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Impaired Erectile Function in CD73-deficient Mice with Reduced Endogenous Penile Adenosine Production

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Abstract

Introduction—Adenosine has been implicated in normal and abnormal penile erection. However, a direct role of endogenous adenosine in erectile physiology and pathology has not been established.

Aim—To determine the functional role of endogenous adenosine production in erectile function.

Methods—CD73-deficient mice (CD73^{-/-}) and age-matched wild-type (WT) mice were used. Some WT mice were treated with alpha, beta-methylene adenosine diphosphate (ADP) (APCP), a

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CD73-specific inhibitor. High-performance liquid chromatography was used to measure adenosine levels in mouse penile tissues. In vivo assessment of intracorporal pressure (ICP) normalized to mean arterial pressure (MAP) in response to electrical stimulation (ES) of the cavernous nerve was used.

Main Outcome Measurement—The main outcome measures of this study were the in vivo assessment of initiation and maintenance of penile erection in WT mice and mice with deficiency in CD73 (ecto-5'-nucleotidase), a key cell-surface enzyme to produce extracellular adenosine.

Results—Endogenous adenosine levels were elevated in the erected state induced by ES of cavernous nerve compared to the flaccid state in WT mice but not in CD73^{-/-} mice. At cellular levels, we identified that CD73 was highly expressed in the neuronal, endothelial cells, and vascular smooth muscle cells in mouse penis. Functionally, we found that the ratio of ES-induced ICP to MAP in CD73^{-/-} mice was reduced from 0.48 ± 0.03 to 0.33 ± 0.05 and ES-induced slope was reduced from 0.30 ± 0.13 mm Hg/s to 0.15 ± 0.05 mm Hg/s (both $P < 0.05$). The ratio of ES-induced ICP to MAP in APCP-treated WT mice was reduced from 0.49 ± 0.03 to 0.38 ± 0.06 and ES-induced slope was reduced from 0.29 ± 0.11 mm Hg/s to 0.19 ± 0.04 mm Hg/s (both $P < 0.05$).

Conclusion—Overall, our findings demonstrate that CD73-dependent production of endogenous adenosine plays a direct role in initiation and maintenance of penile erection.

Keywords

CD73; Erectile Function; Erectile Dysfunction; Penile Adenosine Production

Introduction

Erectile dysfunction (ED) is characterized by the inability to develop or maintain an erection sufficient to permit satisfactory sexual intercourse [1]. ED is increasingly prevalent with age and highly associated with cardiovascular disease and the metabolic syndrome [2,3]. It is estimated that ED affects 30 million men in the United States [2]. Despite intensive research, the specific factors and signaling pathways responsible for ED remain largely undefined. Thus, defining the molecular mechanisms underlying erectile function is essential to advance the understanding of the molecular basis for the pathogenesis of ED. This knowledge will be important for developing novel strategies for the prevention and treatment of this sexual impairment [4].

Penile erection is controlled at multiple levels: neuronal, hormonal, and vascular. Upon sexual stimulation, neuronal activation leads to release of neurotransmitters from cavernous nerves and vasorelaxants from the endothelium, which in turn promote corpus cavernosal smooth muscle relaxation, blood flow into the cavernosum, and subsequent penile erection. Thus, the tone of cavernosal smooth muscle cells is a key regulator of the penile erection and is determined by the balance of vascular relaxants and constrictors [5]. A substantial body of evidence demonstrates that nitric oxide (NO) plays an important role in both initiation and maintenance of penile erection [1,6]. Neuronally derived NO, by activation of neuronal NO synthase, is a well-accepted mediator of smooth muscle relaxation to initiate penile erection by inducing cyclic guanosine monophosphate production in smooth muscle cells [6]. Increased relaxation of cavernosal arterioles allows blood flow into the penis, resulting in shear stress on endothelial cells, the activation of endothelial NO synthase, the production of NO, and sustained cavernosal smooth muscle relaxation. As a result, sinusoid spaces are filled with blood, intracavernosal pressure (ICP) is increased dramatically, and penile erection is sustained [7]. NO is thought to be the principal mediator for penile erections [8–10]. However, it does not exclude a role of other signaling molecules, either

released by neurons, endothelial, and/or smooth muscle cells in the regulation of penile erection.

