Adenosine modulation of neurotransmission in penile erection

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1 Adenosine inhibited the noradrenaline-induced contraction of rabbit corpus cavernosum in a dose-dependent manner. The effect of adenosine was greater in intact corpus cavernosa than in endothelium-denuded preparations. This finding indicates that the relaxing effect of adenosine is partially endothelium-dependent and involved in the release of endothelium-derived relaxing factors.

2 Adenosine and its analogues relaxed the noradrenaline-induced contractile response as well as inhibited the transmural nerve induced contraction with the potency order: NECA > R-PIA > adenosine. These data indicate that adenosine can modulate both the non-adrenergic non-cholinergic and adrenergic neurotransmission. DMPX, an adenosine antagonist selective for the A2 receptors, abolished the electrically elicited relaxation. However, CGS 21680, selective for A2a receptor, had no effect on relaxation. Therefore, adenosine receptors involved in the modulation of neurotransmission in rabbit corpus cavernosum appear to be A2b subtype.

3 Adenosine also induced an increase in human cavernosal arterial velocity and resistive index measured by colour duplex sonography. The combination of adenosine and 10 μg prostaglandin E1 was more effective in resistive index and erection grade than 20 μg prostaglandin E1 alone. Our results suggest that adenosine seems to be an important neuromodulator for penile erection and can be an effective and alternative combination in the treatment of impotence.

Keywords adenosine impotence adenosine receptor colour duplex sonography

Introduction

Many studies have shown that the main neurotransmitter mediating relaxation of corpus cavernosum smooth fibre is a non-adrenergic non-cholinergic (NANC) neurotransmitter [1–7]. Although several endogenous peptides, such as calcitonin-gene-related peptide [3], vasoactive intestinal polypeptide (VIP) [5], neuropeptide [6] and substance P, have been proposed as the NANC neurotransmitter, the main ones responsible for the penile erection are still under investigation. Recent studies [4, 7–9] have also shown that nitric oxide may be involved in the NANC neurotransmission that leads to the smooth muscle relaxation in the corpus cavernosum. However, it has not been determined if nitric oxide is produced from the NANC neuron itself or one of endothelium-derived relaxation factors.

Burnstock [10] described adenosine triphosphate as a likely candidate for NANC neurotransmission. Adenosine is believed to be a purine nucleoside of fundamental importance as a building block in nucleic acid and as a regulator of biological function. Recently, adenosine has been demonstrated to modulate NANC neurotransmission in the rabbit iris sphincter muscle [11], guinea pig atria [12] and guinea pig bronchi [13]. Moreover, adenosine and adenine nucleotide, released during nerve stimulation, probably are capable of inhibiting neurotransmitter release from adrenergic nerve endings in cardiac tissues [14] and fallopian tube [15].

A theory for penile erection was proposed as: (1) endogenous modulators in the inhibitory action of persistent adrenergic tone; (2) withdrawal of the
adrenergic activity resulting in unmasking and activation of NANC neurotransmission; (3) additional support from the endogenous modulators such as acetylcholine and prostaglandin acting on relaxant receptors [16]. The main purpose of this study was to ascertain the role of adenosine in the modulation of adrenergic and NANC neurotransmission in penile erection. We also evaluated the effects of adenosine in human penile erection by colour duplex sonography and assessed its efficacy in the treatment of impotence.

Methods

Drugs

Adenosine, noradrenaline, acetylcholine chloride, atropine sulphate, and guanethidine sulphate were obtained from Sigma Chemical (St Louis, Missouri). R-phenylisopropyl-adenosine (R-PIA), 5’-N-ethylcarboxamidoadenosine (NECA), 3,7-dimethyl-propargylxanthine (DMPX) and CGS 21680 were from Research Biochemicals Incorporated (Wayland, Maryland). Physiological salt solution consisted of (mmol): NaCl, 118.3; KCl, 4.7; MgSO4, 0.6; KH2PO4, 1.2; CaCl2, 2.5; NaHCO3, 25.0; disodium ethylenediaminetetraacetic acid, 0.026; and glucose, 11.1.

