J. Reprod. Fert. (1987) **80**, 607–611
Printed in Great Britain
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Regulation of the motility of fowl spermatozoa by calcium and cAMP

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Summary. Using an objective light-scattering technique, it was confirmed that washed fowl spermatozoa become immotile as the temperature is raised from 30°C to the normal body temperature of 40–41°C. Motility of washed spermatozoa was restored at 40°C by the addition of caffeine or calcium, both stimulating motility to a maximum in a dose-dependent manner. Neither effector stimulated the motility of spermatozoa at 30°C. Caffeine, but not calcium, caused an increase in sperm cAMP levels at 40°C. The concentrations of calcium and cAMP in untreated spermatozoa were not significantly different in samples incubated at 30°C or 40°C.

Introduction

Fowl spermatozoa, when suspended in a simple salt-based medium, become immotile as the incubation temperature is raised to 41°C, the normal avian body temperature (Munro, 1938; Nevo & Schindler, 1968; Ashizawa & Nishiyama, 1978; Takeda, 1982). Motility can be restored by lowering the temperature again (e.g. Takeda, 1982) or by the addition of body fluids, such as seminal plasma (e.g. Nevo & Schindler, 1968). The following work was designed to investigate this phenomenon more rigorously by using an objective assay of the motility of fowl spermatozoa (Wishart & Ross, 1985) and chemically-defined effectors of motility.

Materials and Methods

Animals and semen collection. Male fowls were from a Rhode Island Red control strain from Ross Breeders Ltd, Newbridge, Midlothian. They were caged individually, given 14 h light/24 h and fed a commercial breeders ration *ad libitum*. Semen was collected free from transparent fluid (Lake, 1957) on a 3 times weekly routine. Only males consistently producing spermatozoa with high motility (see Wishart & Palmer, 1986) were used as semen donors.

Preparations of spermatozoa. Semen was diluted 8-fold in 0·15 M-NaCl with 20 mM-TES (*N*-Tris-[hydroxymethyl]-methyl-2-aminoethanesulphonic acid) at pH 7·4 and centrifuged at 700 ***g*** for 12 min at room temperature. The washed spermatozoa were reconstituted in the same buffer to give a final concentration of approximately 1×10^9 cells/ml. Samples of 2–4 ml were incubated aerobically in 25 ml 'Nalgene' flasks.

Assays. Sperm motility was assayed as described by Wishart & Ross (1985), but substituting the NaCl/TES buffer described above. The constant, $\% (\Delta OD)_m$, which correlates with the percentage of motile spermatozoa (Wishart & Ross, 1985), was used to describe 'motility'. Numbers of spermatozoa were established from the constant OD_m.

ATP concentrations in spermatozoa were assayed in boiled extracts (Wishart, 1982). cAMP was estimated in similarly-prepared extracts by a commercially-available protein-binding assay kit (The Radiochemical Centre, Amersham, Bucks, U.K.). Intracellular levels of Ca^{2+} were measured in samples centrifuged in a Beckman 'Microfuge' (10 000 ***g***) for 30 sec. The pellet, including a portion of trapped supernatant, was resuspended in 0·1 M-HCl. After

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storage at 5°C and centrifugation, the acidic supernatants of these samples were assayed for Ca^{2+} by atomic absorption spectroscopy. To assess the quantity of incubation medium in each pellet, samples containing 1% bovine serum albumin were incubated and centrifuged in parallel with test samples. These pellets were resuspended in medium without albumin and recentrifuged. The protein content of the secondary supernatant was compared with that of the original supernatant by the method of Lowry *et al.* (1951) and the extracellular space in each pellet was calculated.

Results

The effects of increasing concentrations of calcium and caffeine on the motility of fowl spermatozoa at 40°C are shown in Fig. 1. Calcium appeared to be more potent, stimulating motility to a maximum of 65·6 ± 2·9% compared with 46·9 ± 3·7% for caffeine. At each concentration, the effect of calcium and caffeine in combination was greater than that of calcium alone and at low concentrations (0·2 and 0·5 mM) their combined effects were aproximately additive.