Adenosine is a well-known signaling nucleoside that, like NO, elicits vascular relaxation [11–14]. Earlier studies in multiple animal species, including humans [15], showed that intracavernous injection of exogenous adenosine resulted in tumescence and penile erection [16–21]. Theophylline, an adenosine receptor antagonist, inhibited adenosine-induced penile tumescence [22]. However, attention was diverted from adenosine signaling to NO by the unexpected discovery of phosphodiesterase type 5 (PDE5) inhibitors, such as sildenafil (i.e., Viagra), that induce penile erection. Although these earlier studies clearly demonstrate that exogenous injection of adenosine enhances penile erection, to our knowledge, the possibility of a direct role of endogenous adenosine in initiation and maintenance of penile erection has not been previously addressed.

Using biochemical, genetic, and physiological approaches, we show here that endogenously generated adenosine plays a direct role in the initiation and maintenance of penile erection. Specifically, we show that mice with a genetic deficiency in ecto-5'-nucleotidase (CD73), a key cell surface enzyme to produce adenosine, or wild-type (WT) mice treated with CD73 specific inhibitor have significantly impaired erectile function. Overall, our findings indicate that endogenous adenosine signaling is a novel player in penile erection.

Materials and Methods

Mice

CD73-deficient mice were obtained from L. Thompson (Oklahoma Medical Research Foundation, Oklahoma City, OK, USA). These mice were backcrossed at least 10 generations onto the C57BL/6 background and were genotyped according to established protocols [23]. For all studies, WT C57BL/6 mice were used as controls. Mice were maintained and housed in accordance with National Institutes of Health guidelines and with the approval of the Animal Care and Use Committee at the University of Texas Health Science Center at Houston.

Cavernous Nerve Stimulation and Intracavernosal Pressure Measurement

Mice were anesthetized by intraperitoneal injection with 250 mg/kg Avertin (Avertin was made by mixing 10 g of 2,2,2-tribromoethyl alcohol with 10 mL of tert-amyl alcohol). Anesthetic depth was verified prior to surgery and during surgery by checking for the absence of toe pinch reflex and verifying normal mucous membrane color and respiration. During the procedure, a heating pad at 37°C was employed to keep the mice warm. After disinfecting the skin of the surgical site, the bladder and prostate were exposed via a midline suprapubic incision. Bilateral cavernous nerve, located posterolateral to the prostate, was identified and isolated. The shaft of the penis was freed of skin and fascia. Small portions of the ischiocavernosus muscles were dissected bilaterally to expose each penile crus. Electric stimulation of the cavernous nerve was carried with a bipolar silver electrode, positioned by a micromanipulator and placed around the cavernous nerve. The electrode cable was attached to a stimulator and the electrical stimulation (ES) was delivered by a Grass Stimulator at 4 V with 16 Hz and 5-ms duration for 4 minutes to induce penile erection (AD Instruments Inc., CO, USA) [7,10,24].

To monitor the ICP, the right corpus cavernosum was penetrated by a 25-gauge needle and a heparinized (150 U/mL) mouse jugular catheter (ALZET Osmotic Pumps, CA, USA) was inserted into the right corpus cavernosum. The cannula inserted into the right corpus cavernosum was connected to a pressure transducer and an amplifier unit (AD Instruments Inc.). The amplifier was connected to a data acquisition module (AD Instruments Inc.). The

ICP before and after ES was recorded on a computer by Chart 5 software (AD Instruments Inc.). The two corporal bodies communicate in the mouse penis and the physiologic effects resulting from the administration of a drug into one corporal body can be successfully monitored via a separate apparatus applied to the other one [25]. For intracavernosal drug administration, a separate cannula (30.5-gauge needle, attached to PE-10 tubing and 25 μ L Hamilton syringe) was inserted into the left corpus cavernosum. Also, 10 μ M APCP (Sigma-Aldrich, St. Louis, MO, USA) was prepared in a volume of 20 μ L of Phosphate Buffered Saline (PBS) for injection. Ten minutes after the injection, ICP was monitored in the right corpus cavernosum with ES.

