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RAPID COMMUNICATION

Potentiation of the cGMP-Induced Guinea Pig Acrosome Reaction by Zinc

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ABSTRACT The addition of 8 bromo cGMP (10 mM) immediately (~2 min) upon suspension of Guinea pig sperm in glucose-free BWW medium induces a significant number of acrosome reactions within minutes. The simultaneous addition of micromolar concentrations of $ZnCl_2$ (25–1000 μM) potentiates the cGMP effects. Mid-micromolar concentrations were able to dramatically increase the percentage of acrosome reactions by a factor of 6.5 over 8 bromo cGMP alone. The induction of acrosome reactions was dependent upon external Ca^{++}, and it is suggested that in this species Zn^{++} functions by affecting cyclic nucleotide metabolism and/or Ca^{++} flux.

Considerable attention has been focused upon the role of Zn^{++} in sperm metabolism. For example, it has been shown that removal of bound Zn^{++} from human sperm increases both oxygen uptake and motility (Huacuja et al., '73), whereas incubation of sperm with Zn^{++} reduces motility (Holland et al., '76). Johnsen and Eliasson ('78) have demonstrated that a reduction of membrane-associated Zn^{++} promotes membrane destabilization in human sperm, as indicated by an increased susceptibility to lipid peroxidation. Results such as these have led some workers to speculate that spermatozoal Zn^{++} depletion may be involved in the capacitation process (Johnsen and Eliasson, '76, '78) and, indeed, some evidence exists in support of this. Aonuma et al. ('78) have shown that mouse sperm preincubated with zinc (250 μM) are unable to fertilize eggs, and by experimental manipulation they related the zinc effects to an inhibition of capacitation. Williams and Lenz ('79) showed that zinc salts were effective vaginal contraceptives in the rabbit. Meizel and Lui ('76) determined that incubation of hamster sperm with zinc (250 μM) as well as synthetic trypsin inhibitors, (Lui and Meizel, '79) dramatically inhibited the acrosome reaction (AR). Thus, zinc may well influence the critical events necessary for egg penetration.

Recently, Santos-Sacchi and Gordon ('80) have demonstrated that the Guinea pig sperm acrosome reaction can be induced by exogenous cyclic guanosine monophosphate (cGMP) analogues, and they suggested that a rise in the cGMP/cAMP ratio is the critical effector of the AR. Because Zn^{++} has been shown to inhibit adenylate cyclase in human sperm (Haesungcharern and Chulavatnatol, '78), it seemed worthwhile to study the effects of zinc upon the cGMP-induced Guinea pig acrosome reaction.

MATERIALS AND METHODS

Guinea pig cauda epididymal spermatozoa were extruded into 0.9% NaCl in H_2O, disaggregated by pipetting, and washed two times by centrifugation (1,000 g, 4 min each). Spermatozoa were then suspended in glucose-free BWW medium (NaCl 94.59 mM, KCl 4.7 mM, $CaCl_2$ 1.71 mM, KH_2PO_4 1.19 mM, $MgSO_4$ 1.19 mM, $NaHCO_3$ 25.07 mM, pyruvate 0.25 mM, lactate 21.58 mM, and bovine serum albumin 1 gm/L) at a final concentration of 2×10^7 spermatozoa/ml and were incubated at 37°C in capped tubes under an air atmosphere. Calcium-free medium was also employed. Media were prepared immediately prior to use.

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The initial pH was adjusted to 7.55 and throughout experiments remained less than 8.20.

Immediately (~2 min) after suspension of sperm in glucose-free BWW, various combinations of the following agents were added to sperm samples: 8 bromo cGMP, 8 bromo cAMP, 8 bromo GMP and $ZnCl_2$ (Table 1). The bromo analogues were chosen because they proved to be more efficacious than dibutyryl cyclic nucleotides (Santos-Sacchi and Gordon, '80). After 20 min incubation with these agents, acrosome reactions were assessed by hemocytometer. At least five chambers (0.5 mm^3 each) were counted for each condition. Care was taken to count all cells through the 0.1 mm counting chamber depth. Standard errors of the mean averaged less than 15%. Results are reported as percentage of acrosome reactions in the total motile sperm population ($\%AR^{tot}$ = [conc. of AR spermatozoa/conc. of total spermatozoa-conc. of immotile spermatozoa] × 100) (Santos-Sacchi and Gordon, '80). The onset of the AR and activated motility were qualitatively evaluated over time by light and differential interference microscopy. Spermatozoa were processed for electron microscopy (Gordon, '73) and examined with a Philips 300EM at 80kV.

