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Adrenergic Stimulation of Fertilizing Ability in Hamster Spermatozoa

LAWRENCE E. CORNETT[1,3], BARRY D. BAVISTER[4] and STANLEY MEIZEL[2,3]

Department of Human Anatomy[3], School of Medicine, University of California, Davis, Dalifornia 95616
and
Department of Pathology[4], Harbor General Hospital, 1000 West Carson Street, Torrance, California 90509

ABSTRACT

Hamster spermatozoa, preincubated for 3 h in a culture medium containing bovine serum albumin (BSA) and a protein-free ultrafiltrate of a bovine adrenal cortex preparation, were unable to fertilize cumulus-free eggs. In contrast, if the culture medium also contained a protein-free ultrafiltrate of a bovine adrenal medulla preparation, 92% of the inseminated eggs were fertilized within 2 h. Replacement of the adrenal medulla preparation with 20.0 μM (-)-epinephrine (BSA and the adrenal cortex preparation also present) resulted in 85% of the inseminated eggs being fertilized. If 20.0 μM (-)-phenylephrine, an α-adrenergic agonist or 20.0 μM (-)-isoproterenol, a β-adrenergic agonist, was used in place of epinephrine, the percentage of fertilized eggs was 11% and 50%, respectively. With both phenylephrine and isoproterenol present, 90% of the eggs were fertilized. Furthermore, fertilization in the presence of 20.0 μM (-)-epinephrine could be inhibited by equimolar concentrations of either phentolamine, an α-adrenergic antagonist, (0% fertilization) or (-)-propranolol, a β-adrenergic antagonist, (18% fertilization). These results, together with our previous work, suggest the involvement of adrenergic receptors in the development of the fertilizing ability of hamster spermatozoa *in vitro*.

INTRODUCTION

Mammalian fertilization can be achieved *in vitro* if spermatozoa first undergo certain physiological and morphological changes, respectively termed capacitation and the acrosome reaction. Capacitation was originally defined as the acquisition by spermatozoa of the ability to penetrate egg investments and to fertilize the egg during residence in the female reproductive tract (Austin, 1951; Chang, 1951). The term capacitation is now used in a more restricted sense to denote those changes, whose nature is still unknown, that must take place in spermatozoa prior to their undergoing an acrosome reaction, a morphological event that is a prerequisite for fertilization. The acrosome reaction involves a progressive, organized membrane fusion occurring between the plasma membrane and the underlying outer acrosomal membrane, leading to vesiculation and the exposure of acrosomal enzymes that appear to be required for penetration of the egg investments (Bedford, 1970).

In spite of intensive research efforts, the mechanisms involved in capacitation and the acrosome reaction are still obscure and the initiators of these events within the female reproductive tract have not yet been identified (Meizel, 1978). It is known, however, that capacitation and the acrosome reaction of hamster spermatozoa can be induced during incubation in a medium containing serum albumin and a protein-free motility factor obtained from several sources including blood serum (Yanagimachi, 1970; Bavister, 1975), follicular fluid (Lui et al., 1977), spermatozoa (Bavister and Yanagimachi, 1977) and adrenal glands (Bavister et al., 1976). Recently, it has

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[1] Present address: University of California, School of Medicine, Department of Obstetrics, Gynecology and Reproductive Sciences, San Francisco, CA 94143.

[2] To whom reprint requests should be sent.

been shown that in the presence of a protein-free ultrafiltrate of the bovine adrenal cortex and bovine serum albumin, epinephrine or α- and β-adrenergic agonists can stimulate activation (the whiplash flagellar movement characteristic of capacitated hamster spermatozoa) and acrosome reactions (Cornett and Meizel, 1978) and motility (Bavister et al., 1979) of hamster spermatozoa. Also, α- and β-adrenergic antagonists inhibited the effects of epinephrine. These observations have now been extended to demonstrate that epinephrine or both an α- and a β-adrenergic agonist are required for the development of fertilizing ability in hamster spermatozoa under our culture conditions.