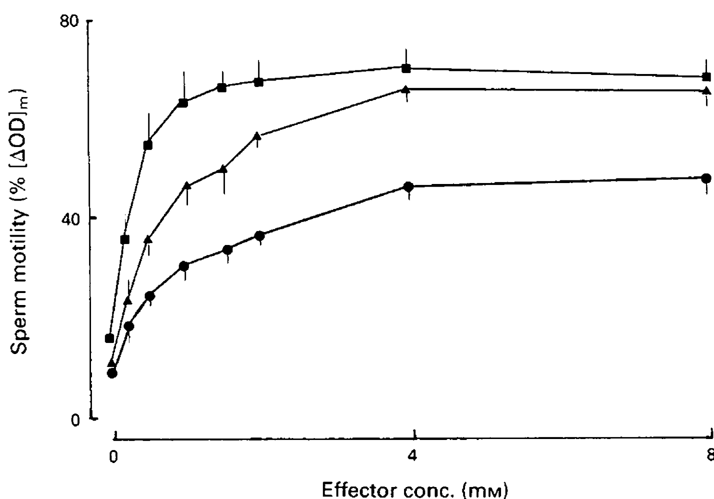

Fig. 1. The effect of caffeine and calcium on the motility of fowl spermatozoa at 40°C. Three samples of spermatozoa were assayed for motility approximately 20 sec after the addition of each effector at each concentration. Points represent the mean (±s.e.m.) motility of samples with added caffeine (●), calcium (▲) and caffeine + calcium (■).

The lack of increase of response to effector at concentrations greater than 4 mM was not, apparently, associated with a limitation of available energy: ATP concentrations of 3 samples (±s.e.m.) in buffer only, with 8 mM-caffeine and with 8 mM-calcium, were 34·4 ± 1·9, 27·2 ± 2·1 and 25·8 ± 0·6 nmol/10^9 spermatozoa, respectively.

Figure 1 shows the stimulation of motility within 15–20 sec of the addition of each effector. The effect of more prolonged exposure is shown in Fig. 2. Stimulation by caffeine was of a short duration (2–3 min) compared with that by calcium. At 30°C, no significant stimulation (Student's *t* test, $P \geq 0{\cdot}05$) of motility by caffeine or calcium could be shown: for 8 mM of each effector, the mean (±s.e.m.) motility of 4 samples was 76 ± 3% with added calcium, and 79 ± 2% with added caffeine, compared to 83 ± 3% for untreated samples. Both calcium and caffeine have been considered to stimulate sperm motility by their action on cyclic nucleotide metabolism (see Garbers & Kopf, 1980; Tash & Means, 1983). Although caffeine could be shown to induce an increase in sperm cAMP concentrations, calcium was quite ineffective (Fig. 3). As the temperature was raised

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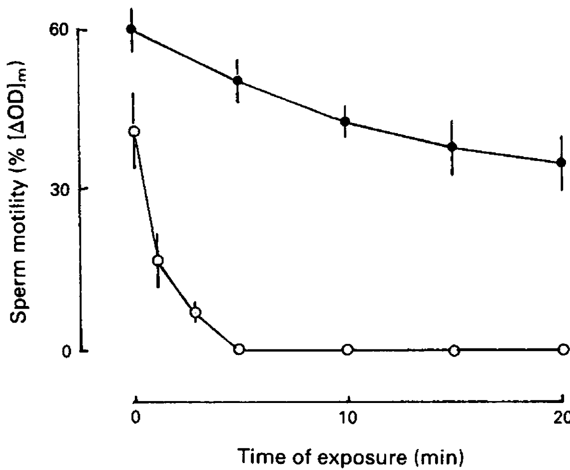

Fig. 2. The time course of motility stimulation by caffeine and calcium. Each point represents the mean (±s.e.m.) motility of three samples of spermatozoa after the addition of 2 mM-caffeine (○) or calcium (●).