RESULTS

Table 1 presents the percentages of acrosome reactions induced by various agents. Whereas 8 bromo cGMP alone was able to induce a significant number of acrosome reactions, the simultaneous addition of micromolar concentrations of Zn^{++} enhanced its effect, and this effect was concentration-dependent. The highest potentiating effects of Zn^{++} were observed at mid-micromolar concentrations, where a 6.5-fold increase in the percent of acrosome reactions was obtained over 8 bromo cGMP alone. The presence of Ca^{++} was necessary for the induction of acrosome reactions. Zinc ions alone (250 μM) or in combination with 8 bromo cAMP or 8 bromo GMP were essentially an ineffective inducer of the acrosome reaction. In addition, zinc ions (250 μM) added at 0 time had no effect upon the percent of acrosome reactions present after 5 hr incubation in glucose-free BWW (Zn^{++}: 5.94 ± 0.91% vs. no Zn^{++}: 5.92 ± 0.61%).

Upon the addition of 8 bromo cGMP alone or in combination with Zn^{++}, sperm become extremely active within minutes. By 5–10 min activated, acrosome-reacted sperm were observed, and by 15–25 min a maximum was reached. Acrosome-reacted sperm remained motile for at least 2 hr, after which increasing numbers became immotile. Differential interference and electron microscopy indicate that the induced reactions are physiological, as previously determined for the cGMP-induced AR (Santos-Sacchi and Gordon, '80).

DISCUSSION

Cyclic GMP analogues are able to induce acrosome reactions in Guinea pig sperm, and this ability was found to be dependent upon the time of addition after initial suspension in media (Santos-Sacchi and Gordon, '80). That is, a dramatic increase in the ability of 8 bromo or dibutyryl cGMP (10 mM) to induce acrosome reactions was observed over a 1 hr period (0 hr: $< 1\%$ vs. 1 hr: $\geq 13\%$). This increased ability was correlated with a decrease in sperm cyclic AMP levels during 1-hr incubation. The present finding that Zn^{++} in micromolar concentrations can potentiate the action of cGMP analogues may be explained by its ability to inhibit adenylate cyclase (Haesungcharern and Chulavatnatol, '78). Thus, the addition of Zn^{++} may further increase the cGMP/cAMP ratio, thereby promoting Ca^{++} influx and the acrosome reaction. Spermatozoa will not acrosome-react in cal-

TABLE 1. $\%AR^{tot}$ score induced by addition of agents immediately after suspension of sperm in glucose-free BWW

		$\%AR^{tot}(\bar{X} \pm SEM)$
8 bromo cGMP(10 mM)	$ZnCl_2$(1 mM)	5.64 ± 0.84%
8 bromo cGMP(10 mM)	$ZnCl_2$(750 μM)	21.54 ± 2.04%
8 bromo cGMP(10 mM)	$ZnCl_2$(250 μM)	23.46 ± 1.92%
8 bromo cGMP(10 mM)	$ZnCl_2$(25 μM)	7.55 ± 1.36%
8 bromo cGMP(10 mM)	—	3.64 ± 0.82%
8 bromo cAMP(10 mM)	$ZnCl_2$(250 μM)	1.31 ± 0.02%
8 bromo GMP(10 mM)	$ZnCl_2$(250 μM)	1.80 ± 0.77%
—	$ZnCl_2$(250 μM)	0.19 ± 0.02%
—	—	0.09 ± 0.03%

cium-free media (Yanagimachi and Usui, '74) nor in calcium-free media containing micromolar concentrations of Zn^{++} and 8 bromo cGMP. Furthermore, the addition of Zn^{++} alone had no effect upon acrosome reaction induction. These data suggest that while Zn^{++} cannot substitute for Ca^{++}, it is possible that optimal concentrations of Zn^{++} affect intracellular Ca^{++} availability and distribution, perhaps by promoting release of bound Ca^{++}, thereby potentiating the effect of a cGMP-induced Ca^{++} influx.

Data from several sources indicate that Zn^{++} profoundly affects sperm function. For example, vaginal inserts of several zinc salts result in the reduction or absence of fertilized ova in mated does (Williams and Lenz, '79). Interestingly, in vitro experiments with hamster sperm gave results that are opposite from those with the Guinea pig. Whereas Zn^{++} (250 μM) in the present study was unable to inhibit acrosome reactions in Guinea pig sperm, it has been reported to inhibit hamster sperm acrosome reactions (Meizel and Lui, '76). Furthermore, it was recently reported that TLCK (a synthetic trypsin inhibitor) actually increased the number of acrosome reactions in Guinea pig sperm as compared to controls (Vitug et al., '79), while the same compound was found to inhibit hamster acrosome reactions (Meizel and Lui, '76; Lui and Meizel, '79). We have observed a potentiation of cGMP-induced Guinea pig acrosome reactions by TLCK (unpublished results). Species differences may be the underlying reason for such disparities.

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