Involvement of calcium in the caffeine stimulation of human sperm motility

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- 1 The shape of the concentration-response curve and the amplitude of maximal motility increase for the stimulatory effect of caffeine on human sperm motility were similar to those of EGTA, a calcium chelator, and lanthanum chloride, a calcium entry blocker.
- 2 On the other hand, the maximal motility stimulation induced by dibutyryl cyclic AMP was similar to that induced by trifluoperazine, a calmodulin antagonist.
- 3 The antagonism of A23187 on the motility-stimulating effect of EGTA and caffeine was competitive in nature, while that on cyclic AMP was non-competitive.
- 4 We proposed that there could be two mechanisms for caffeine to stimulate sperm motility: at higher concentrations, caffeine modified calcium translocation; at lower concentrations, caffeine increased cyclic AMP level.
- 5 The importance of calcium in the regulation of human sperm motility was reconfirmed in this study.

Keywords calcium cyclic AMP caffeine sperm motility calmodulin

Introduction

The stimulatory effect of caffeine on human sperm motility has been demonstrated by several studies (Amelar *et al.*, 1980), including three reports in this journal (Hong *et al.*, 1981a, b; Jiang *et al.*, 1984). Because caffeine is a phosphodiesterase inhibitor, it was proposed that caffeine acts through an increased level of cyclic AMP to stimulate sperm motility (Garber *et al.*, 1971). However, the notion that attributes caffeine stimulation entirely to a specific inhibitory effect on phosphodiesterase has been questioned (Hong *et al.*, 1981b; Jiang *et al.*, 1984; Levin *et al.*, 1981). Caffeine is known to be associated with the translocation of intracellular calcium (Rall, 1980) and we recently found that human sperm motility could be modified by calcium regulating agents (Hong *et al.*, 1984a, 1985). In the present study, we tried to clarify the role of calcium in the stimulatory effect of caffeine. In addition to a better understanding of

sperm physiology and caffeine pharmacology, it is hoped that such an investigation can provide a theoretical basis for future pharmacological management of human fertility problems.

We first compared the concentration-response curves for the motility-stimulating effect of ethyleneglycol-bis-(β -aminoethyl ether)-*N*, *N*, *N'*, *N'*-tetraacetic acid (EGTA), caffeine and dibutyryl cyclic AMP (di-cAMP). EGTA is a calcium chelator while di-cAMP is a membrane-permeable analogue of cyclic AMP. We then studied the sperm immobilizing effect of A23187, a calcium ionophore (Brown & Auerbach, 1982), and examined its interaction with EGTA, caffeine and di-cAMP. Supplementary experiments were also performed to investigate the stimulatory effects of trifluoperazine, a calmodulin antagonist (Cheung, 1980) and lanthanum chloride, a calcium entry blocker (Brown & Auerbach, 1982).

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Methods

All chemicals were purchased from Sigma (USA). A23187 was dissolved in ethanol and then diluted to the desired concentrations in phosphate buffered saline (Dulbecco 'A', pH 7.3). Other chemicals were directly dissolved in phosphate buffered saline. The final concentration of ethanol in semen-drug mixtures containing A23187 was less than 1.6% (v/v) which did not affect sperm motility as measured with the trans-membrane migration method (Hong & Chiang, 1984).

Freshly ejaculated human semen was collected from healthy donors. Each semen sample was divided into several 100 μ l aliquots and the motility of sperm was evaluated with a trans-membrane migration method which measured the proportion of sperm that moved across the 5 μ m pores of a Nuclepore membrane (Nuclepore Ltd, USA) into phosphate buffered saline during 2 h incubation at 37°C. All semen samples fulfilled previous criteria for trans-membrane migration studies (Hong *et al.*, 1981a).

For studying the stimulatory or inhibitory effect of individual drugs, a 100 μ l aliquot of semen was mixed with 50 μ l of drug solution. The motility of sperm in a semen aliquot mixed with phosphate buffered saline was used as control and the amplitude of altered motility was expressed as percentage of the control. For studying the antagonizing or potentiating effect,

a 100 μ l aliquot of semen was first mixed with a 25 μ l solution of either antagonist, potentiator or phosphate buffered saline and 5 min later, 25 μ l of EGTA or caffeine was added to each mixture. P^{A_2} was the negative logarithm of the molar concentration of A23187 in the presence of which the stimulatory potency of EGTA and caffeine was reduced to half. EC_{50} was the concentration of a drug which decreased sperm motility to 50% of the control. Both p^{A_2} and EC_{50} were obtained from the concentration-response curves. A two-tailed *t*-test was used for analyzing the differences of drug potencies. Data of interaction studies were means of three independent experiments. Variation due to differences among basal motility of semen samples were excluded by testing all drugs at different concentrations on each semen samples. Equality of slopes for individual concentration-response curves was tested by analysis of covariance. A computer program (BMDP, Hewlett Packard System 3000) was used for carrying out statistical calculations. $P < 0.05$ was considered to be statistically significant.

