Published in final edited form as:
J Sex Med. 2008 May ; 5(5): 1156–1166. doi:10.1111/j.1743-6109.2007.00752.x.

Adenosine Actions are Preserved in Corpus Cavernosum from Obese and Type II Diabetic db/db Mouse

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Abstract

Introduction—Erectile dysfunction (ED) in diabetes is associated with autonomic neuropathy and endothelial dysfunction. Whereas the nonadrenergic-noncholinergic (NANC)/neurogenic nitric oxide pathway has received great attention in diabetes-associated ED, few studies have addressed sympathetic overactivity.

Aim—To test the hypothesis that adenosine-induced inhibition of adrenergic-mediated contractile responses in mouse corpus cavernosum is impaired in the presence of diabetes.

Methods—The db/db (obesity and type II diabetes caused by a leptin receptor mutation) mouse strain was used as a model of obesity and type II diabetes, and standard procedures were performed to evaluate functional cavernosal responses.

Main Outcome Measures—Increased cavernosal responses to sympathetic stimulation in db/db mice are not associated with impaired prejunctional actions of adenosine.

Results—Electrical field stimulation (EFS)-, but not phenylephrine (PE)-, induced contractions are enhanced in cavernosal strips from db/db mice in comparison with those from lean littermates. Direct effects of adenosine, 2-chloro-adenosine, A_1 receptor agonist C-8031 (N6 cyclopentyladenosine), and sodium nitroprusside are similar between the strips from lean and db/db mice, whereas relaxant responses to acetylcholine and NANC stimulation are significantly impaired in the cavernosal strips from db/db mice. 5′-Iodotubercidin (adenosine kinase inhibitor) and dipyridamole (inhibitor of adenosine transport), as well as the A_1 agonist C-8031, significantly and similarly inhibit contractions induced by stimulation of adrenergic nerves in the cavernosal strips from lean and db/db mice.

Conclusions—Results from this study suggest that corpora cavernosa from obese and diabetic db/db mice display altered neural-mediated responses that would favor penile detumescence, i.e., increased contractile response to adrenergic nerve stimulation and decreased relaxant responses upon activation of NANC nerves. However, increased cavernosal responses to adrenergic nerve stimulation are not due to impaired negative modulation of sympathetic neurotransmission by adenosine in this diabetic model.

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Conflict of Interest: None declared.

Keywords

Adenosine; Obesity; Diabetes; Sympathetic Neurotransmission; Animal Models

Introduction

Autonomic neuropathy and endothelial dys-function are considered the main etiological factors in diabetic erectile dysfunction (ED) [1,2]. Both selective degeneration of nitrergic nerves [3] and decreased cross-sectional area in unmyelinated axons (axonal retraction), including sympathetic postganglionic fibers [4], have been reported in penes from rats with experimental (streptozotocin-induced) diabetes. Morrison and colleagues have recently shown that penes from diabetic animals exhibit increased norepinephrine content as well as greater immunohistochemical staining for tyrosine hydroxylase in the cavernosal nerves, suggesting increased sympathetic tonus [4].

A large number of studies have shown a correlation among hyperinsulinemia, insulin resistance, and overactivity of the sympathetic nervous system [5-7]. Regarding the possible mechanisms, one hypothesis suggests that resistance to insulin represents the initial defect, and that hyperinsulinemia activates the sympathetic nervous system, via actions on the central nervous system, baroreflex-mediated indirect actions, and/or direct effects on norepinephrine metabolism [8].

The balance between contracting and relaxing factors controls the smooth muscle tone of both the penile vasculature and corpora cavernosa and, therefore, determines the functional state of the penis: flaccidity or erection [9,10]. Neurogenic nitric oxide (NO) is considered the most important factor for relaxation of penile vessels and corpus cavernosum and, consequently, penile erection [11,12]. On the other hand, penile arteries and veins, and cavernosal smooth muscle receive a rich adrenergic innervation, and it is generally accepted that the penis is kept in the flaccid state mainly via a tonic activity of the sympathetic nerves [9,10]. Therefore, excessive adrenergic stimulation in the penis may make penile tumescence more difficult to occur, or may be more difficult to overcome in the presence of an erection stimulus.

Adenosine, via prejunctional actions, negatively modulates contractile responses mediated by activation of sympathetic nerves in many organs [13]. We have recently shown that adenosine negatively modulates sympathetic neurotransmission, by A_1 receptor subtype activation, in mouse corpora cavernosa [14]. In addition, adenosine directly relaxes cavernosal smooth muscle cells by the activation of A_{2A}/A_{2B} receptor subtypes, which suggests that adenosine may subserve dual roles in modulating the physiological mechanisms of erection in mice [14,15].