MATERIALS AND METHODS

Spermatozoa recovered from the cauda epididymides of 3- to 4-month-old golden hamsters weighing 130–150 g were washed by a modification (Cornett and Meizel, 1978) of the method of Bavister and Yanagimachi (1977) to remove epididymal fluid which contains a sperm motility stimulating substance (Morton and Chang, 1973). Spermatozoa were incubated in culture medium containing bovine serum albumin at 3 mg/ml, 4.5 mM glucose, 9.0 mM lactate, 0.9 mM pyruvate, penicillin at 100 IU/ml and phenol red at 10 μg/ml in Tyrode's solution as previously described (Bavister and Yanagimachi, 1977). Culture droplets (45 μl) were equilibrated for 2–3 h under mineral oil at 37°C in an atmosphere of 95% air:5% CO_2. Just prior to the addition of spermatozoa, adrenal gland preparations, adrenergic agonists or antagonists were added to bring the volume to 50 μl. The steps involved in making the alumina-treated, chloroform-extracted cortex preparation (AC cortex preparation) and the Florisil-treated medulla preparation have been previously described (Cornett and Meizel, 1978). Stock solutions (1.0 mM) of adrenergic agonists dissolved in Dulbecco's phosphate buffered saline (PBS) and of adrenergic antagonists dissolved in twice distilled H_2O were made immediately before use, passed through Millipore filters (0.45 μm pore diameter) and added in a 1 μl volume. One μl of the suspension of washed spermatozoa was added to each droplet to yield a final concentration of 6 to 8×10^4 sperm/ml. In the droplets used for testing the adrenergic antagonists, epinephrine was added 10 min after addition of the spermatozoa.

Eggs were obtained from superovulated 4- to 6-week-old golden hamsters that had been injected with 20 IU PMSG followed 48–54 h later with 20 IU hCG. Animals were sacrificed 15–16 h after hCG injection and cumulus masses containing eggs were recovered from the oviducts under mineral oil, pooled and incubated to remove cumulus (Bavister and Morton, 1974). Ten to 12 cumulus-free eggs were added to the culture droplets 3–3.5 h after spermatozoa addition. After addition of the eggs, the incubation was continued for 2–2.5 h at which time the eggs were examined by phase contrast microscopy for evidence of sperm penetration.

Chemicals used included (-)-epinephrine and (-)-phenylephrine HCl purchased from Sigma Chemical Co. Phentolamine HCl, (-)-propranolol HCl and (-)-isoproterenol-(+)-bitartrate were gifts from Ciba Pharmaceutical Co., Ayerst Laboratories Inc. and Sterling-Winthrop Research Institute, respectively. Pentex Fraction V bovine serum albumin (BSA) was obtained from Miles Laboratories, Inc.

Due to small sample sizes, significance of the data was determined using nonparametric statistical methods. The Mann-Whitney test was used to compare the fertilizing ability of spermatozoa incubated in the presence of the adrenal gland preparations and paired comparisons using the Kruskal-Wallis Ranked Series were used to compare the fertilizing ability of spermatozoa incubated with the adrenergic agonists and antagonists (Hollander and Wolfe, 1973).

RESULTS

The results in Table 1 show that spermatozoa incubated in the presence of BSA and the AC cortex preparation were not capable of fertilization because no eggs were penetrated. However, sperm penetration was significantly increased to 92% ($P<0.05$) with the addition of the Florisil-treated medulla preparation.

