protein in the paraventricular hypothalamic nucleus (36). CIH was earlier shown to cause apoptosis in the neocortex and hippocampus (37, 38), to induce immediate early gene c-*fos* expression (midline thalamus, epithalamus, cortex) (39–41), and causing behavioral deficits and hypersomnolence (42–44).

Spontaneous erections. Spontaneous erections observed after 1 week of CIH were suppressed to the minimal level and remained stable until the end of exposures (8 wk). The quick suppression suggests involvement of neural mechanisms. The ensuing recovery of spontaneous erectile activity in normoxia for 6 weeks was incomplete, and could suggest either chronic residual deficits after CIH or that full recovery would require longer periods.

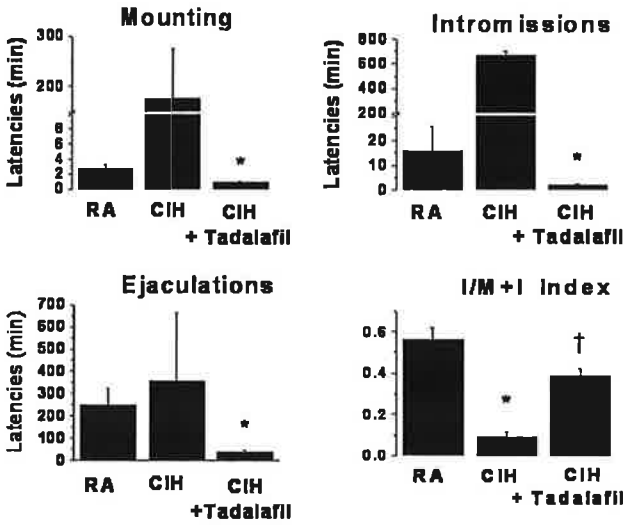

Figure 6. Latencies to mounts, intromissions, ejaculations, and I(I+M) index measured during mating test. CIH = chronic intermittent hypoxia (5 wk); I = intromission; M = mounting; RA = room air. Tadalafil was used at the standard dose of 0.014 mg/25 g body weight; n = 8/group. *$P < 0.001$ CIH versus CIH + tadalafil; †CIH versus RA.

TABLE 2. CORPUS SPONGIOSUM PRESSURES DURING MATING TEST AFTER 5 WEEKS' CHRONIC INTERMITTENT HYPOXIA AND TREATMENT WITH TADALAFIL

Pressures (*mm Hg*)	Baseline	CIH	CIH + Tadalafil
Flaccid pressure	9 ± 2	11 ± 3	9 ± 1
Tumescence pressure	61 ± 3	55 ± 7	77 ± 5*
Maximal pressure (full erection)	376 ± 25	345 ± 48	389 ± 11

Definition of abbreviation: CIH = chronic intermittent hypoxia.
Data are presented as mean ± SE.
* $P < 0.05$ vs. CIH.

Copulatory activity. Sexual behavior after CIH was characterized by increased latencies for mounts and intromissions and decreased I/M+I index. Ejaculation did not occur in five of the seven CIH mice. The increased latencies could reflect a decreased excitability or impairments in the neural circuits, involving central mechanisms (i.e., spinal or supraspinal).

Noncontact sexual activity. The decreased sexual activity in the noncontact tests reflects decreased psychogenic sexual motivation or libido. The limbic system components associated with libido and its modulation are susceptible to hypoxia effects (45), and in turn may influence behavioral patterns such as libido and sexual drive. Responses to a psychogenic stimulation of a male mouse by a receptive female were evaluated by measuring specific CSP as previously described (24). Typical full erections were rare in the mice during noncontact tests as compared with the erections elicited during contact stimulation.

Peripheral Neural Mechanisms

It has been shown that CIH causes sympathetic overactivity (46), and thus can increase smooth muscle tone in the penile arteries and trabecular tissue, preventing erections. CIH evokes c-*fos* expression in cortical (38) and brainstem (39) regions that modulate sympathetic discharge, and reciprocal adrenergic noradrenaline-mediated inhibition of the parasympathetic system (proerectile activity [4]) may also develop. The sympathetic antitumescent effect during oxidative stress may contribute to lowering tumescence pressures in CIH-exposed mice. Tadalafil caused a significant elevation of tumescence pressure in such conditions, and showed slight elevations in maximal pressures during full erection (Table 2).