Preparation of rabbit corpus cavernosum

Strips of corpus cavernosum measuring 0.3 × 0.3 × 1 cm from New Zealand White rabbits (3–4 kg) were carefully dissected from the tunica albuginea and mounted under 2 g resting tension in organ chambers containing physiological salt solution at 37° C gassed with 95% O2-5% CO2; pH was maintained at 7.4. The strip was fixed with metal clips from below and above, connected to a force transducer. Tracings were made on a San-Ei polygraphy (Tokyo, Japan). The strips were allowed to equilibrate under tension for 2 h. The drug was removed by several washes with physiological salt solution, and the tension was allowed to return to the baseline. Strips were allowed to reequilibrate for 30 min after the drug response to ensure maximum washout of the drug and to minimize the possibility of receptor desensitization. The 20 ml organ baths were equipped with platinum electrodes 20 mm in length and 10 mm apart surrounding the middle portion of the strip, a current amplifier, and a simulator (Coulbourn). Transmural electrical stimulation was performed at 10 Hz, 0.5 ms, 50 V at bath electrodes and in trains of 10 s duration. The responses for study of relaxation to field stimulation were evaluated in the presence of 5 μM guanethidine and 1 μM atropine in order to eliminate the adrenergic and cholinergic responses.

Removal of endothelium

The endothelial lining of corpus cavernosum was removed by rubbing the strip between the thumb and index for 20 s [1]. Successful removal and the integrity of the endothelium were confirmed by adding acetylcholine (10^{-3} m) to noradrenaline precontracted strips. The anti-factor VIII peroxidase antiperoxidase technique was also used to detect the integration of endothelium after the experiment.

Colour duplex sonography in the assessment of the effects of adenosine in human penis

Nineteen impotent men (aged 24–75 years) were included in this study. Nine patients also represented various other medical diseases, including diabetes in two, hypertension in three, myocardial infarction in one, parkinsonism in one, CVA in one and neurosis in one. The methods employed in the present study have been described in more detail in our previous work [17]. All patients were extensively informed about the study and the possible side effects and gave written informed consent. Every patient was injected intracorporally with prostaglandin E1 (20 μg). Seven patients received additional intracorporal injection with adenosine (30 or 40 mg). Twelve patients received additional intracorporal injection with adenosine (40 mg) in combination with prostaglandin E1 (10 μg). The interval of each injection was at least 1 week. Peak velocity which is the highest flow rate detected, resistive index (peak systolic velocity—minimum diastolic velocity/peak systolic velocity) and erection grade (1-tumescence, 2-suboptimal erection, 3-full erection) were evaluated during the examination by the same operator (Chiang) [17].

Statistical analysis

The percentage of relaxation refers to the decrease in noradrenaline-induced tone. Data were expressed as mean ± s.e. mean. The concentration of the drug producing 50% of the relaxation (EC50 value) was estimated using a statistical package for the personal microcomputer. The concentration-response curves were compared by a repeated-measure analysis of variance. The peak velocity and resistive index of cavernosal arteries were compared by Wilcoxon matched paired signed-rank test for non-parametric data and paired t-test for parametric data.