Therefore, we hypothesized that adenosine-induced inhibition of adrenergic-mediated contractile responses in mouse corpus cavernosum is impaired in the presence of diabetes. If our hypothesis is correct, greater contractile responses upon stimulation of adrenergic nerves, as well as decreased inhibitory effects of adenosine on these responses, will be observed in cavernosum from diabetic mice. Using the db/db (obesity and type II diabetes caused by a leptin receptor mutation) mouse strain as a model of obesity and type II diabetes, we have evaluated, in corpus cavernosum from lean and db/db mice, responses induced by adrenergic and nonadrenergic-noncholinergic (NANC) nerves stimulation, alpha-adrenergic receptors activation, acetylcholine (endothelium-dependent vasodilator), and sodium nitroprusside (endothelium-independent vasodilator). We have also determined

the effects of adenosine on the adrenergic-mediated contractile responses as well as the direct effects of adenosine in the cavernosal smooth muscle cells.

Methods

Animals

Male C57BL/KsOlaHsd-lepr^{db}/lepr^{db} mice (db/db, mice with obesity and type II diabetes caused by a leptin receptor mutation) and their lean, non-diabetic heterozygote (db/+) C57bl/6kso littermates (14–16 weeks old, Harlan, Indianapolis, IN, USA) were used in the study. All procedures were performed in accordance with the Guiding Principles in the Care and Use of Animals, approved by the Medical College of Georgia Committee on the Use of Animals in Research and Education. The animals were housed four per cage on a 12-hour light/dark cycle, and were fed a standard chow diet with water *ad libitum*.

Drugs and Solutions

Physiological salt solution of the following composition was used: 130 mM NaCl, 14.9 mM $NaHCO_3$, 5.5 mM dextrose, 4.7 mM KCl, 1.18 mM KH_2PO_4, 1.17 mM $MgSO_4.7H_2O$, 1.6 mM $CaCl_2.2H_2O$, and 0.026 mM ethylene-diaminetetraacetic acid (EDTA). Acetylcholine, atropine, Nω-nitro-L-arginine methyl ester (L-NAME), adenosine, 2-chloro-adenosine, phenylephrine (PE), sodium nitroprusside, 5′-iodotubercidin (4-amino-5-iodo-7-(b-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine), C-8031 (N6 cyclopentyladenosine), bretylium tosylate [(o-bromobenzyl) ethyldimethylammonium p-toluenesulfonate] were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dipyridamole (2,6-bis(diethanolamino)-4,8-dipiperidinopyrimido [5,4-d]pyrimidine) was from Tocris (Ellisville, MO, USA). All reagents used were of analytical grade. Stock solutions were prepared in deionized water, ethanol (2-chloro-adenosine), or dimethylsulfoxide (DMSO) (C-8031), and were stored in aliquots at −20°C; dilutions were made up immediately before use.

Functional Studies in Cavernosal Strips

After euthanasia, penes were excised, transferred into ice-cold buffer, and dissected to remove the tunica albuginea, as previously described [14]. One crural strip preparation (1 × 1 × 10 mm) was obtained from each corpus cavernosum (two crural strips from each penis). Cavernosal strips were mounted in 4-mL myograph chambers (Danish Myo Technology, Aarhus, Denmark) containing the buffer at 37°C, continuously bubbled with a mixture of 95% O_2 and 5% CO_2. The tissues were stretched to a resting force of 2.5 mN, and were allowed to equilibrate for 60 minutes. Changes in isometric force were recorded using a PowerLab/8SP data acquisition system (Chart software, version 5.0, ADInstruments, Colorado Springs, CO, USA). To verify the contractile ability of the preparations, a high potassium chloride (KCl) solution (120 mM) was added to the organ baths at the end of the equilibration period. Cumulative concentration-response curves to acetylcholine (10^{-9} to 10^{-5} M); sodium nitroprusside (10^{-9} M to 3×10^{-5} M), adenosine (10^{-8} M to 3×10^{-4} M), 2-chloro-adenosine (10^{-9} M to 3×10^{-4} M; stable analog of adenosine), and C-8031 (10^{-8} M to 3×10^{-4} M; adenosine A_1 receptor agonist [16]) were obtained in cavernosal strips contracted with PE (10^{-5} M; alpha1-adrenergic receptor agonist). Cumulative concentration-response curves to PE (10^{-9} M to 5×10^{-5} M) were performed both in the absence or presence of L-NAME, 10^{-4} M. Electrical field stimulation (EFS) was applied to the strips placed between the platinum pin electrodes attached to a stimulus splitter unit (Stimu-Splitter II), which was connected to a Grass S88 stimulator (Astro-Med West Warwick, RI, USA). EFS was conducted at 50 V, 1-ms pulse width, and trains of stimuli lasting for 10 seconds at varying frequencies (1–32 Hz). To evaluate adrenergic nerve-mediated responses, the strips were incubated with L-NAME, 10^{-4} M, plus atropine, 10^{-6} M, before EFS was

