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Adenosine Stimulates Human Sperm Motility via A2 Receptors

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Abstract—The effects of adenosine and its analogues on human sperm motility were studied using a transmembrane migration method. Specific binding sites for adenosine in human sperm were also investigated. Adenosine and 5′-*N*-ethylcarboxamidoadenosine (NECA) stimulated human sperm motility with similar efficacies and the maximal amplitudes of motility increases were both about 70%. 3,7-Dimethyl-1-propargylxanthine (DMPX), a potent A_2 antagonist, competitively antagonized NECA-induced motility stimulation. Successively higher concentrations of DMPX shifted the dose–response curve of NECA to the right in a nearly parallel fashion. Dipyridamole, an inhibitor of adenosine uptake, does not reduce the ability of adenosine to stimulate human sperm motility. In radioligand-binding studies, adenosine A_1 selective analogues, cyclopentyl-1,3-dipropylxanthine and 1-methyl-2-phenylethyl adenosine, have little competitive effect on [^{3}H]NECA binding in human sperm membrane. These results provide evidence that adenosine enhances human sperm motility via adenosine A_2 receptors on the surface of sperm membranes.

Adenosine modulates a variety of biological functions, including nervous and cardiovascular systems, immune responses, and metabolism (Williams 1987; Belardinelli et al 1990; Linden 1991). On the basis of pharmacological and biochemical studies, adenosine receptors have been subdivided into two major subtypes, termed A_1 and A_2 receptors (Van Calker et al 1979; Linden 1991). The A_1 receptor mediates an inhibition, whereas the A_2 receptor mediates a stimulation of adenylyl cyclase activity.

Cyclic (c) AMP appears to play a critical role in the regulation of sperm motility (Aitken et al 1983, 1986). The validation of the existence of functional adenosine receptors in human sperm has not yet been reported. In the present study we have investigated the effects of adenosine and its analogues on human sperm motility. It has been reported that adenosine is transported by facilitated diffusion via the nucleotide transporter in mammalian cells. While recent evidence suggests that adenosine uptake in mammalian spermatozoa appears to be slight (Brown & Casillas 1984), it may be important to exclude the possibility of an intracellular site of action. Therefore, the possible effect of dipyridamole, an inhibitor of adenosine transport systems (Cass et al 1987), was also evaluated. Finally, we investigated specific binding sites for adenosine in human spermatozoa.

Materials and Methods

Chemicals

5′-*N*-Ethylcarboxamidoadenosine (NECA), N^6-[(*R*)-1-methyl-2-phenylethyl] adenosine (R-PIA), and 3,7-dimethyl-1-propargylxanthine (DMPX) were purchased from Research Biochemicals Inc. (Natick, MA). Adenosine deaminase was from Boehringer Mannheim (Mannheim, Germany). [^{3}H]NECA (sp. act., 15 Ci mmol^{-1}) was purchased from New England Nuclear (Boston, MA). The other reagents were from Sigma (St Louis, MO).

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Measurement of human sperm motility

Semen samples were collected from normal volunteers after three days of sexual abstinence. All these semen samples had spermatozoa concentrations higher than 20×10^6 mL^{-1} and more than 20% progressive forward motility. Our previous studies (Hong et al 1981, 1984; Shen et al 1991, 1992) have shown that these values were essential criteria for the transmembrane migration method (Hong et al 1981) which was used to examine the in-vitro effects of adenosine and its analogues on sperm motility in this study. This method has been found to be most suitable for studying drug effects on straight and rapid sperm motility, which is the most important sperm quality for predicting the fertilizing capacity of a semen sample (Hong et al 1991). Each semen sample was divided into several 100 μL aliquots, which were mixed with either 50 μL of drug solution or phosphate-buffered saline. After a 20 min preincubation at room temperature (21°C), semen–drug or semen–buffer mixture (100 μL) was pipetted into the upper chamber of the transmembrane apparatus. The proportion of sperm that moved across the 5 μm pores of a Nucleopore membrane (Nucleopore, USA), from the upper chamber into a lower chamber containing phosphate-buffered saline during a 2 h incubation at 37°C, was designated the trans-membrane migration ratio (TMMR). Previous studies have shown that the TMMR is a quantitative and reproducible parameter for sperm motility (Hong et al 1984; Shen & Chen 1990; Shen et al 1991, 1992; Chen et al 1992).

