Cyclic CMP (cytidine 3′,5′-monophosphate) suppresses changes in human sperm amplitude of lateral head displacement and hyperactivation

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Summary. The dibutyryl analog of cCMP suppressed sperm amplitude of lateral head displacement and hyperactivation. Sperm motility was inhibited by dibutyryl cCMP with a shift toward less linear trajectory sperm movements. The results suggest a role of cCMP as an inhibitory signal on sperm motility patterns related to sperm capacitation.
Key words. cCMP; sperm motility; capacitation; videomicrograph.

An important sperm motility parameter that has been correlated to successful in vitro fertilization of human eggs is the mean amplitude of lateral head (ALH) displacement[1]. The ALH parameter measures the extent of the oscillations of the sperm head as it swings from side to side during movement along a sperm trajectory. Rising ALH values are indicative of sperm undergoing the capacitation process, a necessary process involving alterations in sperm head membrane whereby the sperm acquires the capacity to fertilize an egg[2]. The ALH measurements are objectively measured using a computerized videomicrograph image analyzer system.

Cyclic CMP is an endogenous[3] pyrimidine compound and has been implicated as an intracellular modulator or signal[4]. The role of cCMP in other cell processes such as cancer cell proliferation has been reported[5]. A study done on pigeon crop-sac mucosal epithelial cells suggests a role of cCMP as a mediator in the proliferation of cells[6]. Cyclic CMP has been implicated in activation of protein kinases[7], initiation of hemoglobin synthesis[8], regulation of hepatoma cell proliferation[9]. In the reproductive system, cCMP has been shown to promote the attachment process of hatched mouse blastocysts, alter the size of trophoblast giant cells[10] and promote development[11]. Other studies implicate cCMP in lymphokine serine protease activity, olfactory receptor cilia activity, ischemic heart fibrillations and as a marker for ovarian cancer[12–15]. While another cyclic nucleotide, cAMP (cyclic adenosine 3′5′-monophosphate), has been shown to accelerate sperm capacitation[16] and to enhance sperm motility[17], the action of cCMP on sperm function has not been examined. By measuring the changes in the ALH parameter, the effects of cyclic CMP (cCMP) on sperm motility parameters can be studied with greater accuracy than by traditional subjective visual methods. The purpose of the present study was to define the role of cCMP in the sperm cell and to determine its effect on sperm motility parameters assessed through the Cellsoft computerized automated semen analyzer.

Materials and methods

Fresh sperm cells derived from a donor of proven fertility conforming to W.H.O. normal standards[18] were evenly divided and centrifuge-washed once at 300 × g for 10 min. The medium used was filter-sterilized Ham's F-10 (with L-glutamine, 5.6 mM D-glucose; GIBCO, Grand Island, NY) supplemented with 3.5% heat-inactivated fetal cord serum, 2.1 g/l sodium bicarbonate, 245.2 mg/l calcium lactate, 75 mg/l penicillin G and 75 mg/l streptomycin. The initial pH and osmolarity were 7.4 and 282 mOsm, respectively. The centrifuged pellets were resuspended (final concentration 25 million/ml) in either control medium or medium containing 10 μM dibutyryl cCMP (dbcCMP; Sigma Chemical Co., St. Louis, MO, Cat # D-7392). A 'delayed' dbcCMP treatment was also carried out which consisted of resuspended sperm cells in control medium added with 10 μM dbcCMP only after 4 h of incubation. The 4-h preincubation period was based on the reported time for maximum sperm capacitation[19]. The dibutyryl analog of cCMP was used instead of cCMP or cytosine because the dibutyryl group facilitates penetration into the cell[20] and is more resistant to phosphodiesterase degradation[21]. All cultures were incubated at 37 °C in a moist 5% CO_2 air mixture.

Aliquots of the sperm cells were placed in a Makler chamber on a heated (37 °C microscope stage and examined at 0, 1, 4, 6 and 24 h after incubation using the Cellsoft (Cryo Resources, Ltd., New York) computerized videomicrographic image analysis system (CASA). The set-up calibrations were: 30 frames/s, 15 frames/analysis, minimum sampling 1 motile and 7 velocity, maximum velocity 200 f/s, threshold velocity 10 f/s, grey level 70, 0.688 f/pixel, cell size 4 to 25 pixels. The lateral head displacement settings were: minimum 7 points, minimum velocity 20 μm/s and minimum linearity 0 (preset for system with forward progression module).