Results

Relaxing effect of adenosine in corpus cavernosum with intact and denuded endothelium

Strips of corpus cavernosum, with and without the presence of endothelium, were contracted with noradrenaline (10^{-5} m). Adenosine (10^{-5}-10^{-2} m), added cumulatively, relaxed both types of corpus cavernosum in a dose-dependent manner. Figure 1 reveals the dose-response curves for the action of adenosine in both preparations. The EC50 value of adenosine on the inhibition of contractions induced by noradrenaline was 5 × 10^{-4} ± 4 × 10^{-5} m (n = 10) in intact and
1.53 \times 10^{-3} \pm 2.3 \times 10^{-4} \text{ M} (n = 7) \text{ in denuded endothelium, respectively.}

**Pharmacological properties of adenosine receptor subtypes**

The dose-response curves for the action of adenosine, R-PIA and NECA are shown in Figure 2. The EC\textsubscript{50} values of R-PIA and NECA on the inhibition of contractions were $1.46 \times 10^{-4} \pm 8.6 \times 10^{-5} \text{ M} (n = 10)$ and $1.03 \times 10^{-5} \pm 5.3 \times 10^{-6} \text{ M} (n = 11)$ respectively. Adenosine and its analogues inhibited contractile responses which were induced by noradrenaline with the potency order: NECA > R-PIA > adenosine. When transmural nerve stimulation was applied to the strip of corpus cavernosum, adenosine and its analogues inhibited the nerve induced contraction with the potency order: NECA > R-PIA > adenosine (Figure 3). Transmural nerve stimulation of the strips treated with guanethidine and atropine caused frequency-dependent relaxation on contractile responses induced by noradrenaline. DMPX, an adenosine antagonist selective for the A2 receptor, abolished the electrically elicited relaxation (Figure 4) in a dose-dependent manner. However, CGS 21680, an adenosine agonist selective for the A2a receptor, had no effect of relaxation on contractile responses induced by noradrenaline. In conclusion, adenosine receptors involved in the modulation of relaxation of non-adrenergic non-cholinergic neurotransmission in rabbit corpus cavernosum belong to the A2b subtype.

**Effects of adenosine in human penile erection**

Direct injection of adenosine (30 or 40 mg in 2 ml of normal saline) into corpus cavernosum caused dilation of vascular spaces immediately. The effect of adenosine is faster than that of prostaglandin E\textsubscript{1}. However, there was no significant difference between the injection of PGE\textsubscript{1} (20 µg) and adenosine (30 or 40 mg) in peak velocity and resistive index (Table 1). In the initial seven patients the intracorporeal injection of adenosine alone induced a suboptimal erection in two and evident tumescence in five (Table 1). The same seven patients receiving the injection of PGE\textsubscript{1}...
alone induced a full erection in one, suboptimal erection in three and tumescence in three. There was no difference of cavernosal arterial peak velocity in patients receiving injection of PGE₁ (20 µg) alone and adenosine (40 mg) combined with PGE₁ (10 µg) (Table 2), whereas, it was better for patients receiving adenosine combined with half-dose of PGE₁ in resistive index (Table 2). For the erection grade, improvements were noted in five patients receiving adenosine (40 mg) combined with PGE₁ (10 µg) (Table 2). Seven patients remained stationary. Two patients suffered from palpitation and dizziness for about 10 s when receiving injection of PGE₁ in combination with adenosine. Many patients felt pain when injected with PGE₁ alone, but felt no pain when injected with PGE₁ plus adenosine or adenosine alone (Tables 1 and 2).

Discussion

After the concept of ATP as a neurotransmitter in the non-adrenergic and non-cholinergic nerves was first described [18], Ginsborg & Hirst [19] discovered that adenosine could modulate the release of the transmitter from the phrenic nerve of the rat. Since then intensive studies about the effects of adenosine have received careful attention. As a local hormone, neurotransmitter, or neuromodulator, adenosine is a ubiquitous biologic compound, being present in every cell of the human body. It has also been established as an efficient local chemical regulatory signal to address communication between cells and to exert a wide spectrum of effects on various tissues and organs under physiologic and pathophysiologic conditions [20]. However, little is known about the effects of adenosine on penile erection.