Systemic Arterial Pressure Measurement

ICP data were normalized by mean arterial pressure (MAP) and the MAP was monitored simultaneously with ICP monitoring. The right carotid artery was dissected via a midline cervical incision under the microscope, and then a mouse jugular catheter was inserted into the carotid artery. The catheter was connected to a pressure transducer and an amplifier unit. The amplifier was connected to a data acquisition module, and the MAP was recorded simultaneously with ICP monitoring on a computer by Chart 5 Software (AD Instruments Inc.).

High-Performance Liquid Chromatography (HPLC) Analysis of Tissue Extracts of Adenosine

The penes of mice were rapidly removed before and immediately after ES and quick frozen in liquid nitrogen containing a cocktail including 10 μ M 2'-deoxycoformycin (Sigma-Aldrich) to inhibit adenosine deaminase, 10 μ M alpha, beta-methylene ADP (APCP) to inhibit CD73, and 10 μ M dipyridamole to inhibit equilibrium nucleotide transporter activity [26]. Nucleosides were extracted from frozen penes using 0.4 N perchloric acid as described previously [27]. Tissue extracts and adenosine and adenine nucleotides were separated and quantified by reverse-phase HPLC (Waters, Millipore Corp., Billerica, MA, USA) analysis on a Partisphere-bonded phase C18 (reverse-phase) cartridge column at a flow rate of 1.5 mL/min [28].

Adenosine 5'-Triphosphate Measurement

The penile tissues before and after ES were collected and frozen in liquid nitrogen. Adenosine 5'-triphosphate (ATP) was extracted from frozen penes using 0.4 N perchloric acid (Sigma-Aldrich). One hundred μ L supernatant of the extraction was transferred to a new tube and neutralized with 50 μ L 0.6 N KHCO_3 /0.72 N KOH. Then, the sample was centrifuged at 20,000 g for 5 minutes and the supernatant was transferred to a new tube and stored at -20°C for ATP measurement. The ATP level was assessed by ATP Bioluminescent Assay Kit (Sigma-Aldrich) [29].

CD73 Immunohistochemistry

CD73 antibody is a gift from Michael Blackburn (University of Texas—Medical School at Houston). This antibody has been used to determine expression of CD73 in the lung [30]. To localize the expression of CD73 in penile tissue, immunohistochemistry was performed on 5- μ m sections cut from formalin-fixed, paraffin-embedded penes. Slides were processed following the ABC Elite Staining Reagents instruction (Vector Laboratories, Burlingame, CA, USA) and incubated with a 1:50 dilution of monoclonal antibody to CD73 overnight at 4°C [30]. The sections were counter-stained with hematoxylin.

Statistical Analysis

All data were expressed as the mean $\pm$ SEM. Data were analyzed for statistical significance using GraphPad Prism 4 software (GraphPad Software, San Diego, CA, USA). Student's *t*-tests (paired or unpaired as appropriate) were applied in two-group analysis. Differences between the means of multiple groups were compared by the one-way analysis of variance, followed by Tukey's multiple comparisons test. A value of $P < 0.05$ was considered significant and was the threshold to reject the null hypothesis.

Results

Cavernous Nerve Stimulation Induces Adenosine Production in Penile Tissue

In an effort to determine whether endogenous adenosine plays a direct role in penile erection, we used a well-established method to mimic normal physiological erection by ES of the cavernous nerve to induce an erectile response [7,10,24]. Flaccid penile tissue was obtained as a control from sham-operated unstimulated mice. Tissue extracts from flaccid and erected penile tissue were fractionated by high-pressure liquid chromatography (HPLC) to measure endogenous adenosine levels. The results show that the concentration of adenosine in penile tissue doubled as a result of cavernous nerve stimulation (Figure 1).