Hyperactivation[19] characterized by star-spin and transitional trajectory motility with wide amplitude head and tail oscillatory movements was measured using the 'freeze motion' method[22] which consisted of using the motion pattern module (option 4) of the CellSoft analyzer to plot the sperm tracks on the video screen, freezing the screen and recording the number of sperm exhibiting hyperactivation patterns.

The other parameters measured were mean velocity (velocity calculated from the sum of trackpoint-to-track-

point velocity), beat cross frequency (BCF; crossing of sperm over computer-calculated mean path), forward progression index (4 = greatest forward motility, 0 = lowest) and mean linearity (indicates track straightness, 0 = circular track, 10 = straight path). Each set of experiments was conducted in duplicates. Five readings were done for each hyperactivation mean. The data were analyzed using the t-test statistic. A $p < 0.05$ value was considered significant.

Results

The data (table 1) showed a significant ($p < 0.05$) suppression of mean amplitude of lateral head displacement in sperm incubated in dbcCMP at hour 4. Hyperactivation was also significantly ($p < 0.05$) suppressed in the dbcCMP-treated sperm group. Percent sperm motility was significantly ($p < 0.05$) lower throughout the 24-h incubation period in the dbcCMP-treated group compared with the control. The addition of dbcCMP at hour 4 to the 'delayed' treatment group did not affect sperm motility and the results were similar to the control.

The mean velocity of sperm significantly decreased ($p < 0.05$) in the dbcCMP treatment group at the end of the incubation period. The addition of dbcCMP after 4 h of preincubation ('delayed' group) reduced the sperm mean velocity considerably ($p < 0.05$). At hour 4 of incubation, dbcCMP inhibited ($p < 0.05$) the characteristic depression on BCF, forward progression index and mean linearity. Delayed addition of dbcCMP to control sperm did not produce significant changes compared to the control for these 3 sperm motility parameters.

Table 1. The effect of cyclic cytidine 3′,5′-monophosphate on the amplitude of lateral head displacement, hyperactivation and percent motility in human sperm.

Treatment	Period of incubation Hour 0	Hour 1	Hour 4	Hour 6	Hour 24
Mean ALH (μm)					
Control	3.5 ± 0.2	3.2 ± 0	5.3 ± 1.2	3.0 ± 0.3	3.5 ± 0.3
10 μM dbcCMP		2.7 ± 0.7	2.9 ± 0.7[a]	3.8 ± 0.1	1.9 ± 0.6[a]
Delayed dbcCMP				3.5 ± 0.3	3.1 ± 0.4
Hyperactivation (%)					
Control	31.5 ± 3.6	41.2 ± 2.1	64.3 ± 5.8	19.5 ± 4.7	8.6 ± 9.5
10 μM dbcCMP		39.3 ± 2.7	39.1 ± 9.7[a]	11.5 ± 12.1[a]	21.1 ± 6.9[a]
Delayed dbcCMP				22.2 ± 6.3	23.8 ± 13.7[a]
Motility (%)					
Control	65.9 ± 0.2	66.0 ± 0	61.7 ± 3.8	54.9 ± 0.1	52.5 ± 4.3
10 μM dbcCMP[a]		51.2 ± 1.2	44.9 ± 7.4[a]	47.4 ± 0	42.9 ± 2.2[a]
Delayed dbcCMP				52.8 ± 2.0	50.6 ± 5.1

Values expressed as mean ± 1 standard deviation, minimum 100 cells per point. [a]Significantly different from control ($p < 0.05$). Delayed dbcCMP group consisted of donor sperm added with 10 μM dbcCMP at hour 4.

Table 2. The effect of cyclic cytidine 3′,5′-monophosphate on human sperm motility parameters.