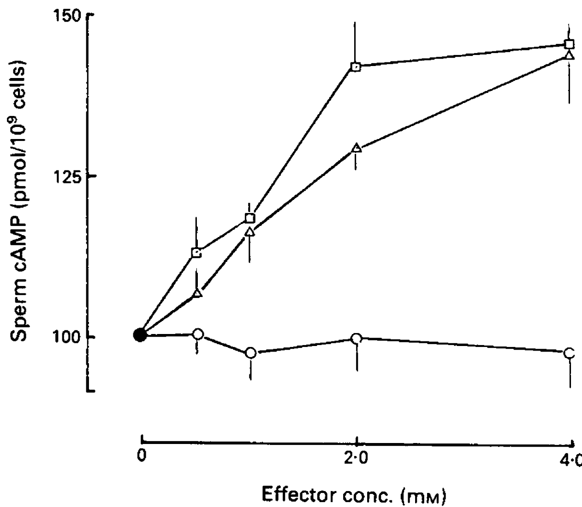

Fig. 3. The effect of caffeine and calcium on fowl sperm cAMP concentrations. The mean (±s.e.m.) concentrations of cAMP in 3 suspensions are expressed as a percentage of control (no additions) levels following the addition of caffeine (△), calcium (○) or caffeine + calcium (□). Control cAMP concentrations were 103 ± 5 pmol/10^9 spermatozoa.

from 30 to 40°C, the mean (±s.e.m.) motility of 5 samples of fowl spermatozoa fell from 78·9 ± 7·5% to 9·7 ± 2·7%. However, sperm cAMP concentrations in samples incubated at 30 and 40°C were, at levels of 94 ± 9 and 93 ± 9 pmol cAMP per 10^9 spermatozoa, respectively, not significantly different (Student's *t* test, $P \geq 0{\cdot}01$); nor were the sperm concentrations of calcium, at 130 ± 6 and 126 ± 6 nmol/10^9 spermatozoa, respectively.

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Discussion

Methylxanthines such as caffeine have been shown to stimulate the motility of spermatozoa of many vertebrate and invertebrate species (see Garbers & Kopf, 1980). This effect has been mainly attributed to a rise in sperm cAMP levels (Garbers *et al.*, 1971, 1973; Hoskins *et al.*, 1975) resulting from the property of these compounds to inhibit cAMP phosphodiesterase (see Jost & Rickenberg, 1971).

The effect of calcium on sperm motility is, however, less straightforward. Depending on the species, calcium may stimulate (Morita & Chang, 1970; Young & Nelson, 1974; Davis, 1978; Cooper, 1984), inhibit (Bredderman & Foote, 1971; McGrady *et al.*, 1974) or have little effect (Quinn *et al.*, 1970; Hyne & Garbers, 1979). Most evidence suggests that stimulation of motility by calcium is mediated through cyclic nucleotide metabolism since calcium involves a rise in sperm cAMP concentrations (Hyne & Garbers, 1979; Kopf & Garbers, 1980; Kopf *et al.*, 1983) and can be shown to activate adenylate cyclase activity in broken cell preparations of spermatozoa (Braun, 1975; Hyne & Garbers, 1979; Hyne & Lopata, 1982; Kopf & Vacquier, 1984). However, calcium does have a more direct effect on phosphorylation of motility-associated proteins in both actin- (Aldestein & Eisenberg, 1980) and tubulin- (Schulman, 1985) based systems. The present work offers the first evidence in spermatozoa of an effect of calcium on motility in the absence of any demonstrable change in cyclic nucleotide metabolism.

The inhibition of motility of fowl spermatozoa which occurs as the temperature is raised from 30 to 40°C could not be shown to be associated with a change in the cellular concentration of cAMP or calcium. If, as with spermatozoa of other species, calcium and cAMP are the main regulators of fowl sperm motility, then this anomalous temperature-dependent inhibition is probably the result of different compartmentation of these effectors.

We thank the AFRC Underwood Fund for financial assistance to K.A. and Dr P. E. Lake for helpful discussion.

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Received 2 December 1986

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