Genetic Deletion of CD73 Abolishes Cavernous Nerve Stimulation-Induced Adenosine Production in Penile Tissue

In many circumstances, a cell-surface ecto-5'-nucleotidase termed CD73 catalyzes the rate-limiting step in the conversion of extracellular adenine nucleotides to adenosine [31]. To assess the functional role of CD73 in adenosine production, we measured adenosine levels in penile tissue in WT and CD73-deficient mice with or without cavernous nerve stimulation. We found that adenosine levels in penile tissue of CD73-deficient mice were significantly lower than that of WT mice and that no increase in adenosine levels resulted from cavernous nerve stimulation of CD73-deficient mice (Figure 1). These findings demonstrate that CD73 is essential for adenosine production within penile tissues in response to cavernous nerve stimulation *in vivo*.

CD73 is Highly Expressed in the Neuronal and Endothelial Cells of Mouse Penile Tissues

Next, we used immunohistochemistry staining to localize CD73 expression in WT mouse penile tissue. We found that CD73 was widely expressed in penile tissue with remarkably high expression levels in the nerve bundles, smooth muscle, and endothelium (Figure 2), suggesting that these are the major cell types involved in adenosine production within penile tissues.

Neuronal Activation-Mediated ATP Release is Responsible for CD73-Dependent Adenosine Elevation during Penile Erection

Neuronal activation is responsible for penile erection [5]. Knowing that the activation of certain presynaptic neurons results in ATP release [32–34] and in view of our finding that CD73 is highly expressed in cavernous nerve bundles (Figure 2) and is important for adenosine production during penile erection (Figure 1), we hypothesize that during penile erection, extracellular ATP is sequentially dephosphorylated into ADP and adenosine monophosphate (AMP), while adenosine is generated from subsequent catabolism of AMP by CD73. In support of this hypothesis, we found that total ATP levels (i.e., intracellular and extracellular) were reduced to 75% of baseline in penile tissues of WT mice following cavernous nerve stimulation ($N = 5-7$). The decrease in total ATP levels presumably reflects the rapid conversion of released ATP to ADP and AMP that subsequently led to elevated extracellular adenosine production in penile tissues (Figure 1).

Genetic Deficiency of CD73 Leads to Impaired Erectile Function in Mice

To evaluate the role of CD73-mediated adenosine production in the initiation and maintenance of penile erection, ICP was monitored in WT and CD73-deficient mice following cavernous nerve stimulation. We found that the initial increase in ICP with time (the initiation phase) in the CD73-deficient mice was 0.15 ± 0.05 mm Hg/s, which was significantly less than that in the WT mice (0.30 ± 0.13 mm Hg/s, $P < 0.05$, $N = 8-10$ for each group of mice) (Figure 3A,D), suggesting that CD73-mediated production of adenosine contributed to the initiation of penile erection. In addition, the ratio of ES-induced ICP to MAP in CD73-deficient mice reduced from 0.48 ± 0.03 to 0.33 ± 0.05 ($P < 0.05$) and the total ICP (i.e., area under the curve) in these mice reduced from $5,016 \pm 1,262$ mm Hg*s to $2,994 \pm 1,265$ mm Hg*s ($P < 0.05$) (Figure 3), demonstrating that CD73-induced adenosine production also contributes to the maintenance of penile erection.

Pharmacologic Inhibition of CD73 Activity Leads to Decreased Initiation and Maintenance of Penile Erection in WT Mice

Subsequently, to determine the significant role of CD73 in penile erection in WT mice, we pre-injected APCP, a specific inhibitor of CD73, in WT mice prior to ES of cavernous nerve. The WT mice pre-injected with equal volume of PBS served as control. We found that the initial slope was reduced from 0.29 ± 0.11 mm Hg/s to 0.19 ± 0.04 mm Hg/s in APCP-treated mice ($P < 0.05$, $N = 5-7$) (Figure 4). The ratio of ICP to MAP and total ICP in APCP-treated WT mice were reduced from 0.49 ± 0.03 to 0.38 ± 0.06 and from $5,151 \pm 1,150$ mm Hg*s to $2,994 \pm 1,265$ mm Hg*s, respectively (both $P < 0.05$). Thus, by using genetically deficient mice and pharmacologic inhibitors, we determined that CD73, a key enzyme responsible for endogenous adenosine generation, contributes to the initiation and maintenance of normal penile erection.

