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The stimulatory effects of caffeine, theophylline, lysine-theophylline and 3-isobutyl-1-methylxanthine on human sperm motility

C. S. JIANG[1], S. A. KILFEATHER[1], R. M. PEARSON[2] & P. TURNER[1]
[1]Department of Clinical Pharmacology, St Bartholomew's Hospital, London EC1A 7BE and [2]Harold Wood Hospital, Romford, Essex

The potencies of caffeine, theophylline, lysine-theophylline and 3-isobutyl-1-methylxanthine (IBMX) in stimulating sperm motility have been compared, and we have found IBMX to be significantly more potent than the other three compounds, which did not exhibit significant differences in potency from each other.

Keywords sperm motility caffeine theophylline IBMX

Introduction

Methylxanthines such as caffeine and theophylline increase sperm motility (Burge, 1973; De Turner *et al.*, 1978; Garbers *et al.*, 1971; Haesungcharern & Chulavatnatol, 1973; Hicks *et al.*, 1972; Levin *et al.*, 1980; Makler *et al.*, 1980; Schoenfield *et al.*, 1973). This effect has been attributed to their potent phosphodiesterase (PDE) inhibitory action and elevation of adenosine 3'5-cyclic monophosphate (cAMP) levels within the sperm, following inhibition of cAMP-PDE (Tash & Means, 1983). We have measured the effects of theophylline and caffeine at stimulating sperm motility and compared them with IBMX and lysine-theophylline. IBMX exhibits a higher degree of PDE inhibitory activity than either caffeine or theophylline (Davis & Kuo, 1977; Glass & Moore, 1978; Helfman & Kuo, 1982) and lysine-theophylline is a more soluble theophylline preparation with a pharmacokinetic profile after oral administration almost identical to that of aminophylline in normal subjects (Johnston *et al.*, 1983).

Methods

Fresh semen samples were obtained from 27 healthy volunteers aged between 18–24 years. Only samples with sperm concentrations higher than 25×10^6 ml^{-1} and a percentage of progressive forward moving sperms higher than 20% were used. All drugs were dissolved in phosphate buffered saline (PBS, Oxoid Ltd) at pH 7.3. Sperm motility was measured using the method developed by Hong *et al.* (1981). Semen aliquots (100μl) were mixed with 50μl of methylxanthine dissolved in PBS at pH 7.3. The semen-drug mixture (100μl) was immediately placed onto a Nucleopore 5μm diameter membrane within the motility measuring apparatus and incubated for 2 h at 37°C, as previously described (Hong *et al.*, 1981). Change in sperm motility was measured from the percentage of sperm crossing the membrane compared with that of the control (2:1 semen:PBS mixture). Measurement of the effect of the methylxanthines was conducted on at least six different semen samples and in each sample the response to each methylxanthine concentration was estimated in duplicate.

Materials

All methylxanthines were obtained from Sigma Chemicals Ltd, phosphate buffered saline from Oxoid Ltd.

Results

The responses to theophylline and caffeine were measured on the same semen samples in six cases and comparison of responses to caffeine and theophylline were made using a paired *t*-test. The responses to IBMX and lysine-theophylline were determined on different semen samples from those used to examine theophylline and caffeine and comparison of responses to IBMX and lysine theophylline with theophylline, was made, therefore, using the Mann Whitney U test. The response to IBMX differed significantly from that of theophylline ($P<0.004$), but the concentrations of caffeine, theophylline and lysine-theophylline producing half maximal increase in motility (EC_{50}) did not differ significantly. Dose-response curves of theophylline, caffeine and IBMX are illustrated in Figure 1 and the data are in Table 1.

Table 1 Effect of caffeine, theophylline, lysine theophylline and IBMX on human sperm motility.