Results

Figure 1 shows the concentration-response curves for the stimulatory effect of EGTA, caffeine and di-cAMP on human sperm motility. The amplitude of maximal motility increase by

Figure 1 Concentration-response curves for the stimulatory effects of EGTA ($\blacktriangle$), caffeine ($\bullet$) and dibutyryl cyclic AMP (di-cAMP) ($\blacksquare$) on human sperm motility. All points are mean $\pm$ s.e. mean of six samples collected from different donors. Sperm motility was measured with a trans-membrane migration method. Motility of sperm in semen aliquots mixed with phosphate buffered saline were used as the controls.

0.05 mM EGTA, 7.5 mM caffeine and 2.5 mM di-cAMP were $79.3 \pm 17.5\%$, $84.0 \pm 10.2\%$ and $31.8 \pm 5.5\%$ respectively (mean $\pm$ s.e. mean, $n = 6$). The maximal increment in motility caused by di-cAMP was smaller than those by EGTA and caffeine ($P < 0.05$, t -test).

Figure 2 shows the concentration-dependent inhibitory action of A23187. The EC_{50} for A23187 in this inhibition was 20 μ M.

Figure 3 shows that A23187 competitively antagonized the stimulatory effect of EGTA. A23187 shifted the concentration-response curve of EGTA to the right and the degree of shift was proportional to A23187 concentration. The pA_2 value for A23187 as an EGTA antagonist was 5.3; that is, in the presence of 5 μ M A23187, the stimulatory potency of EGTA was reduced to half. We also found that A23187 parallelly shifted the concentration-response curve of caffeine to the right and the pA_2 value for A23187 as a competitive caffeine antagonist was 5.2. A23187 antagonized di-cAMP (Figure 4), however, the shifted concentration-response curves were not parallel; the latter was proved by a statistically significant difference among slopes of these curves (analysis of covariance, $P < 0.05$). Both EGTA and di-cAMP parallelly shifted the concentration-response curves of caffeine to the left. This finding indicated that both EGTA and di-cAMP potentiated the motility-stimulating effect of caffeine. Figure 5 shows EGTA potentiated caffeine.

In the supplementary experiments, we found that trifluoperazine and lanthanum chloride stimulated sperm motility. The amplitude of maximal motility increase was $31.6 \pm 4.2\%$ for trifluoperazine and $71.5 \pm 15.2\%$ for lanthanum chloride (mean $\pm$ s.e. mean, $n = 6$). The former

took place at 0.5 μ M of trifluoperazine while the latter at 2 mM of lanthanum chloride. The shape of the concentration-response curve and the amplitude of maximal motility increase induced by lanthanum chloride were similar to those of EGTA and caffeine. On the other hand, trifluoperazine showed a pattern of stimulation similar to di-cAMP.

Discussion

Measuring human sperm motility with a laser doppler spectroscopy, Hartmann and colleagues (1983) found that the motility stimulated by di-cAMP was relatively small as compared with caffeine. In the present study, we demonstrated that the stimulatory effect of caffeine was more close to EGTA and lanthanum chloride than to cyclic AMP. That EGTA and caffeine could, while di-cAMP could not, be antagonized competitively by A23187 further supported our suggestion that there is a discrepancy between the stimulatory effects of caffeine and cyclic AMP.

In addition to an inhibitory action on phosphodiesterase, caffeine may influence calcium flux and block adenosine receptor (Rall, 1980). Involvement of adenosine receptor in the regulation of human sperm motility could be excluded because we found that adenosine at concentrations ranged from 10^{-7} to 10^{-3} M did not change human sperm motility (Hong, unpublished). On the other hand, the relationship between calcium and sperm motility has been demonstrated by several studies (Gibbons & Gibbons, 1980), including our recent report that two calcium chelators and calcium antagonists stimulate human sperm motility (Hong *et al.*,

Figure 2 Concentration-response curve for the inhibitory effect of A23187 on human sperm motility. All points are mean of three samples.