Discussion

In this study, we demonstrate that endogenously produced adenosine contributes to the initiation and maintenance of penile erection. First, we have provided the *in vivo* evidence that cavernous nerve stimulation induces endogenous adenosine elevation during penile erection. We then demonstrate that CD73, which is highly expressed in the neuronal and endothelial cells in the penile tissues, is essential for endogenous adenosine production during penile erection. Functionally, we provide both genetic and pharmacologic studies that CD73-dependent endogenously produced adenosine contributes to the initiation and maintenance of penile erection. Thus, our studies reveal a significant role of endogenous-generated adenosine in normal penile erection and offer underlying mechanisms for its generation.

Penile erection is a complex physiologic process that involves many factors and signaling pathways. NO is considered to be a dominant player in the erectile response [6,8,10] and NOS-deficient mice display abnormalities in penile erection [35,36]. However, these findings do not exclude other factors that contribute to penile erection. Here, we provide both genetic and pharmacological evidence that neuronal-mediated increase in endogenous adenosine contributes to penile erection. Although CD73-deficient mice have impaired ED, these deficient mice, as with NOS-deficient mice, do not show reduced fertility. These studies implicate that adenosine and NO can compensate for each other and contribute to normal penile erection. Of note, the importance of endogenous adenosine in normal penile erection is strongly supported by earlier studies in multiple animal species, including humans [15], showing that intracavernous injection of exogenous adenosine resulted in tumescence and penile erection [16–21]. However, attention was diverted from adenosine signaling to NO by the unexpected discovery of PDE5 inhibitors, such as sildenafil, that

induce penile erection. Attention has recently returned to adenosine signaling with reports indicating that impaired A2B adenosine receptor (A2BR) is associated with ED in men [37] and that excess adenosine contributes to priapism in mice [27]. Overall, these studies significantly strengthen our findings that endogenously generated adenosine is a previously unrecognized player in normal penile erection and abnormal levels of adenosine or signaling may contribute to erectile disorders.

Penile erection is initiated by activation of cavernous nerves as a result of signals delivered through the central nervous system in response to sexual excitation. We show here that the activation of cavernous nerves is accompanied by the release of ATP that is dephosphorylated to AMP which is subsequently converted to adenosine through the rate-limiting enzyme, ecto-5'-ectonucleotidase, CD73. While numerous earlier studies have shown that ATP is released from presynaptic neurons [32–34,38], a role for ATP release at neurovascular junctions and for the initiation of penile erection has not been previously identified. Here, we demonstrate that CD73, a key regulator of extracellular adenosine production from AMP is highly expressed in the membranes of cavernous nerves. The localization of CD73 in the neuronal cell membrane suggests that ATP released from cavernous neurons may be dephosphorylated to AMP which quickly converted to adenosine and subsequently engage adenosine receptors on the surrounding cells, such as endothelial cells or smooth muscle cells to induce cavernosal smooth muscle relaxation. Supporting this hypothesis, we showed that genetic deletion of CD73 in mice prevented the increase in adenosine levels following cavernous nerve stimulation. Functionally, we provide genetic and pharmacologic evidence that CD73 contributes to the initiation and maintenance of penile erection. Although our studies revealed a previously unrecognized role for CD73 in endogenous adenosine production and normal penile erection, we do realize that other potential candidates or sources, besides CD73, are likely involved in regulating endogenous adenosine levels. For example, extra-cellular adenosine levels could be influenced by the activity of equilibrative transporters that shuttle adenosine in and out of cells, by the level of adenosine deaminase, which converts adenosine to the presumably inactive nucleoside inosine. Taken together, our studies revealed the importance of CD73-dependent endogenous adenosine production in normal penile erections and suggested that CD73 likely works with other components involved in adenosine production and subsequent penile function.