performed. To determine the relaxant responses to NANC nerve stimulation, the strips were treated with bretylium tosylate, 3×10^{-5}, and atropine, 10^{-6} M, for 45 minutes, were contracted with PE, 10^{-5} M, and then EFS was performed. When antagonists or inhibitors were used, drugs were introduced 30–45 minutes before the concentration-response or frequency-response curves were performed. Time control experiments were performed to determine the force development of cavernosal strips not related to the effects of each antagonist/inhibitor. Control solutions containing vehicle levels of ethanol and DMSO were also used through the experimental protocols.

Statistical Analysis

Contractions were recorded as changes in the displacement from baseline and were represented as millinewtons (mN) for N experiments. Relaxation was expressed as percentage change from the PE-contracted levels. Agonist concentration-response curves were fitted using a nonlinear interactive fitting program (Graph Pad Prism 4.0, GraphPad Software Inc., San Diego, CA, USA). Agonist potencies and maximum responses were expressed as pD_2 (negative logarithm of the molar concentration of agonist producing 50% of the maximum response) and Emax (maximum effect elicited by the agonist), respectively. Constraining curve-fit parameters were used to fit a sigmoidal curve and to determine pD_2 values for adenosine, 2-chloro-adenosine, C-8031, and acetylcholine. Statistically significant differences were calculated by one-way analysis of variance or Student's *t*-test. $P < 0.05$ was considered as statistically significant.

Results

C57BL/KsOlaHsd-lepr^{db}/lepr^{db} (db/db) mice were overweight, displayed hyperinsulinemia and hyperglycemia in comparison with their lean, nondiabetic littermates (Table 1). The average dry weights (milligram) of the cavernosal strips from db/db and lean mice were 1.71 ± 0.2 (N = 18) and 1.97 ± 0.2 (N = 18), respectively. Stimulation with 120 mM KCl induced contractile responses (mN) of 1.58 ± 0.18 (N = 10) and 1.48 ± 0.06 (N = 10) in the strips from db/db and lean mice, respectively.

Contractile Effects Induced by Adrenergic Nerve Stimulation and the Alpha-Adrenergic Receptor Agonist, PE

After 45 minutes of incubation with atropine (a muscarinic receptor antagonist, 10^{-6} M) plus L-NAME (nonselective inhibitor of nitric oxide synthase [NOS], 10^{-4} M), EFS (1–32 Hz) produced frequency-dependent contractions in the cavernosal smooth muscle strips (Figure 1A). In the absence of L-NAME and atropine, contractile responses to EFS were observed only at higher frequencies (16 and 32 Hz) and cannot be accurately used to make comparisons between experimental groups. In the present study, e.g., in the absence of L-NAME and atropine, no contractile responses to EFS (1–8 Hz) were observed in the strips from either lean or db/db mice (graph not shown). At 16 Hz, EFS produced contractile responses as follows (mN): in the absence of L-NAME and atropine, lean, 0.05 ± 0.02, and db/db, 0.18 ± 0.05; in the presence of L-NAME and atropine, lean, 0.55 ± 0.09, and db/db, 1.06 ± 0.15 (Figure 1A).

EFS-dependent contractions were virtually abolished by the sympathetic nerve blocking agent bretylium tosylate (3×10^{-5} M) and by the alpha-adrenergic antagonist terazosin (10^{-6} M), confirming that these responses are neuronal in origin and adrenergic in nature (data not shown). As shown in Figure 1A, EFS-induced contractions are enhanced in the cavernosal strips from db/db mice (N = 8) in comparison with those in the strips from lean littermates (N = 10; $P < 0.05$). However, PE-induced contractile responses were similar

between the strips from db/db and lean mice, both in the absence (Figure 2A) or presence (Figure 2B) of L-NAME 10^{-4} M (N = 5 in all groups).

Effects of Inhibitors of Adenosine Metabolism or Uptake on EFS-Induced Contraction

To evaluate the effects of endogenous adenosine on the contractions induced by EFS of sympathetic nerves, the following compounds, which are known to increase adenosine levels, were used: 5′-iodotubercidin (adenosine kinase inhibitor; 10^{-6} and 10^{-5} M) and dipyridamole (inhibitor of adenosine transport; 10^{-7} and 10^{-6} M). The concentrations were chosen based on our recent report on the effects of these drugs on EFS-induced contractile responses of mouse cavernosal strips.