Preparation of human sperm membranes

Semen samples, obtained from normal volunteers, were left at room temperature (21°C) for liquefaction after ejaculation. Spermatozoa were washed twice by centrifugation at 800 *g* for 10 min with 50 mL Ham's F10 medium and frozen at −20°C overnight. After thawing, the spermatozoa were Dounce homogenized in a small volume of 0·1 M HEPES (pH 8·0) and then centrifuged at 13000 *g* for 10–15 min. The pellet was resuspended in HEPES buffer containing (mM):

EDTA 1, benzamidine 0·1, HEPES 10, 0·02% sodium azide, pH 7·4 for radioligand-binding assays.

Radioligand-binding assays

Radioligand binding to adenosine receptors was assessed with [^{3}H]cyclopentyl-1,3-dipropylxanthine (DPCPX), ^{125}I-aminobenzyladenosine, ((2-*p*-carboxyethyl)phenylamino-5′-*N*-carboxamidoadenosine); and [^{3}H]NECA, but specific binding (competition by 10 mM theophylline) was observed only with the latter radioligand. Binding assays were initiated by adding 50 μL HEPES buffer (pH 7·4), containing 2·5 units of adenosine deaminase, 100 000–160 000 counts min^{-1} [^{3}H]NECA, and 5 mM $MgCl_2$, to 100 μL sperm membrane (100–200 μg membrane protein). Adenosine agonists (NECA, R-PIA) and antagonists (DPCPX, theophylline, DMPX) were used to compete with [^{3}H]NECA binding. Following incubation at 0°C (little specific binding could be detected to membranes incubated at room temperature (21°C)), assays were terminated after 3 h by filtration over Whatman GF/C glass fibre filters using a modified cell harvester (Brandel Cell Harvester M-24R, Brandel Scientific, Gaithersburg, MD). The filters were washed with 3 × 4 mL of ice-cold Tris buffer composed of 10 mM Tris-HCl, pH 7·4, and counted in a Beckman 5500 gamma-counter at a counting efficiency of 70%.

Data analyses

Sperm motility in semen mixed with phosphate-buffered saline was used to normalize the motility of drug-treated sperm. Binding parameters (K_i of competing compound) were fitted by Marquardt's nonlinear least squares interpolation to equations for one or multiple binding sites (Patel et al 1988), and the number of affinity sites was determined by use of F tests evaluated at $P < 0·001$. To calculate binding parameters, the concentrations of radioligands and competing ligands were corrected to account for their fractional binding to receptors.

Results

Effect of adenosine analogues on human sperm motility

As shown in Fig. 1, adenosine and NECA stimulate human sperm motility in a dose-dependent manner with EC50 values of 100 and 0·3 μM, respectively. Both adenosine and NECA caused a similar maximal amplitude of motility stimulation; i.e. about 70% of control. The ability of DMPX to antagonize NECA-induced motility stimulation is shown in Fig. 2. Successively higher concentrations of DMPX shifted the dose–response curve of NECA to the right in a nearly parallel fashion.

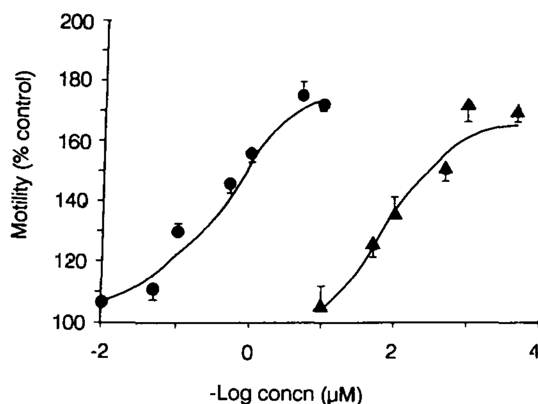

FIG. 1. Log concentration–response curves of adenosine and NECA on human sperm motility. ▲ Adenosine, ● NECA. Each point represents mean ± s.e.m. of six different normal samples. Sperm motility in semen mixed with phosphate-buffered saline was used as control.