The stimulatory effect of penetration by the Florisil-treated medulla preparation was duplicated by the addition of epinephrine to the incubation media. In the presence of BSA, the AC cortex preparation and 20.0 μM epinephrine, 85% of the inseminated eggs were penetrated (Table 2). The AC cortex preparation is required for sperm viability because spermatozoa incubated in the presence of BSA and epinephrine are immotile after 3 h (Cornett and Meizel, 1978; Bavister et al., 1979). The percentage of penetrated eggs obtained (90%) in the presence of 10.0 μM phenylephrine, an α-adrenergic agonist, together with 10.0 μM isoproterenol, a β-adrenergic agonist, was not significantly different from that obtained with epinephrine (Table 2). The percentage of penetrated eggs obtained in the prescence of 20.0 μM phenylephrine (11%) was significantly less ($P<0.05$) than that obtained with epinephrine. With 20.0 μM isoproterenol, the percentage of penetrated eggs (50%) was also less ($P<0.10$) than that obtained with epinephrine.

The effect of 20.0 μM phentolamine, an α-adrenergic antagonist and 20.0 μM propranolol, a β-adrenergic antagonist, on the ability of hamster spermatozoa to penetrate eggs in the presence of 20.0 μM epinephrine is shown in Table 3. The percentage of penetrated eggs obtained with spermatozoa incubated in the presence of 20.0 μM epinephrine served as the

TABLE 1. Effect of bovine adrenal gland preparations on the fertilization of hamster eggs *in vitro*.[a]

Type of adrenal gland preparation	Eggs inseminated (no. exp.)	%Penetrated median (range)	Total no. penetrated eggs		
			SI[b]	SII[c]	SIII[d]
Cortex	42 (4)	0	0	0	0
Cortex and medulla	35 (3)	92 (90–100)[e]	2	14	17

[a]Incubations carried out as described in text. Fifty μl drops of culture medium (with 3 mg/ml Fraction V BSA) contained 3 μl AC cortex preparation and either 2 μl PBS or 2 μl Florisil-treated medulla preparation.

[b]Stage I: Penetration of spermatozoa into the perivitelline space.

[c]Stage II: Penetration of the vitellus with sperm nuclear decondensation.

[d]Stage III: Sperm components in the vitellus with 2 well formed pronuclei and 2 polar bodies.

[e]Significantly different from cortex at $P<0.05$ level.

control. Both phentolamine and propranolol significantly ($P<0.05$) reduced sperm fertilizing ability to 0% and 18% penetration, respectively. The percentage of penetrated eggs (25%) was also significantly ($P<0.05$) reduced when the 2 antagonists were used in combination.

DISCUSSION

In this paper we have demonstrated that epinephrine stimulates the fertilizing ability of hamster spermatozoa *in vitro*. The data obtained with the adrenergic agonists and antagonists suggests that concomitant α- and β-adrenergic stimulation is required for hamster spermatozoa to acquire the ability to fertilize. Epinephrine is known to stimulate both α- and β-mediated processes (Innes and Nickerson, 1975).

In these experiments, we have assumed that those changes observed in the incidence of fertilization *in vitro* that were brought about by the adrenergic agonists and antagonists arose from the effects of these substances on sperm

TABLE 2. Effect of α- and β-adrenergic receptor agonists on the fertilization of hamster eggs *in vitro*.[a]

Treatment	Eggs inseminated (no. exp.)	%Penetrated Median (range)	Total no. penetrated eggs		
			SI[b]	SII[c]	SIII[d]
20.0 μM epinephrine	61 (6)	85 (67–92)	9	21	20
20.0 μM phenylephrine	31 (3)	11 (0–42)[e]	2	3	1
20.0 μM isoproterenol	32 (3)	50 (20–64)[f]	6	2	7
10.0 μM phenylephrine and 10.0 μM isoproterenol	33 (3)	90 (58–91)	4	10	12

[a]Incubations carried out as described in text. Fifty μl drops of culture medium (with 3 mg/ml Fraction V BSA) contained 3 μl AC cortex preparation and either 1 μl epinephrine or adrenergic agonist solution (final concentration 20.0 μM) and 1 μl PBS.

[b]Stage I: Penetration of spermatozoa into the perivitelline space.

[c]Stage II: Penetration of the vitellus with sperm nuclear decondensation.

[d]Stage III: Sperm components in the vitellus with 2 well formed pronuclei and 2 polar bodies.