Because in mouse corpora cavernosa the inhibitory effects of adenosine on sympathetic nerve-mediated contractile responses are mediated by adenosine A_1 receptors, we also evaluated the effects of the adenosine A_1 receptor agonist, C-8031 (10^{-7} and 10^{-6} M), on contractile responses induced by EFS in the cavernosal strips from lean and db/db mice.

As shown in Figure 1, each agent (5′-iodotubercidin [10^{-5} M, Figure 1B]; dipyrida-mole [10^{-6} M, Figure 1C]; and C-8031 [10^{-7} M, Figure 1D]) had a significant inhibitory effect on EFS-induced contractions over the full range of the frequency-response curve. However, similar inhibitory effects of 5′-iodotubercidin, dipyridamole, and C-8031 were observed in the cavernosal strips from lean and db/db mice, and the differences in the cavernosal contractile responses between lean and db/db were not abolished by these drugs (Figure 1, Table 2). The A_1 agonist at the dose of 10^{-7} M had no relaxant effects when tested directly on 10^{-5} M PE-contracted cavernosal strips, as can be observed in Figure 3C.

Relaxing Effects of Adenosine and Its Analogs, NANC Nerves Stimulation, Acetylcholine, and Sodium Nitroprusside

The addition of PE (10^{-5} M) to the bathing medium caused a submaximal contraction (mN) of cavernosal segments from db/db and lean mice, and generated active forces of 1.05 ± 0.1 (N = 18) and 1.06 ± 0.2 (N = 18), respectively, which consisted of a rapid rise in force, followed by a slower rise to a sustained level within 10 minutes. The cumulative addition of adenosine (10^{-8} to 3×10^{-4} M, N = 4–6, Figure 3A), 2-chloroadenosine (10^{-9} to 3×10^{-4} M, N = 5, Figure 3B), or C-8031 (10^{-8} to 3×10^{-4} M, N = 5, Figure 3C) produced concentration-dependent relaxations of PE-contracted tissues ($P < 0.05$). Emax and pD_2 values, which represent the maximum response and potency for each agonist, are listed in Table 3. No differences in the effects of adenosine or its analogs were observed between the strips from lean and db/db mice (Figure 3).

Similarly, EFS stimulation (1–32 Hz, N = 5, Figure 4A), as well as the cumulative addition of acetylcholine (10^{-9} to 10^{-5} M, N = 5, Figure 4B) or sodium nitroprusside (10^{-9} to 3×10^{-5} M, N = 5, Figure 4C), produced a relaxation of PE-contracted cavernosal strips. However, relaxant responses to acetylcholine and EFS, but not to sodium nitroprusside, were significantly impaired in the strips from db/db mice (Figure 4). Incubation of the cavernosal strips with L-NAME (10^{-4} M) completely abrogated NANC and acetylcholine-induced relaxations (data not shown).

Discussion

The present study shows that isolated corpora cavernosa from obese and diabetic db/db mice display altered neural-mediated responses that would favor penile detumescence or that would make tumescence more difficult to occur: increased contractile response to adrenergic nerve stimulation and decreased relaxant responses upon activation of NANC nerves. This study also shows that agents known to increase adenosine levels, such as 5′-iodotubercidin

and dipyridamole, as well as adenosine A_1 receptor activation, similarly inhibit cavernosal contractile responses induced by electrical stimulation of sympathetic nerves in lean and db/db mice. In addition, direct relaxant effects of adenosine in the cavernosal smooth muscle cells are preserved in this experimental model of diabetes, which does display impaired relaxant responses upon stimulation with acetylcholine.

In the first set of experiments, we observed that the cavernosal strips from db/db mice display increased contractile responses to EFS, but not to the alpha1-adrenergic receptor agonist PE. To determine contractile responses due to stimulation of adrenergic nerves, EFS experiments were conducted in the presence of L-NAME and atropine to abolish any effects due to the activation of NANC and cholinergic nerves, respectively. In mouse cavernosal strips, adrenergic nerves-mediated contractile responses are clearly evidenced only in the presence of L-NAME and atropine, as we previously demonstrated [12]. Accordingly, PE-induced contractions were evaluated both in the absence and in the presence of L-NAME, and no differences were observed between the responses from the cavernosal strips from lean and db/db mice in any of these experimental conditions. These data suggest that increased contractile responses to EFS in the strips from db/db mice are due to changes in prejunctional events, most likely to increased sympathetic nerve transmission.