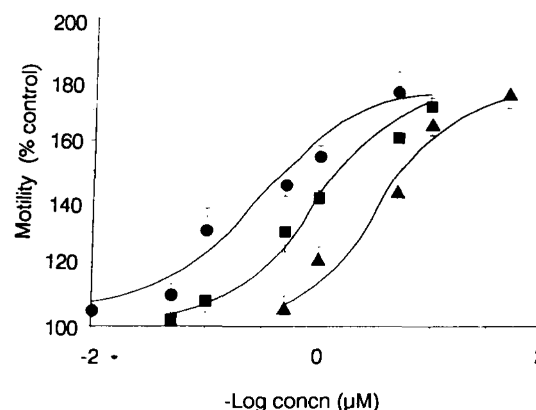

FIG. 2. Competitive antagonism by DMPX of NECA-stimulated sperm motility. Each point represents mean ± s.e.m. of six different normal samples. ● NECA only, ■ NECA + 30 μM DMPX, ▲ NECA + 100 μM DMPX.

Effect of dipyridamole on adenosine-stimulated sperm motility

The continuous presence of dipyridamole at 0·01 and 0·1 μM was assessed for the effect on sperm motility after a 20 min

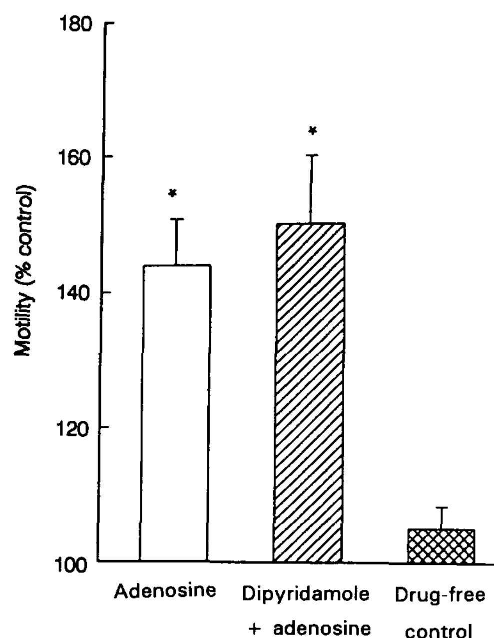

FIG. 3. Effect of dipyridamole on the ability of adenosine to stimulate human sperm motility, evaluated after a 20 min preincubation. Each column is for six different normal samples. *$P < 0·01$, paired Student's *t*-test, compared with drug-free control. Adenosine 500 μM; Dipyridamole 0·1 μM.

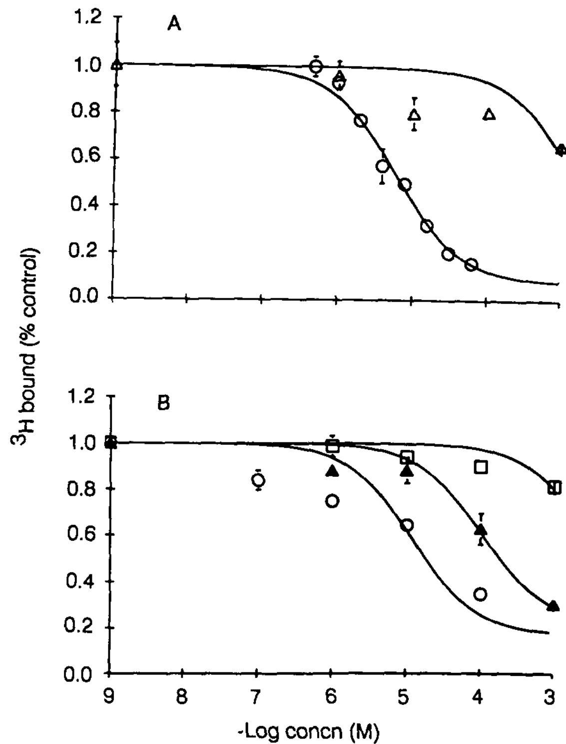

FIG 4. Competition for [^{3}H]NECA binding to human sperm membranes by A. R-PIA (△) and NECA (○) and B. DPCPX (□) theophylline (▲) and DMPX (○). Each point represents the mean of duplicate determinations.

preincubation with semen samples (n = 6). No significant differences in motility were noted among the control and dipyridamole samples; that is, dipyridamole alone did not affect sperm motility. Adenosine-stimulated motility was also evaluated in the presence or absence of 0·1 μM dipyridamole which may inhibit more than 90% of adenosine uptake in somatic cells (Pearson et al 1983). The sperm motility of 20 min preincubated samples was significantly higher in the presence of adenosine alone ($P < 0{\cdot}01$, paired Student's t-test) or adenosine with 0·1 μM dipyridamole, compared with drug-free control groups (Fig. 3). The amplitude of motility stimulation was not significantly different in the presence of adenosine alone or adenosine with 0·1 μM dipyridamole. These results suggested that adenosine-induced stimulation of human sperm motility does not require the involvement of a nucleoside transporter.

