J. Reprod. Fert. (1978) **53**, 59–61

Inhibitors of adenylate cyclase from ejaculated human spermatozoa

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Summary. Adenylate cyclase from ejaculated human spermatozoa was inhibited by fluoride, Cu^{2+}, Zn^{2+}, Ni^{2+} and several carboxylic acids.

Introduction

The characteristics of adenylate cyclase from the spermatozoa of several species have been investigated (Casillas & Hoskins, 1970, 1971; Hoskins & Casillas, 1975; Gray, Drummond, Luk, Hardman & Sutherland, 1976; Towns & Luke, 1976; Garbers, 1977), and some properties of the sperm enzyme have been shown to be different from those of other tissues. The sperm enzyme seems to be insensitive to stimulation by hormones (for review, see Harrison, 1975) and by NaF (Gray *et al.*, 1976; Garbers, 1977). Adenylate cyclase from sea urchin spermatozoa is inhibited by phosphorylated nucleosides but not by a number of carbohydrates and fatty acids (Garbers, 1977). The present study was of the inhibition of human sperm adenylate cyclase by NaF, metal ions and carboxylic acids.

Materials and Methods

Fresh human semen was collected by masturbation into a sterile bottle and delivered to the laboratory within 2–3 h. The samples were pooled and the spermatozoa were collected by centrifuging at 1000 *g* for 40 min at 4°C. Subsequent procedures were carried out at 0–4°C. The sperm pellet was suspended in the tris-based salt buffer, pH 7·4, described by Peterson & Freund (1970) and recentrifuged at 270 *g* for 10 min. The washing was repeated twice more. The final sperm pellet was resuspended in the buffer and sonicated for 5 min in a Branson sonifier, model J-17A, at the setting of 55–60. The unbroken cells and debris were removed by centrifugation at 755 *g* for 10 min. The supernatant fluid was further centrifuged at 100 000 *g* for 1 h and the resulting pellet was resuspended in the buffer by sonication for 30 sec and used as the 'particulate' fraction while the clear supernatant fluid was the 'soluble' fraction.

The activity of adenylate cyclase was assayed by measurement of the amount of [^{14}C]cyclic AMP formed from [^{14}C]ATP (sp. act. 53–61 mCi/mmol: Radiochemical Centre, Amersham, England). The assay mixture contained 0·03 M-tris–HCl, pH 7·4, 2×10^{-3} M-[^{14}C]ATP (approx. 1·8 Ci/mol), 0·01 M-$MnCl_2$, 0·02 M-KCl, 0·01 M-caffeine, 0·1 % bovine serum albumin (fraction V: Sigma Chemical Co.), 0·02 M-phosphoenolpyruvate, 20 μg pyruvate kinase (400 units/mg), the appropriate concentration of one of the inhibitors listed in Table 1 and the enzyme extract in the total volume of 0·15 ml. Mn^{2+} was used instead of Mg^{2+} because it gave much better activity (Gray *et al.*, 1976). The reaction mixture was incubated at 37°C for 1 h with gentle shaking. The reaction was terminated by addition of 0·1 ml 3×10^{-3} M-cyclic AMP in 0·01 M-tris–HCl, pH 7·4, and 0·05 ml 0·06 M-EDTA. The incubation tube was immediately transferred to a boiling water bath for 3 min. (Braun & Dods (1975) suggested the use of EDTA to prevent non-enzymic formation of cyclic AMP in the presence of Mn^{2+} during the boiling process.) The tube was then allowed to cool. The separation of the cyclic AMP

formed was accomplished by using a small column of neutral aluminium oxide as described by Ramachandran (1971). The radioactivity of the cyclic AMP fraction was determined in a liquid scintillation counter. In these conditions, the cyclase reaction was linear up to 1 h and proportional with protein in the 'particulate' fraction up to 300 μg. The protein content was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) with bovine serum albumin as standard. The protein in the 'particulate' fraction was digested in 0·1 M-NaOH at 80°C for 15 min before determination.

The specific activities of the enzyme in the crude extract, the 'particulate' fraction and the 'soluble' fraction of our best preparation were 27, 100 and 22 pmol/min.mg protein^{-1} respectively. The specific activity of the 'particulate' fraction varied widely from 26 to 100 pmol/min.mg protein^{-1} in 11 preparations. However, the total activity in the 'particulate' fraction was usually three times that in the 'soluble' fraction and therefore the 'particulate' fraction was chosen as the source of the enzyme for the present study.

Results and Discussion

The results in Table 1 show that adenylate cyclase from human spermatozoa was inhibited by NaF, a finding at variance with those of other workers. Gray *et al.* (1976) showed that 0·01 M-NaF did not inhibit adenylate cyclase of intact spermatozoa of various species when assayed at a Mn^{2+}/ATP ratio of 25. Similarly, the enzyme from sea urchin spermatozoa was not inhibited by 0·08 M-NaF at the Mn^{2+}/ATP ratio of 12 (Garbers, 1977). The fluoride inhibition in our study, therefore, was not due to high Mn^{2+} because the Mn^{2+}/ATP ratio of 5 in our assay was lower than the values cited above. Of the metal ions tested, Cu^{2+}, Zn^{2+} and Ni^{2+} showed pronounced inhibition, similar to the metal ion effects on the bovine sperm enzyme (Braun, 1975), but the human sperm enzyme was insensitive to Ca^{2+}, ions which activated the bovine enzyme (Braun, 1975) and inhibited or activated the enzyme from sea urchin spermatozoa, depending on the ratio of Mn^{2+} to ATP (Garbers, 1977).

Table 1. Inhibition of adenylate cyclase of human spermatozoa by NaF, metal ions and carboxylic acids

Addition*	Conc. (mM)	Inhibition (%)†
NaF	1	17 ± 1
	3	38 ± 5
	5	60 ± 2
	10	67 ± 2
$CuCl_2$	2·5	86 ± 1
	5·0	91 ± 1
$ZnSO_4$	2·5	63 ± 2
	5·0	82 ± 1
$Ni(CH_3COO)_2$	2·5	65 ± 2
	5·0	64 ± 2
Citrate	10	84 ± 1
Malate	10	78 ± 3
Fumarate	10	74 ± 3
Succinate	10	74 ± 8
α-Ketoglutarate	10	63 ± 2
Lactate	10	36 ± 5
Pyruvate	10	32 ± 1

* Each inhibitor solution was neutralized before use and tested on samples of pooled semen.

† From the total activity of 32–49 pmol/min.mg^{-1}. Mean ± s.d. for 3 assays.

The mono- and di-carboxylic acids also inhibited the adenylate cyclase of human spermatozoa (Table 1). Although the concentration used was anticipated to be higher than the physiological value, the effect was comparable to that of the metal ions. Apart from citrate, these carboxylic acids do not act as strong chelating agents for metal ions, and these effects were probably not, therefore, due to chelation of Mn^{2+} in the assay system. In contrast, pyruvate, acetate and glucose (at 2 mM) were not inhibitory to the enzyme from sea urchin spermatozoa (Garbers, 1977). The inhibitory effects of the carboxylic acids may be another aspect in which the human sperm adenylate cyclase differs from that of other spermatozoa.

This work was supported by World Health Organization and the Rockefeller Foundation.

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Received 29 July 1977