A number of studies have shown that diabetic patients are afflicted with ED at a much higher incidence and prevalence than normal men [17,18]. These patients, as well as experimental animals with diabetes, exhibit autonomic neuropathy, normally manifested as a sympathetic overdrive, which can be associated with a sympatho-vagal imbalance [8]. Although unlikely, a relationship between increased sympathetic drive and impairment of cholinergic nerve activity in the penis is possible. It is known that in the smooth muscle septa surrounding the cavernous spaces, and around the central and helicine arteries, there are a large number of sympathetic nerve terminals and few-to-moderate numbers of parasympathetic terminals [19]. In penes from both human and rats, immunoreactivities for vesicular acetylcholine transporter, neuronal NOS, and vasoactive intestinal peptide are generally found in the same varicose nerve terminals, indicating that these terminals comprise a distinct population of parasympathetic, cholinergic nerves that are capable of forming NO and that functionally behave as nitrergic nerves [19-22]. In addition, relaxant responses to EFS are minimally modified in the presence of atropine, physiostigmine (acetylcholinesterase inhibitor), hexamethonium (ganglionic blocker), or vesamicol (inhibitor of vesicular acetylcholine transporter), whereas they are completely abolished in the presence of tetrodotoxin and L-NAME [23,24]. However, a functional penile neuropathic condition of the cholinergic nerves, represented by impairment in acetylcholine synthesis, has been reported in corpus cavernosum of diabetic impotent patients [25], and diminished parasympathetic fiber size was also described in penis from spontaneously diabetic BioBreeding rat [26].

On the other hand, hyperinsulinemia itself increases resting sympathetic output [27-32]. Sympathetic activation seems to occur at an early stage of diabetes, as evidenced by increased vascular contractile responses to EFS as well as greater immunoreaction intensity for neuropeptide Y and tyrosine hydroxylase in nonobese early diabetic mice compared with control animals without diabetes [33]. Sympathetic hyperactivation also occurs in normal nondiabetic offspring of patients with type II diabetes [34] and in Wistar fatty rats, a model of insulin resistance-related hypertension associated with obesity, hyperglycemia, and hyper-insulinemia [35]. Because the db/db mouse strain, a model of obesity and type II diabetes caused by a leptin receptor mutation, exhibits hyperinsulinemia, it is possible that sympathetic overactivity is directly related to increased insulin levels. Interestingly, a causal relationship between diseases commonly associated with increased insulin levels, such as obesity and dyslipidemias, with the development of ED in humans has been suggested [36].

In accordance with our results and further supporting increased sympathetic activity in penes from diabetic animals, a recent study of Morrison and colleagues showed increased norepinephrine content as well as greater immunohistochemical staining for tyrosine hydroxylase in cavernosal nerves of diabetic animals [4]. It is important to mention, however, that a study with six diabetic patients revealed a marked reduction in vasoactive intestinal polypeptide-like immunoreactivity in nerves associated with the cavernous smooth muscle, as well as reduced acetylcholinesterase-positive staining and decreased norepinephrine content in the corpus cavernosum from diabetic patients in comparison with the cavernosal tissue from patients with non-neuropathic-related impotence [37].

It is well known that adenosine modulates norepinephrine release from sympathetic nerve endings [13]. Most commonly, the adenosine A_1 receptor subtype negatively modulates norepinephrine release, whereas the A_2 receptor subtypes enhance neurotransmitter release [13]. We have recently shown that adenosine, via A_1 receptor subtype activation, negatively modulates contractile responses elicited by adrenergic nerves stimulation in mouse corpora cavernosa [14], and therefore, we hypothesized that this inhibitory action is impaired in the cavernosal strips from db/db mice, contributing to increased adrenergic-mediated contractile responses in the corpus cavernosum from db/db mouse. However, both 5′-iodotubercidin and dipyridamole, which are known to increase adenosine levels [38,39], as well as the A_1 agonist C-8031, similarly right-shifted the contractile responses to EFS in the strips from both lean and db/db mice. These results reinforce our suggestion that adenosine/A_1 receptor activation negatively modulates sympathetic neuro-transmission in mouse corpora cavernosa, but discharges the idea that enhanced adrenergic-mediated responses in the cavernosal strips from db/db mice are due to impairment in adenosine-mediated effects.

We have further evaluated the direct effects of adenosine and its analog, 2-chloro-adenosine, as well as the effects of the A_1 agonist C-8031, in the cavernosal smooth muscle cells from lean and db/db mice. No differences in adenosine-, 2-chloro-adenosine-, or C-8031-induced relaxation were observed between the strips from lean and db/db mice. We have previously shown that 2-chloro-adenosine, as well as adenosine-induced relaxation in mouse corpora cavernosa, is mediated by A_{2A} and A_{2B} receptor subtypes [14]. In this study, we observed that A_1 receptors also induce relaxation of cavernosal strips. Because experiments were performed in the presence of L-NAME and atropine, relaxant responses to adenosine, 2-chloro-adenosine, and C-8031 do not rely on NO release. In accordance with our results, adenosine-induced relaxation in cavernosal tissues from diabetic men and rats is also considered NO-independent [40]. In addition, relaxant responses to acetylcholine and to NANC nerves stimulation, but not to sodium nitroprusside, were decreased in the cavernosal strips from db/db mice, demonstrating that diabetes-associated changes in the cavernosal smooth muscle cells reactivity are not generalized or do not occur indistinctly.