Caffeine (n = 9)			*Theophylline* (n = 6)			*Lysine theophylline* (n = 8)			*IBMX* (n = 10)		
Concentration (*M*)	*Motility* (% basal ± s.d.)	P	*Concentration* (*M*)	*Motility* (% basal ± s.d.)	P	*Concentration* (*M*)	*Motility* (% basal ± s.d.)	P	*Concentration* (*M*)	*Motility* (% basal ± s.d.)	P
1.0×10^{-4}	102.2 ± 6.0		1.0×10^{-4}	98.3 ± 6.4		1.0×10^{-4}	99.7 ± 4.8		1.0×10^{-7}	101.7 ± 1.6	<0.05
3.3×10^{-4}	103.1 ± 4.5	<0.05	3.3×10^{-4}	103.0 ± 5.6		2.0×10^{-4}	100.8 ± 5.4		1.0×10^{-6}	103.8 ± 4.1	<0.05
1.0×10^{-3}	104.6 ± 11.4		1.0×10^{-3}	107.3 ± 2.9	<0.05	5.0×10^{-4}	102.2 ± 6.5		1.0×10^{-5}	109.8 ± 4.1	<0.01
3.3×10^{-3}	110.0 ± 9.6	<0.01	3.3×10	112.3 ± 6.9	<0.05	1.0×10	108.1 ± 11.6	<0.05	1.0×10	112.2 ± 3.2	<0.01
1.0×10^{-2}	113.2 ± 9.3	<0.01	1.0×10^{-2}	112.0 ± 7.8	<0.05	2.0×10^{-3}	109.8 ± 11.3	<0.05	1.0×10^{-3}	112.2 ± 9.5	<0.01
						5.0×10^{-3}	116.7 ± 7.6	<0.01			
						1.0×10^{-2}	102.1 ± 10.7				

P = significance level of increase above basal compared with a paired *t*-test.)

Discussion

During the past decade there have been several reports indicating that methylxanthine derivatives can exert a facilitatory effect on sperm motility. Our findings are in agreement with these reports and extend the generalisation to include IBMX as a stimulator of sperm motility. The mechanism of this action of methylxanthines is not established. Some investigators have emphasised the primary involvement of phosphodiesterase inhibition and consequent elevation of basal cAMP levels as a mediator of methylxanthine induced increase in sperm motility. Direct application of dibutyryl-cAMP on to sperm produces an increase in sperm motility (Hartmann *et al.*, 1983; Hoskins *et al.*, 1975) and from the observed increase in sperm cAMP content during stimulation of motility by methylxanthines (Garbers *et al.*, 1971; 1973; Hoskins *et al.*, 1975) and other factors (reviewed by Tash & Means, 1983) it appears that a positive relationship exists between sperm cAMP content and motility stimulation with methylxanthines.

In addition to phosphodiesterase inhibition, however, the methylxanthines exhibit at least two other major pharmacological actions in various tissues, namely facilitation of Ca^{2+} mobilisation and direct antagonism of adenosine receptors (Bergstrand, 1980; Fredholm *et al.*, 1979; Rall, 1980; Svedmyr & Simonsson, 1978). The role of adenosine receptor interaction in the movement of sperm has not been investigated, but the effects of externally applied Ca^{2+} and internal Ca^{2+} have received considerable