[e]Significantly different from 20.0 μM epinephrine at $P<0.05$ level.

[f]Significantly different from 20.0 μM epinephrine at $P<0.10$ level.

TABLE 3. Inhibition of the fertilization of hamster eggs *in vitro* by α- and β-adrenergic receptor antagonists in the presence of (-)-epinephrine.[a]

Treatment	Eggs inseminated (no. exp.)	%Penetrated median (range)	Total no. penetrated eggs		
			SI[b]	SII[c]	SIII[d]
Control (20.0 μM epinephrine)	61 (6)	85 (67–92)	9	21	20
20.0 μM phentolamine (+20.0 μM epinephrine)	30 (3)	0 (0–40)[e]	2	1	1
20.0 μM propranolol (+20.0 μM epinephrine)	31 (3)	18 (0–38)[e]	5	0	0
10.0 μM phentolamine and 10.0 μM propranolol (+20.0 μM epinephrine)	33 (3)	25 (8–33)[e]	7	0	0

[a]Incubations carried out as described in text. Fifty μl drops of culture medium (with 3 mg/ml Fraction V BSA) contained 3 μl AC cortex preparation, 1 μl epinephrine solution and either 1 μl adrenergic antagonist solution or 1 μl PBS (control drops).

[b]Stage I: Penetration of spermatozoa into the perivitelline space.

[c]Stage II: Penetration of the vitellus with sperm nuclear decondensation.

[d]Stage III: Sperm components in the vitellus with 2 well formed pronuclei and 2 polar bodies.

[e]Significantly different from control at $P<0.05$ level.

fertilizing ability. We cannot rule out the possibility that the adrenergic antagonists may also have interacted with the inseminated eggs rendering them incapable of fertilization. However, in light of the previously published results which demonstrate that phentolamine and propranolol inhibit capacitation and/or acrosome reactions of hamster spermatozoa (Cornett and Meizell, 1978), it is clear that the fertilizing ability of spermatozoa incubated in the presence of these antagonists would be decreased. Furthermore, penetration was not reduced to 0 in the presence of phentolamine and propranolol, indicating that the eggs were viable and capable of being fertilized.

Since it has been established that epinephrine stimulates fertilization by hamster spermatozoa *in vitro*, it will be important to determine if epinephrine or another catecholamine is required for fertilization *in vivo*. Although no report of the catecholamine content of female reproductive tract fluid has been made, the uterus and oviduct (the sites of capacitation *in vivo*) of several mammalian species have been shown to contain catecholamines (Bodkhe and Harper 1972; Dujovne et al., 1976). In addition, catecholamines have been demonstrated in unfertilized rat ova (Burden and Lawrence, 1973).

The presence of adrenergic receptors has not been established in mammalian spermatozoa, but the expression of α- and β-adrenergic receptors has been studied in several other cell types. Activation of α-adrenergic receptors leads to a calcium influx in hepatocytes (Assimacopoulos-Jeannet et al., 1977) and in the parotid gland (Peterson et al., 1977). Calcium is required for the acrosome reaction of mammalian spermatozoa (Yanagimachi and Usui, 1974; Talbot et al., 1976) as it is for other cellular events involving membrane fusions. Activation of β-adrenergic receptors usually leads to increased intracellular cyclic AMP levels (Wolf et al., 1977) and there is evidence that cyclic AMP may be involved in the capacitation of mammalian spermatozoa (Hoskins and Casillas, 1975).

Because the fertility of hamster spermatozoa *in vitro* is stimulated by catecholamines and it appears the effect is mediated through adrenergic receptors, it is not unreasonable to suggest the possibility that catecholamines might also play a similar role in the stimulation of fertility *in vivo*. Further studies of the mechanism of this effect of catecholamines on hamster spermatozoa *in vitro*, extension of these studies to spermatozoa of other mammalian species and eventual studies *in vivo* may add to our understanding of the molecular events essential for fertilization and may ultimately lead to new approaches to the control of fertility.

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