In conclusion, our work identified a previously unrecognized role of adenosine signaling in penile erection and revealed the underlying mechanisms accounting for adenosine production. It is likely that the significance of our findings is not limited to penile physiology and pathology but extends to a more general role for adenosine signaling in the regulation of vascular tone. For example, the regulated release of ATP by peripheral nerves, and its contribution to adenosine production, may play an important role in neurovascular physiology and vascular tone. Thus, our studies not only discover a new player contributing to normal penile erection but are likely to have substantial relevance and implications for the mechanisms by which adenosine regulates vascular tone. Our findings also suggest that adenosine signaling pathway may serve as a novel pathway which can be targeted for the treatment of ED.

References

1. Lue TF. Erectile dysfunction. *N Engl J Med*. 2000; 342:1802–13. [PubMed: 10853004]
2. Burnett AL. Erectile dysfunction. *J Urol*. 2006; 175:S25–31. [PubMed: 16458737]
3. Gratzke C, Angulo J, Chitaley K, Dai YT, Kim NN, Paick JS, Simonsen U, Uckert S, Wespes E, Andersson KE, Lue TF, Stief CG. Anatomy, physiology, and pathophysiology of erectile dysfunction. *J Sex Med*. 2010; 7:445–75. [PubMed: 20092448]

4. Burnett AL, Goldstein I, Andersson KE, Argiolas A, Christ G, Park K, Xin ZC. Future sexual medicine physiological treatment targets. *J Sex Med.* 2010; 7:3269–304. [PubMed: 21029380]
5. Burnett AL. Neurophysiology of erectile function: Androgenic effects. *J Androl.* 2003; 24:S2–5. [PubMed: 14581490]
6. Burnett AL, Lowenstein CJ, Bredt DS, Chang TS, Snyder SH. Nitric oxide: A physiologic mediator of penile erection. *Science.* 1992; 257:401–3. [PubMed: 1378650]
7. Hurt KJ, Musicki B, Palese MA, Crone JK, Becker RE, Moriarity JL, Snyder SH, Burnett AL. Akt-dependent phosphorylation of endothelial nitric-oxide synthase mediates penile erection. *Proc Natl Acad Sci U S A.* 2002; 99:4061–6. [PubMed: 11904450]
8. Burnett AL, Nelson RJ, Calvin DC, Liu JX, Demas GE, Klein SL, Kriegsfeld LJ, Dawson VL, Dawson TM, Snyder SH. Nitric oxide-dependent penile erection in mice lacking neuronal nitric oxide synthase. *Mol Med.* 1996; 2:288–96. [PubMed: 8784782]
9. Burnett AL. Nitric oxide regulation of penile erection: Biology and therapeutic implications. *J Androl.* 2002; 23:S20–6. [PubMed: 12236169]
10. Champion HC, Bivalacqua TJ, Takimoto E, Kass DA, Burnett AL. Phosphodiesterase-5A dysregulation in penile erectile tissue is a mechanism of priapism. *Proc Natl Acad Sci U S A.* 2005; 102:1661–6. [PubMed: 15668387]
11. Fredholm BB, IJzerman AP, Jacobson KA, Klotz KN, Linden J. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol Rev.* 2001; 53:527–52. [PubMed: 11734617]
12. Wen J, Jiang X, Dai Y, Zhang Y, Tang Y, Sun H, Mi T, Kellems RE, Blackburn MR, Xia Y. Adenosine deaminase enzyme therapy prevents and reverses the heightened cavernosal relaxation in priapism. *J Sex Med.* 2010; 7:3011–22. [PubMed: 19845544]
13. Phatarpekar PV, Wen J, Xia Y. Role of adenosine signaling in penile erection and erectile disorders. *J Sex Med.* 2010; 7:3553–64. [PubMed: 19889148]
14. Dai Y, Zhang Y, Phatarpekar P, Mi T, Zhang H, Blackburn MR, Xia Y. Adenosine signaling, priapism and novel therapies. *J Sex Med.* 2009; 6(3 suppl):292–301. [PubMed: 19267852]
15. Kilic S, Salih M, Anafarta K, Baltaci S, Kosar A. Adenosine: A new agent in the diagnosis of impotence. *Int J Impot Res.* 1994; 6:191–8. [PubMed: 7795719]
16. Lin CS, Lin G, Lue TF. Cyclic nucleotide signaling in cavernous smooth muscle. *J Sex Med.* 2005; 2:478–91. [PubMed: 16422842]
17. Yonezawa A, Sakurada S, Furukawa K, Kimura Y. Adenosine and adenosine triphosphate. *Nippon Rinsho.* 2002; 60(6 suppl):52–6. [PubMed: 12166216]
18. Noto T, Inoue H, Mochida H, Kikkawa K. Role of adenosine and P2 receptors in the penile tumescence in anesthetized dogs. *Eur J Pharmacol.* 2001; 425:51–5. [PubMed: 11672574]
19. Filippi S, Mancini M, Amerini S, Bartolini M, Natali A, Mancina R, Forti G, Ledda F, Maggi M. Functional adenosine receptors in human corpora cavernosa. *Int J Androl.* 2000; 23:210–7. [PubMed: 10886423]
20. Shalev M, Staerman F, Allain H, Lobel B, Saiag B. Stimulation of P2y purinoceptors induces, via nitric oxide production, endothelium-dependent relaxation of human isolated corpus cavernosum. *J Urol.* 1999; 161:955–9. [PubMed: 10022733]
21. Sharifzadeh M, Zarrindast MR, Samini M. Effects of adenosine analogues on apomorphine-induced penile erection in rats. *Gen Pharmacol.* 1995; 26:1785–90. [PubMed: 8745169]
22. Chiang PH, Wu SN, Tsai EM, Wu CC, Shen MR, Huang CH, Chiang CP. Adenosine modulation of neurotransmission in penile erection. *Br J Clin Pharmacol.* 1994; 38:357–62. [PubMed: 7833226]
23. Chen JF, Huang Z, Ma J, Zhu J, Moratalla R, Standaert D, Moskowitz MA, Fink JS, Schwarzschild MA. A(2A) adenosine receptor deficiency attenuates brain injury induced by transient focal ischemia in mice. *J Neurosci.* 1999; 19:9192–200. [PubMed: 10531422]
24. Bivalacqua TJ, Musicki B, Hsu LL, Gladwin MT, Burnett AL, Champion HC. Establishment of a transgenic sickle-cell mouse model to study the pathophysiology of priapism. *J Sex Med.* 2009; 6:2494–504. [PubMed: 19523035]