It has recently been observed that penile NANC neurotransmission is regulated by several endogenous substances such as calcitonin-gene-related peptide [3], vasoactive intestinal polypeptide [5], neuropeptide Y [6], substance P, prostaglandins and nitric oxide [4, 7–9]. In the present study, we demonstrated that exogenous adenosine and its analogues had relaxing effects on the corporeal smooth muscle submaximally contracted with noradrenaline as well as

Table 1  Comparison of the effects of prostaglandin E₁ and adenosine on peak velocity, resistive index and erection grade

<table>
<thead>
<tr>
<th>Patient</th>
<th>Prostaglandin E₁ (20 µg)</th>
<th>Adenosine (30 or 40 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak velocity (cm s⁻¹)</td>
<td>Resistive index</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>36</td>
<td>0.62</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>0.75</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>0.88</td>
</tr>
<tr>
<td>4</td>
<td>47</td>
<td>1.33</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>0.66</td>
</tr>
<tr>
<td>6</td>
<td>47</td>
<td>0.70</td>
</tr>
<tr>
<td>7</td>
<td>13</td>
<td>0.75</td>
</tr>
<tr>
<td>Mean ± s.d.</td>
<td>30.6 ± 14.5</td>
<td>0.8 ± 0.2</td>
</tr>
</tbody>
</table>

*1. tumescence; 2. suboptimal erection; 3. full erection.  
²P > 0.05 Wilcoxon matched paired signed-rank test.

Table 2  Comparison of the effects of prostaglandin E₁ (PGE₁) and PGE₁ plus adenosine on peak velocity, resistive index and erection grade

<table>
<thead>
<tr>
<th>Patient</th>
<th>PGE₁ (20 µg)</th>
<th>PGE₁ (20 µg) + Adenosine (40 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak velocity (cm s⁻¹)</td>
<td>Resistive index</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>22</td>
<td>0.82</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>0.69</td>
</tr>
<tr>
<td>3</td>
<td>27</td>
<td>0.67</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>0.66</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>0.77</td>
</tr>
<tr>
<td>6</td>
<td>36</td>
<td>0.84</td>
</tr>
<tr>
<td>7</td>
<td>17</td>
<td>0.70</td>
</tr>
<tr>
<td>8</td>
<td>36</td>
<td>0.88</td>
</tr>
<tr>
<td>9</td>
<td>41</td>
<td>1.00</td>
</tr>
<tr>
<td>10</td>
<td>17</td>
<td>0.40</td>
</tr>
<tr>
<td>11</td>
<td>15</td>
<td>0.63</td>
</tr>
<tr>
<td>12</td>
<td>55</td>
<td>0.80</td>
</tr>
<tr>
<td>Mean ± s.d.</td>
<td>28.9 ± 11.7</td>
<td>0.74 ± 0.15**</td>
</tr>
</tbody>
</table>

*P > 0.05 paired t-test.  
**P < 0.05 paired t-test.

1. tumescence; 2. suboptimal erection; 3. full erection.

Painful.
the inhibitory effects on transmural nerve stimulation induced contractions. The rank order of potency between the adenosine and its analogues on the relaxing effects of noradrenaline-induced contractions and the inhibitory effects of transmural nerve stimulation was: NECA > R-PIA > adenosine. This rank order is compatible with an action at A2 adenosine receptor [21]. These findings suggest that the inhibitory effect of adenosine and its analogues was mediated via postjunctional action at adenosine A2 receptors. Moreover, DMPX, an adenosine antagonist selective for the A2 receptor, abolished the electrically elicited relaxation. The blockade of electrically elicited relaxation of corporeal smooth muscle with the addition of tetrodotoxin is consistent with the hypothesis that electrically elicited relaxation is mediated by the non-adrenergic, non-cholinergic neuronal pathway [1]. Thus, the present observations indicate non-adrenergic, non-cholinergic neurotransmission is involved with adenosine modulation. In addition, adenosine and its analogues can inhibit the electrical stimulation-induced contraction. Since the electrically induced contractions are probably mediated by postjunctional α1-adrenoceptors [1], our results suggest that adenosine and its analogues can modulate adrenergicneuroeffector transmission in corporeal smooth muscle via action at postjunctional receptors stimulating contractile activity. This is in agreement with previous findings in the human fallopian tube [15] and guinea pig uterine smooth muscle [22].