Whereas our results are in accordance with those of Ayan and colleagues, who observed similar adenosine-induced relaxation responses of cavernosal strips from control and alloxan-induced diabetic rabbits [41], they differ from those obtained by Gür and Oztürk, who reported that cavernosal tissues from diabetic men and rats display increased responses to adenosine [40]. Whereas an increased sensitivity was detected in cavernosum from diabetic men, increased maximal relaxation was observed in cavernosal strips from diabetic rats [40]. It is not clear whether species variation regarding the actions of adenosine or adenosine receptor subtypes is involved in these differences.

Adenosine-induced increases in coronary flow rate are lower in hearts from diabetic db/db mice when compared with hearts from nondiabetic animals [42]. In addition, adenosine-induced relaxations in the detrusor muscle from streptozotocin-induced diabetic rats are preserved in normoxic conditions, but the enhancement of adenosine responsiveness in

hypoxic conditions observed in tissues from control animals is impaired in the detrusor muscle from diabetic rats [43]. Adenosine-induced relaxation is also decreased in aortic rings of diabetic (streptozotocin-induced) Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats compared with nondiabetic WKY and SHR. Because endothelium removal decreases adenosine-induced relaxation and abolishes differences in the vascular responses between the groups, and similar responses are observed with sodium nitroprusside, the authors suggested that vascular endothelial dysfunction leads to impaired vascular responses in diabetes [44].

Further support to this suggestion is derived from studies showing that endothelium-dependent relaxation, produced, e.g., by acetylcholine or the calcium ionophore, A23187, is significantly attenuated in diabetic vessels, whereas relaxations produced by endothelium-independent vasodilators, e.g., sodium nitroprusside or adenosine, are comparable in control and diabetic vessels [45,46]. Similar observations are extended to human cavernosal smooth muscle strips from diabetic patients, which display impairment of endothelium-mediated, but not of endothelium-independent, relaxation of cavernosal smooth muscle [47].

Although adenosine does not cause penile erection in men [48], as it does in other species [49], it increases corporal peak blood flow velocity (via Doppler analysis) [48], suggesting that adenosine may be an important modulator of erection. This modulatory role becomes even more relevant within the concept that the balance between contracting and relaxing factors controls smooth muscle tone of both the penile vasculature and corpora cavernosa and, therefore, determines the functional state of the penis (flaccidity or erection). In addition, because of the diverse range of physiological actions of adenosine, a beneficial effect in ED might be observed when adenosine is used in in vivo conditions.

In summary, we have shown that cavernosal smooth muscle from type II diabetic db/db mouse display increased responses to adrenergic nerve stimulation. However, the dual effects of adenosine on mouse corpora cavernosa, direct relaxation of cavernosal smooth muscle cells and negative modulation of sympathetic neurotrans-mission, are not impaired in this diabetic model. Whereas impaired NANC neurotransmission and endothelial dysfunction seem to play major roles in diabetic ED, increased sympathetic drive should also be considered as a contributing factor.

Statement of Authorship

Category 1

a. Conception and Design

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Category 2

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Category 3

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Acknowledgments

This study was supported by grants from the National Institutes of Health (HL71138 and HL74167), Fundacao de Amparo a Pesquisa do Estado de Sao Paulo—FAPESP, and Coordenacao de Aperfeicoamento de Pessoal de Nivel Superior—CAPES, Brazil.

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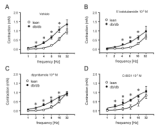

Figure 1.

Effects of 5′-iodotubercidin (adenosine kinase inhibitor), dipyridamole (inhibitor of adenosine transport), and C-8031 (A_1 adenosine receptor subtype agonist) in the frequency-response curves elicited by electrical field stimulation (EFS) (1–32 Hz) in cavernosal strips from lean (○) and db/db (●) mice. Cavernosum strips were preincubated with Nω-nitro-L-arginine methyl ester, 10^{-4} M, and atropine, 10^{-6} M. After the completion of a control curve to EFS, the tissues were incubated in the presence of (A) vehicle (N = 10 and 8, respectively), (B) 5′-iodotubercidin (10^{-5} M, N = 5 and 6, respectively), (C) dipyridamole (10^{-6} M, N = 7 in each group), or (D) C-8031 (10^{-7} M, N = 8 and 6, respectively), and a second curve to EFS was performed. Experimental values of contraction are in millinewton, and data represent the mean ± SEM of N experiments. * = $P < 0.05$ compared with the values of cavernosal strips from lean mice; db/db = obesity and type II diabetes caused by a leptin receptor mutation.