25. Sezen SF, Burnett AL. Intracavernosal pressure monitoring in mice: Responses to electrical stimulation of the cavernous nerve and to intracavernosal drug administration. *J Androl.* 2000; 21:311–5. [PubMed: 10714826]
26. Zhang Y, Dai Y, Wen J, Zhang W, Grenz A, Sun H, Tao L, Lu G, Alexander DC, Milburn MV, Carter-Dawson L, Lewis DE, Zhang W, Eltzschig HK, Kellems RE, Blackburn MR, Juneja HS, Xia Y. Detrimental effects of adenosine signaling in sickle cell disease. *Nat Med.* 2011; 17:79–86. [PubMed: 21170046]
27. Mi T, Abbasi S, Zhang H, Uray K, Chunn JL, Xia LW, Molina JG, Weisbrodt NW, Kellems RE, Blackburn MR, Xia Y. Excess adenosine in murine penile erectile tissues contributes to priapism via A2B adenosine receptor signaling. *J Clin Invest.* 2008; 118:1491–501. [PubMed: 18340377]
28. Blackburn MR, Datta SK, Kellems RE. Adenosine deaminase-deficient mice generated using a two-stage genetic engineering strategy exhibit a combined immunodeficiency. *J Biol Chem.* 1998; 273:5093–100. [PubMed: 9478961]
29. Gorman MW, Marble DR, Ogimoto K, Feigl EO. Measurement of adenine nucleotides in plasma. *Luminescence.* 2003; 18:173–81. [PubMed: 12701093]
30. Volmer JB, Thompson LF, Blackburn MR. Ecto-5'-nucleotidase (CD73)-mediated adenosine production is tissue protective in a model of bleomycin-induced lung injury. *J Immunol.* 2006; 176:4449–58. [PubMed: 16547283]
31. Colgan SP, Eltzschig HK, Eckle T, Thompson LF. Physiological roles for ecto-5'-nucleotidase (CD73). *Purinergic Signal.* 2006; 2:351–60. [PubMed: 18404475]
32. Yegutkin GG. Nucleotide- and nucleoside-converting ectoenzymes: Important modulators of purinergic signalling cascade. *Biochim Biophys Acta.* 2008; 1783:673–94. [PubMed: 18302942]
33. Burnstock G. The past, present and future of purine nucleotides as signalling molecules. *Neuropharmacology.* 1997; 36:1127–39. [PubMed: 9364468]
34. Burnstock G. Physiology and pathophysiology of purinergic neurotransmission. *Physiol Rev.* 2007; 87:659–797. [PubMed: 17429044]
35. Bivalacqua TJ, Liu T, Musicki B, Champion HC, Burnett AL. Endothelial nitric oxide synthase keeps erection regulatory function balance in the penis. *Eur Urol.* 2007; 51:1732–40. [PubMed: 17113219]
36. Burnett AL. Nitric oxide in the penis: Physiology and pathology. *J Urol.* 1997; 157:320–4. [PubMed: 8976289]
37. Faria M, Magalhaes-Cardoso T, Lafuente-de-Carvalho JM, Correia-de-Sa P. Corpus cavernosum from men with vasculogenic impotence is partially resistant to adenosine relaxation due to endothelial A(2B) receptor dysfunction. *J Pharmacol Exp Ther.* 2006; 319:405–13. [PubMed: 16837560]
38. Burnstock G. Historical review: ATP as a neurotransmitter. *Trends Pharmacol Sci.* 2006; 27:166–76. [PubMed: 16487603]