Figure 3 A23187 competitively antagonized the stimulatory effect of EGTA on human sperm motility. A 100 μ l aliquot of semen sample was first mixed with 25 μ l of either A23187 ($\blacksquare$ 5 μ M, $\circ$ 10 μ M, $\blacktriangle$ 20 μ M) or phosphate buffered saline (PB, $\bullet$) and 5 min later, 25 μ l of EGTA was added to each mixture. All concentrations are final concentrations of drugs in semen-drug mixtures. Maximal stimulation indicates amplitude of motility increase induced by 50 μ M EGTA. Data are mean of three independent experiments.

Figure 4 Non-competitive antagonism of the stimulatory effect of dibutyryl cyclic AMP (di-cAMP) ($\bullet$) by A23187 at concentrations of 5 μ M ($\blacktriangle$), 10 μ M ($\triangle$) and 20 μ M ($\blacksquare$). Details of experimental procedure were similar to Figure 3.

1984a, 1985). In the present study, we observed the inhibitory action of A23187 and the stimulatory effect of lanthanum chloride. Because A23187 is a calcium ionophore that increases intracellular calcium concentration while lanthanum chloride is a calcium entry blocker that blocks calcium flux into the cell (Brown & Auerbach, 1982), we consider that an increased level of intracellular calcium concentration could be detrimental to human sperm motility. Additionally, because caffeine has been shown to induce calcium efflux in skeletal muscle (Huddart & Syson, 1975) and theophylline inhibits calcium uptake in boar and human sperm (Peterson *et al.*, 1979), we proposed that caffeine

Figure 5 10 μ M ($\bullet$), 20 μ M ($\blacktriangle$) and 30 μ M ($\blacksquare$) EGTA potentiated the stimulatory effect of caffeine ($\blacktriangledown$) on human sperm motility. Details of experimental procedure were similar to Figure 3.

stimulated human sperm motility at least in part by a mechanism which decreased intracellular calcium concentration.

Calcium plays an important role in the regulation of cyclic nucleotide metabolism. It could be argued that an interaction between calcium and cyclic AMP may be involved in the regulation of sperm motility (Tash & Means, 1983). We found an overlap in the concentration-response curves of caffeine and di-cAMP, and such overlap occurred at lower concentration of caffeine (Figure 1). It is known that the inhibitory effect of caffeine on phosphodiesterase required a caffeine concentration lower than that is needed for modifying calcium translocation (Rall, 1980). We also found that trifluoperazine, a calmodulin antagonist, stimulated human sperm motility with an amplitude of maximal stimulation similar to that of di-cAMP. This finding is consistent with an earlier report which showed a calmodulin-dependent cyclic nucleotide phosphodiesterase in sperm (Wasco & Orr, 1984). It appears that caffeine may stimulate sperm motility through two interrelated pathways. At lower concentrations, caffeine inhibits a calmodulin-dependent phosphodiesterase, which in turn increased intracellular cyclic AMP contents. At higher concentrations, caffeine modifies calcium translocation, which results in a decreased intracellular calcium levels. These two pathways, one is calcium dependent and the other is cyclic AMP dependent, may interact in a way so as to potentiate each other. This may explain our observation that the stimulatory effect of caffeine could be potentiated by both EGTA and di-cAMP.

The role of calcium in the regulation of sperm function has received considerable attention in the recent years (Hong *et al.*, 1984b). Our present study further suggests its being an important mediator for pharmacological agents to modify sperm motility. Sperm number, morphology and motility in ejaculated semen are three major determinants of male fertility. Among them, number and morphology are closely related to spermatogenesis and thus difficult to be changed once sperm are formed. Motility, however, is more accessible for pharmacological manipulation even if semen has been ejaculated. Systematic screening for motility stimulating agents has just begun; we consider it a work with great clinical implication. It should be admitted that improved sperm motility is not necessarily asso-

ciated with enhanced male fertility (Harrison, 1978), and the potential mutagenicity of caffeine has been considered as a caution against the practice of mixing sperm with caffeine for improving the successful rate of artificial insemination (Ronen & Marcus, 1978). Researches on motility stimulating agents, including attempts to elucidate their mechanism, can nevertheless provide invaluable information based on which human fertility problems could be managed better in the future.

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