Treatment	Period of incubation Hour 0	Hour 1	Hour 4	Hour 6	Hour 24
Beat cross frequency (Hz)					
Control	14.1 ± 1.6	17.3 ± 0.3	12.9 ± 2.8	18.8 ± 0.3	15.9 ± 0.2
10 μM dbcCMP		16.9 ± 1.1	15.0 ± 0.6[a]	16.7 ± 0.7[a]	16.6 ± 2.0
Delayed dbcCMP				17.5 ± 1.8	17.0 ± 0.1[a]
Forward progression index					
Control	2.7 ± 0.3	3.3 ± 0.3	2.6 ± 0.4	3.4 ± 0.2	3.1 ± 0.1
10 μM dbcCMP		3.5 ± 0.1	3.5 ± 0.2[a]	3.3 ± 0.1	2.6 ± 1.1
Delayed dbcCMP				3.2 ± 0.4	3.2 ± 0.2
Mean linearity					
Control	4.8 ± 0.5	6.3 ± 0.7	4.4 ± 0.8	6.4 ± 0.5	5.4 ± 0.2
10 μM dbcCMP		7.0 ± 0.8	7.3 ± 1.1[a]	6.1 ± 0.5	5.3 ± 1.8
Delayed dbcCMP				6.1 ± 0.9	5.9 ± 0.5
Mean velocity (μm/s)					
Control	63.1 ± 3.0	71.4 ± 6.1	78.1 ± 6.5	73.6 ± 2.6	88.3 ± 1.3
10 μM dbcCMP		67.0 ± 7.8	69.9 ± 3.3[a]	76.7 ± 15.9	50.8 ± 20.0[a]
Delayed dbcCMP				73.1 ± 8.0	69.1 ± 51.6

Values expressed as mean ± 1 standard deviation, minimum 100 cells per point. [a]Significantly different from control ($p < 0.05$). Delayed dbcCMP group consisted of donor sperm added with 10 μM dbcCMP at hour 4.

Discussion

The amplitude of lateral head displacement [1] and hyperactivation [19] parameters are both positively correlated to the fertilizing potential of the sperm cell. The suppression of these 2 important parameters by dbcCMP around the reported time of maximal sperm capacitation while decreasing sperm motility suggest that cCMP may act as an inhibitory signal on sperm function related to capacitation. Cyclic AMP has been shown to be a stimulatory signal [16–17] for sperm function and it is postulated that cCMP and cAMP act as an opposing regulatory signalling mechanism on sperm function. In simple terms, sperm intracellular cCMP, elevated by seminal plasma polyamines such as spermidine [23] may act to prevent premature capacitation until the sperm comes in contact with reproductive tract fluids that elevate cAMP with resultant capacitation and increased sperm motility. To understand the mechanisms involved, consider the sperm flagellar movement. The flagellar movement increases with increases in sperm intracellular ATP concentration, the energy source for motility. Calcium influx during capacitation results in ATP being generated through a cAMP-creatine kinase action on ADP and phosphocreatine [24]. This ATP energy has been hypothesized to be used in sliding the sperm tail tubulin filaments through a dynein arm rachet system, similar to skeletal muscle contraction [24]. It is proposed that cCMP prevents activation of the G-protein and decreases sperm motility by inhibiting the calcium-ion-associated ATP generating system. More studies are needed to elucidate the action of cCMP on motility.

It is interesting to note that control sperm showed a depression in the mean BCF measurement at hour 4, the time of maximal sperm capacitation [19]. Since this parameter provides an indirect measure of sperm metabolism [25], the data suggest either a transient decrease in sperm metabolism just prior to capacitation or a shift in sperm motility pattern that produced an artefact decrease in the BCF measurements. The latter explanation appears more likely since the shift in sperm motility pattern may be verified by the decrease in linearity around hour 4 towards more circular motility patterns related to hyperactivation in the control group. The higher linearity and BCF value noted in the dbcCMP-treated group suggest that this cyclic nucleotide inhibits changes in sperm motility patterns related to capacitation.

DbcCMP treatment produced a gradual increase in sperm BCF while reducing both sperm velocity and forward progression throughout the incubation period indicating a settling down of the sperm from rapid progressive grade A movements towards nonprogressive movements. This is regarded as an inhibitory effect as studies have shown that grade A movements are necessary for sperm fertilization function [26].

The results of the present study document an effect of dbcCMP on the various sperm motility parameters and suggest the possibility of cCMP as an inhibitory signal on sperm motility. It is very likely that cCMP is effective only on the sperm when it is at the pre-capacitation phase as evidenced by a lack of response in the delayed dbcCMP treatment group. The results are preliminary and more studies are needed to resolve the mechanism of cCMP action in sperm.

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