Radioligand binding studies

Fig. 4 shows the competition by various adenosine receptor agonists and antagonists for [^{3}H]NECA binding sites in human sperm membrane. The K_i values for each adenosine analogue were as follows: NECA, 6 μM; R-PIA, 1·4 mM; DPCPX > 10 mM; theophylline, 102 μM; DMPX, 11 μM. R-PIA and DPCPX, which are known to be highly selective adenosine A_1 analogues have K_i values > 1 mM. Since DPCPX binds with over 740 times higher affinity to A_1 than to A_2 receptors (Linden 1991), it is unlikely that any of the specific [^{3}H]NECA binding can be attributed to A_1 receptors.

Discussion

This study provides four lines of evidence to suggest that adenosine enhancement of human sperm motility is mediated via an action on adenosine A_2 receptors in human sperm membranes. First, adenosine and NECA stimulate human sperm motility with similar efficacies (the maximal amplitudes of motility increases were both about 70%), suggesting the possibility that they share a common mechanism of action. Second, DMPX, a potent A_2 antagonist (Linden 1991), competitively antagonizes NECA-induced stimulation of motility. Successively higher concentrations of DMPX shifts the dose–response curve of NECA to the right in a nearly parallel fashion. Third, dipyridamole, an inhibitor of adenosine uptake, does not reduce the ability of adenosine to stimulate human sperm motility. These results indicate that the motility response to adenosine is due to an action at an extracellular rather than an intracellular site. This conclusion is further supported by the evidence that NECA is a very potent stimulator of motility. This compound is quite stable, generally being a poor substrate for adenosine transport systems (Daly et al 1987). Fourth, in radioligand binding studies, adenosine A_1 selective analogues, DPCPX and R-PIA, have little competitive effect on [^{3}H]NECA binding in sperm membranes.

Adenosine was first implicated in the stimulation of bovine sperm motility about thirty years ago (Bernstein & Steberl 1959), but this observation was not pursued until 1986 when Vijayaraghavan & Hoskins (1986) showed that adenosine and a number of analogues elevate cAMP levels of bovine spermatozoa and can initiate motility of sperm isolated from the bovine caput epididymidis. However, the mechanism of action for these adenosine analogues was unclear. Aitken et al (1986) reported that 2′-deoxyadenosine significantly increased the percentage of motile sperm, the linear velocity of progression and the frequency of sperm head rotation; but a high concentration (2·5 mM) was required to achieve a maximal response. Since 2′-deoxyadenosine is a weak activator of adenosine receptors, those authors (Aitken et al 1986) proposed that this response might involve an intracellular purine (P) site. One problem with this hypothesis is that P site agonists inhibit adenyl cyclase activity whereas stimulation of sperm motility appears to involve elevated cAMP. Recently, the molecular cloning of a novel putative G-protein-coupled receptor was expressed in rat spermatocytes. This novel gene encodes a receptor that is homologous with canine adenosine A_1 (47% identical) and canine adenosine A_{2a} (42%) receptors (Meyerhof et al 1991).

It is well-established that adenosine and a series of its analogues could increase the intracellular cAMP levels of mammalian spermatozoa (Vijayaraghavan & Hoskins 1986; Aitken et al 1986), a response that is characteristic of A_2 receptors (Bruns et al 1986; Linden 1991). Several groups have observed differences among A_2 receptor regional distributions and affinities for adenosine. On the basis of these observations, Daly et al (1983) proposed that brain A_2 receptors be subdivided into a receptor with high affinity for adenosine (EC50, 0·1–1 μM) which is localized mainly in the striatum and which stimulates adenyl cyclase in broken cell preparations, and a receptor with a low affinity for adenosine (EC50, 5–10 μM) which exists throughout the brain and

stimulates cAMP accumulation in brain slices, but apparently does not stimulate broken cell adenyl cyclase. Bruns et al (1986) proposed that high affinity A_2 receptors be designated A_{2a} and low affinity receptors be designated A_{2b}. However, our present results can not clearly indicate which A_2 receptor subtypes exist in the human sperm membrane.

In conclusion, these results support the interpretation that the stimulatory effects of adenosine and adenosine analogues on human sperm motility are receptor-mediated, and that the binding characteristics of adenosine receptors in human sperm membranes have characteristics more similar to the A_2 than to the A_1 receptors.

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