CGS 21680, an adenosine agonist selective for A2a receptors, had no relaxing effect on noradrenaline-induced contraction. This result suggests that the adenosine receptors involved in the modulation of relaxing effects in rabbit corpus cavernosum belong to the A2b subtype. It is compatible with the findings that most tissues consist mostly of the A2b subtype and the density of A2a receptors on most tissues except for central nervous system is too low to measure [23].

There are contradictory reports about the role of vascular endothelium in the response of arteries to adenosine. Some investigators indicated that adenosine was not affected by the removal of endothelium for dog femoral arteries [24]. On the other hand, adenosine was reported to be endothelium-dependent [25] for the pig aorta. In the present study we found the relaxing effect of adenosine was greater in intact corpus cavernosum than in preparations denuded with endothelium. These results suggest that the relaxing effects of adenosine were partially endothelium-dependent. Since nitric oxide is thought to be responsible for the corporeal smooth muscle relaxation elicited by endothelium-derived relaxing factor (EDRF) [4, 7, 8], ATP which is rapidly degraded to adenosine by highly active ectonucleotidases enhanced vasal release of nitric oxide and cGMP [26]. In addition, ADP is also demonstrated to provoke nitric oxide synthesis [9]. According to our previous observations [27] the relaxing effect of ATP and ADP is mainly due to their rapid catabolism to adenosine. In rat aorta, adenosine has been reported to induce the production of EDRF via endothelial A2 receptors and thus stimulate smooth muscle soluble guanylate cyclase [26]. The present results suggest that the relaxing effects of adenosine may be due to both the release of EDRF or nitric-oxide factor and direct effects on the A2 receptors of corporeal smooth muscle resulting in stimulation of adenylylate-cyclase and increasing intracellular level of cAMP.

We also performed the same study for human corpus cavernosum from impotent men receiving penile prostheses and renal donor cadaver (unpublished data) and found that the effects of adenosine were the same with those of rabbit corpus cavernosum. Since adenosine has been used as an antiarrhythmic agent and has short half-life in humans (<1 s) with only mild and transient side effects, we performed intracorporeal injection with adenosine and obtained more objective data evaluated by colour duplex sonography. The intracorporeal injection of adenosine alone caused an increase of cavernosal arterial flow immediately. These findings are supported by a study [28] indicating that adenosine is a potent inhibitor of exocytotic noradrenaline release in the heart. The injection of adenosine alone could not induce a full erection in our initial seven patients, but achieved suboptimal erection in two patients. Though adenosine increased the arterial flow as well as PGE1, it did not appear to result in an effective penile erection as PGE1. This may be due to the short half-life of adenosine. However, compared with the intracorporeal injection of 20 μg PGE1, the combination of adenosine and 10 μg PGE1 resulted in improvement in resistive index and erection grade. In our series, eight of the 19 consecutive patients with erectile dysfunction felt pain after administration of 20 μg PGE1. However, only one reported pain after the intracorporeal injection of combined adenosine and PGE1, and none experienced pain after administration of adenosine alone. The improvement of resistive index and erection grade with combination of adenosine and PGE1 may result from the direct effect of adenosine to relax both helicine and trabecular smooth muscle and the lesser intensity of pain which can induce the adrenergic tone and impede penile erection. The incidence of prolonged erection and side effects such as dizziness, palpitation will presumably be low, since adenosine is probably quickly metabolized by deaminase within the cavernous tissue. Our present study suggests that the combination of adenosine and PGE1 may be used for the treatment of erectile dysfunction. Further studies involving a greater number of cases and long-term follow-up are needed to confirm the role of adenosine in the treatment of impotence.

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References


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