Figure 1.

Adenosine levels in penile tissues of wild-type (WT) mice and CD73-deficient mice with or without electrical stimulation of cavernous nerve. (A) Representative profiles of reverse-phase HPLC analysis of penile extracts isolated from WT mice and CD73-deficient mice with or without electrical stimulation (ES) of cavernous nerve. (B) Adenosine was significantly decreased in the mouse penile tissues in CD73-deficient mice with or without ES. Data are expressed as means $\pm$ SEM. (N = 6–7). * P < 0.05 vs. WT mice without ES. ** P < 0.05 vs. WT mice with ES.

Figure 2.

Immunohistochemical localization of CD73 in mouse penis. CD73 is highly expressed in neuronal (black arrows), endothelial cells (black triangles), and smooth muscle cells (black arrows) in wild-type (WT) mouse penile tissues. Penile section from WT mice and CD73-deficient mice (CD73^{-/-}) were incubated with primary anti-CD73 antibody and secondary antibody, respectively. While the negative control refers to the penile section from WT mice without incubation of primary anti-CD73 antibody and with incubation of secondary antibody. No CD 73 immunostaining was detected in either CD73^{-/-} or negative control section. Scale bars, 100 μ m.

Figure 3. Impaired erectile function in CD73-deficient mice. (A) Representative intracavernous pressure (ICP) tracing after cavernous nerve stimulation at 4 V for 4 minutes. CD73-deficient mice had a reduced maximum ICP (B), total ICP (C), and displayed a reduced initial rate of increase in ICP (i.e., slope, D). Data are expressed as means $\pm$ SEM (N = 8–10). * P < 0.05 versus WT mice.

Figure 4. CD73 inhibitor reduced erectile function in wild-type mice. (A) Representative intracavernous pressure (ICP) tracing after cavernous nerve stimulation at 4 V for 4 minutes in the presence or absence of APCP, a CD73-specific inhibitor. (B–D) APCP reduced maximum ICP (B), total ICP (C), and the initial rate of increase in ICP (slope, D). Data are expressed as means $\pm$ SEM. (N = 5–7). * P < 0.05 vs. WT mice with PBS treatment.