Intrinsic Erectile Tissue

Eight-week CIH exposures were not associated with light microscopic evidence of structural damage in the testes. Similarly, there was no evidence of reduced circulating total testosterone levels, which could lead to reduced intromission times and ejaculatory events (47). Testosterone is secreted by the Leydig cells of the testes and metabolized in target cells to either estradiol (by aromatization) or dihydrotestosterone by 5α reduction (48). Testosterone is more effective than either estradiol or dihydrotestosterone in restoring precopulatory and copulatory behaviors in castrated mice (49), whereas estradiol is the major hormone to activate sexual behavior in male rodents (19). Therefore, the increases in estradiol levels after 6-month CIH exposures could represent compensatory mechanisms in the restoration of some aspects of sexual behavior or, alternatively, underlie preferential metabolisms of testosterone by aromatization.

In this study, 8 weeks of CIH reduced the expression of eNOS protein levels in erectile tissues. In the latter, NO derived from endothelial sources is a major source of the total NO released during erection, and reductions in the expression of this enzyme could significantly affect the maintenance of the erection initiated by nNOS. eNOS uncoupling, rapid inactiva-

tion of NO by reactive oxygen species, and generation of a highly toxic molecule, peroxynitrite ($ONOO^-$) in penile tissue may indeed underlie vasculogenic ED. We found only one other published paper on the down-regulation of eNOS by CIH in a cell culture model using umbilical vein endothelial cells (50).

After 8 weeks of exposure to CIH, there were no effects on nNOS protein levels in the penile tissue, suggesting that a substantial level of disruption of the noncholinergic, nonadrenergic system did not occur after CIH, and as such, did not alter NO release from peripheral nerve endings. In contradistinction, CIH has now been extensively demonstrated as a paradigm that increases sympathetic activity (51). It is now well established that sympathetic pathways are antierectile, sacral parasympathetic pathways are proerectile, and contraction of the perineal striated muscles upon activity of the pudendal nerves improves penile rigidity (35). Therefore, the increased level of sympathetic tone and reactivity associated with CIH would be expected to adversely affect erectile function.

There were no changes in iNOS protein in the penile tissue after 8 weeks of CIH. We should, however, consider the fact that the penile tissue is subjected to short-lasting periodic ischemic episodes during the rigid phase of erection, and that blood within the penile corpus cavernosum, as compared with peripheral venous blood, is characterized by an increased total antioxidant capacity (52). Notwithstanding such considerations, elevated mitochondrial superoxide production during CIH could influence the oxidative–antioxidative balance and increase the susceptibility of penile tissue to oxidative stress, which in turn would promote ED (53). The absence of increased iNOS expression may reflect heterotopic responses of this gene to CIH within penile tissues, because cavernosal iNOS immunoreactivity was increased in the presence of atherosclerosis (54).

In patients with OSAS, ED treatment failures may occur after administration of PDE5 inhibitors and may underlie impairments in NO-generating mechanisms. Treatment of OSAS using CPAP improved sexual performance from 20 to 75% of patients at 1-month follow-up (55–57). However, treatment of ED with the PDE5 inhibitor sildenafil and/or with CPAP did not completely eliminate ED, even though the PDE5 inhibitor appeared to be superior to CPAP (56). In the present study, tadalafil restored CIH-induced impairments of latencies to mounts, intromissions, and ejaculation, significantly improving performance during spontaneous erections, and during mating and noncontact activity. Thus, the effects of tadalafil were not only limited to the erectile tissue but extended to behavioral components, suggesting a possible role for PDE5 in central nervous system mechanisms that control sexual behavior (58). Of course, the contributions of coexisting morbidities in patients with OSAS could also lower the effectiveness of PDE5.

In summary, we conclusively demonstrate that even relatively short periods of CIH, one of the key components of OSA, are associated with significant effects on sexual activity and erectile function in mice, and that such changes are most likely attributable, at least in part, to reductions in eNOS expression. Furthermore, administration of the long-acting PDE5 inhibitor tadalafil is associated with substantial improvements in erectile functioning.

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