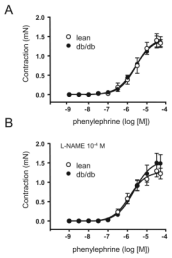

Figure 2.

Contractile responses to phenylephrine, alpha1-adrenergic receptor agonist, in cavernosal strips from lean (○) and db/db (●) mice. Phenylephrine concentration-response curves were performed in the absence (A) or presence (B) of Nω-nitro-L-arginine methyl ester (L-NAME), 10^{-4} M(N = 5 in all groups). Experimental values of contraction of cavernosal strips are in millinewton, and data represent the mean ± SEM of N experiments. db/db = obesity and type II diabetes caused by a leptin receptor mutation.

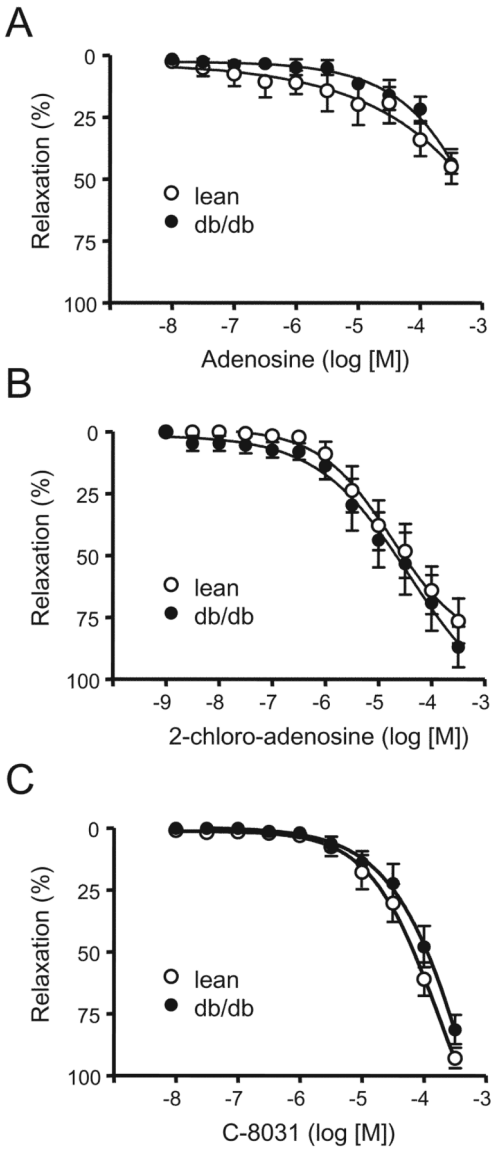

Figure 3.

Effects of adenosine (A), 2-chloro-adenosine (B), and A_1 agonist C-8031 (C) in phenylephrine (PE)-contracted cavernosal strips from lean (○) and db/db (●) mice. Experimental values of the relaxations induced by adenosine (N = 4 and 6, respectively), 2-chloro-adenosine (N = 5 in each group), and C-8031 (N = 5 in each group) were calculated relative to the maximal changes from the contraction produced by PE in each tissue, which was taken as 100%. Data represent the mean ± SEM of N experiments. db/db = obesity and type II diabetes caused by a leptin receptor mutation.

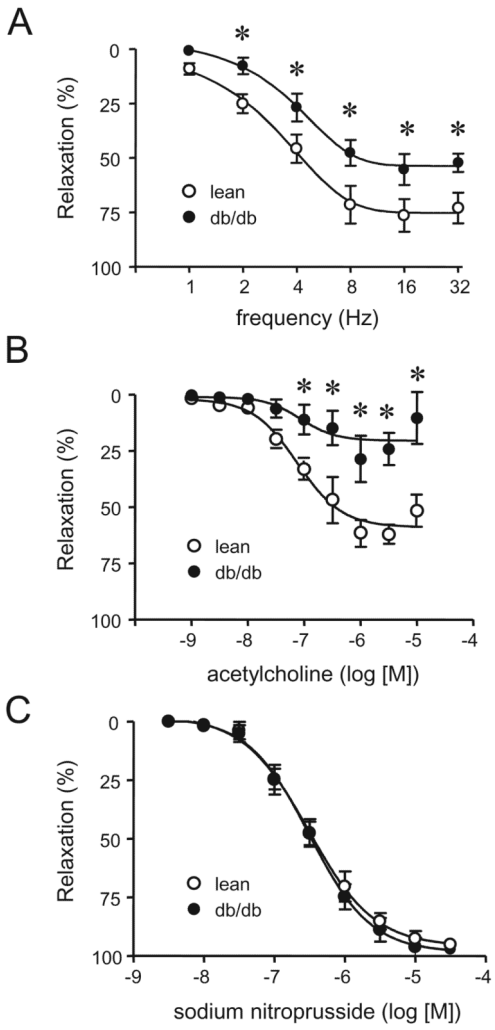

Figure 4.