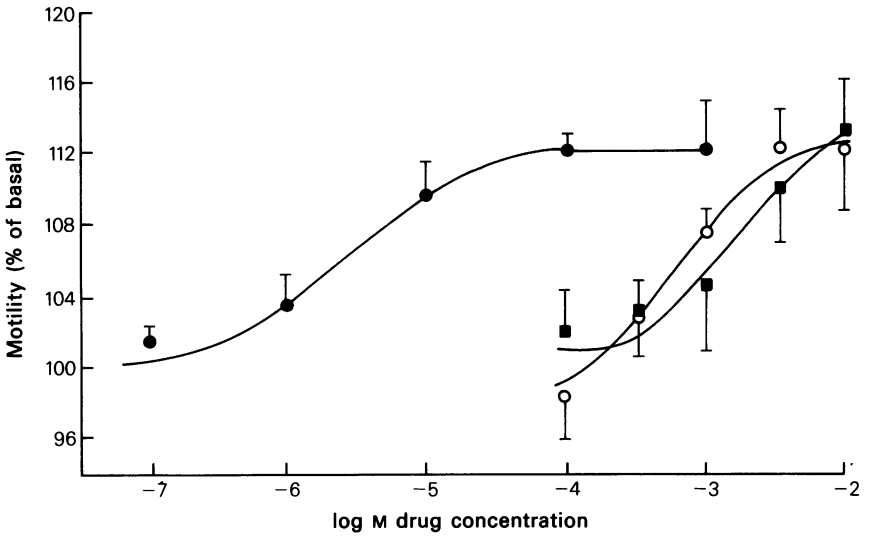

Figure 1 Effect (mean ± s.e. mean) of IBMX (●, $n=10$), theophylline (○, $n=6$) and caffeine (■, $n=9$) on human sperm motility.

attention. A correlation between the dependency of sperm on Ca^{2+} for initiation of motility and the epididymal Ca^{2+} concentration has been found (Morton *et al.*, 1978). This relationship, however, is species specific: in some species external Ca^{2+} appears to play an inhibitory role (McGrady *et al.*, 1974). Maintenance of low intracellular Ca^{2+} levels seems to be important in many species for optimum motility (Tash & Means, 1983) and the presence of Ca^{2+} pumps in the plasma membrane support this concept (Peterson *et al.*, 1979). Furthermore, stimulation of Ca^{2+} pump activity may be mediated through cAMP following the stimulation of adenylate cyclase by Ca^{2+} (Garbers & Kopf, 1980), and stimulation of the acrosome reaction.

In view of the important effects of Ca^{2+} on sperm motility it should be noted that in this study measurements were conducted with a total Ca^{2+} concentration estimated to be between 5.0×10^{-3} M at the start of incubation to 2.4×10^{-4} M during the measurement. This inference is valid if it is assumed that free Ca^{2+} equilibrates across the membrane separating the two chambers in the incubation apparatus. The free Ca^{2+} concentration, however, would be influenced by the degree of interaction between Ca^{2+} and proteins of seminal plasma. It is difficult, therefore, without accurate measurements of the free Ca^{2+} concentrations to interpret our findings in terms of involvement of methylxanthine effects on Ca^{2+} mobilisation. It is possible that the reduction of free Ca^{2+} concentration during the 2 h incubation period assisted the effect of the methylxanthines on sperm motility.

The considerably higher potency of IBMX compared with caffeine and theophylline observed in this study, may reflect the large difference in phosphodiesterase inhibitory activity of these compounds. IBMX has been shown to be a more potent inhibitor of phosphodiesterase in many tissues. Little is known, however, of the relative potencies of these compounds to directly increase intracellular free Ca^{2+} levels and, therefore, the possibility that a proportion of the difference in capacity of these compounds to stimulate sperm motility can be attributed to effects on Ca^{2+} or adenosine activity cannot be excluded.

Caffeine has been demonstrated to enhance the fertilising ability of human sperm (Rogers 1981; Aitken *et al.*, 1983) and could, therefore, be of clinical benefit in men with reduced sperm motility. Aitken *et al.* (1983) reported, however, that increases in motility and fertilising ability of human sperm were dependent on consistent presence of caffeine and were not present in sperm washed free of stimulatory concentrations of caffeine: indicating that caffeine must be present at the point of conception to maintain enhanced fertilising ability. Caffeine has been reported to have teratogenic potential (Fujii & Nishmura, 1974; Morris & Weinstein, 1981; Nishmura & Nakal, 1960) and other methylxanthines could have teratogenic properties limiting their clinical use in improving sperm motility and fertilising ability.

Further studies with compounds having specific effects on cAMP and Ca^{2+} activity should, however, yield a better understanding of the mechanisms governing sperm motility and thus increase our capacity to influence sperm motility in either direction for purposes of fertility control or contraception.

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