Effects of nonadrenergic-noncholinergic nerves stimulation (A), acetylcholine (B), and sodium nitroprusside (C) in phenylephrine (PE)-contracted cavernosal strips from lean (○) and db/db (●) mice. Experimental values of the relaxations induced by electrical field stimulation stimulation (1–32 Hz) (N = 5 in each group), acetylcholine (N = 5in each group), and sodium nitroprusside (N = 5 in each group) were calculated relative to the maximal changes from the contraction produced by PE in each tissue, which was taken as 100%. Data represent the mean ± SEM of N experiments. * = $P < 0.05$ compared with the values of cavernosal strips from lean mice; db/db = obesity and type II diabetes caused by a leptin receptor mutation.

Table 1

Blood glucose, insulin levels, and lipid profile of db/db and lean mice

	Lean	db/db
Body weight (g)	33.8 ± 2.1	59.6 ± 2.3*
Glucose (mg/dl)	119.3 ± 29.1	524.1 ± 36.3*
Insulin (ng/ml)	5.96 ± 0.85	15.81 ± 3.51*

*$P < 0.05$ vs. lean (*t*-test).

Values are means ± SEM for N = 6 in each group.

db/db = obesity and type II diabetes caused by a leptin receptor mutation.

Table 2

Effects of 5′-iodotubercidin (adenosine kinase inhibitor), dipyridamole (inhibitor of adenosine transport), and C-8031 (adenosine A_1 receptor agonist) on contractile responses induced by EFS (adrenergic stimulation) in cavernosal strips from lean and db/db mice

	Lean		db/db	
EFS	**8 Hz**	**16 Hz**	**8 Hz**	**16 Hz**
Vehicle	0.21 ± 0.04	0.55 ± 0.09	0.64 ± 0.09†	1.06 ± 0.15†
5′-iodotubercidin (10^{-5} M)	0.12 ± 0.02* (42.9%)	0.32 ± 0.05* (41.8%)	0.39 ± 0.05*† (39.1%)	0.63 ± 0.11*† (40.6%)
Dipyridamole (10^{-6} M)	0.19 ± 0.04 (9.5%)	0.44 ± 0.02* (20.0%)	0.52 ± 0.09† (18.8%)	0.79 ± 0.06*† (25.5%)
C-8031 (10^{-7} M)	0.19 ± 0.13 (9.5%)	0.48 ± 0.15 (12.7%)	0.58 ± 0.03† (9.4%)	0.88 ± 0.05*† (17.0%)

*$P < 0.05$ vs. vehicle (*t*-test).

†$P < 0.05$ vs. lean.

Values are means ± SEM for N experiments in each group. Vehicle (N = 10 lean, N = 8 db/db); 5′-iodotubercidin (N = 5 lean, N = 6db/db); dipyridamole (N = 7 lean and db/db); and C-8031 (N = 8 lean, N = 6 db/db). The numbers in parentheses indicate the percentage of inhibition in contractile responses induced by EFS in control conditions (vehicle incubation).

EFS = electrical field stimulation; db/db = obesity and type II diabetes caused by a leptin receptor mutation.

Table 3

Emax and pD_2 values for acetylcholine, adenosine-, 2-chloro-adenosine, C-8031, and sodium nitroprusside-induced relaxation of cavernosal strips from lean and db/db mice

	Lean		db/db	
Agonist	**Emax (%)**	**pD_2**	**Emax (%)**	**pD_2**
Acetylcholine (N = 5)	62.1 ± 4.3	7.12 ± 0.13	24.2 ± 7.1*	7.07 ± 0.43
Adenosine (N = 4–6)	45.0 ± 7.1	3.05 ± 0.34	43.7 ± 4.2	3.18 ± 0.13
2-chloro-adenosine (N = 5)	76.6 ± 9.1	4.47 ± 0.09	86.9 ± 8.2	4.27 ± 0.11
C-8031 (N = 5)	92.9 ± 4.1	4.23 ± 0.05	81.4 ± 5.9	4.02 ± 0.05
Sodium nitroprusside (N = 5)	95.2 ± 2.41	6.51 ± 0.06	97.2 ± 1.9	6.50 ± 0.03

* $P < 0.05$ vs. lean (*t*-test).

Values are means ± SEM for N experiments in each group.

Emax = maximum effect elicited by the agonist; pD_2 = negative logarithm of the molar concentration of agonist producing 50% of the maximum response; db/db = obesity and type II diabetes caused by a leptin receptor mutation.