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Delta-9-tetrahydrocannabinol and human spermatozoa

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Abstract. The *in vitro* effect of Δ^9-tetrahydrocannabinol on adenosine triphosphatase and phosphodiesterase activities as well as on the cyclic-AMP content of human spermatozoa has been studied. At a concentration of 1·0 μg, sperm metabolism may be increased as shown by increased cyclic AMP and adenosine-triphosphatase activity while at a higher concentration (10 μg tetrahydrocannabinol), it may be reversed.

Keywords. Delta-9-tetrahydrocannabinol; energy metabolism; spermatozoa.

Introduction

It is well known that cannabis and related compounds affect the function of the gonads (Howes and Osgood, 1976; Stefanis and Issidorides, 1976). Delta-9-tetrahydrocannabinol (Δ^9-THC) causes changes in the sperm head proteins (Hembree *et al*., 1976: Dixit *et al*., 1974; Stefanis and Issidorides, 1976). *In vitro* studies using testicular slices by Jakubovic and McGeer (1976) indicate that the drug acts directly on testicular protein and nucleic acid synthesis, These observations suggest that Δ^9-THC may have a direct effect on the spermatozoal cells. The present paper describes the *in vitro* effects of Δ^9–THC on the level of cyclic AMP, phosphodiesterase (EC 3·1·4·17) and adenosine triphosphatase (ATPase) (E C 3·6·1·4) activity in the human spermatozoa.

Materials and methods

Pure Δ^9-THC was obtained from United Nations Narcotics Laboratory, New York, in the resinous form. It was dissolved in saline containing trace amounts of Tween-80. For the control, a saline and Tween-80 suspension of equivalent proportion was prepared. The stock solution of Δ^9-THC (20mg/ml) was diluted with normal saline to the proper concentration prior to use.

Preparation of samples

The semen was collected from normal, healthy human donors and centrifuged at 800 g at 4°C for 30 min in a Damon I.E.C. centrifuge. The seminal plasma was drained off, and the sperms were suspended in the Kreb-Ringer buffer (pH 7·1) and washed a few times in the same buffer. The spermatozoa were adjusted to a concentration of 10^8 cells/ml. The estimation of adenyl cyclase and adenosine triophosphatase activities was carried out in the whole cells, whereas for estimating phosphodiesterase activity, the cells were sonicated in MES ultrasonic disintegrator at the frequency at 20 kHz prior to estimation. The sonication was done under ice for 10 sec followed by a pause of 20 s. This process was repeated for 10-12 times. The method of Winer *et al*. (1971) was essentially followed.

In all cases, spermatozoa from the same person served as control as well as all the experimental groups. Triplicate assays of samples from ten individuals were performed.

Adenyl cyclase activity of spermatozoa

The incubation mixture contained sperms at a concentration of 20×10^6 suspended in 0·04 M tris-HCl buffer (pH 7·1), 0·073 M $MgSO_4$, 10 mM theophylline, 1 mM ATP and different doses of Δ^9-THC. The total volume of the reaction mixture was 0·4 ml and the incubation was carried out at 37° C for 10 min. The reaction was stopped by placing the tubes in a boiling water bath. The samples were then deproteinised with ethanol and centrifuged. The supernatant solution was evaporated to dryness at 65° C and the cyclic AMP formed was estimated according to the method of Tsang *et al*. (1972).

Phosphodiesterase activity of the spermatozoa

The phosphodiesterase activity was determined according to the method of Butcher and Sutherland (1962). The sonicated supernatant solution of the human spermatozoa, was incubated with 1 mM cyclic AMP at pH 7·5 at 37° C. After 20 min of incubation at 37° C, 0·1 ml of Russel viper venom (1 mg/ml) was added and the incubation was continued for a further 10 min. The incubation mixture was deproteinised with trichloroacetic acid and the released inorganic phosphate in the supernatant was estimated by the method of Fiske and Subba Row (1925).

Adenosine triphosphatase activity of spermatozoa

ATPase activity was measured by the method of Quinn and White (1968).
The sperm suspension (20×10^6 cells) in tris-HCl buffer 0·05 M, pH 7·4, sucrose 0·25 M, was incubated with ATP (3 mM, pH 7·4) in presence of Na^+ (150 mM), K^+ (30 mM) and Mg^{2+} (3 mM) for 30 min at 37° C. The reaction was stopped by adding 0·2ml of 10% (w/v) trichloroacetic acid at the end of incubation period. The orthophosphate was estimated in the supernatant of the incubation mixture by the method of Fiske and Subba Row (1925).

Results

Effect of Δ^9-THC on adenyl cyclase activity of spermatozoa

Figure 1 shows the cAMP levels in pmol/10^8 spermatozoa. The control values of cAMP were 3·7 pmol which increased to 16·1 pmol on addition of 0·1 μg of Δ^9-THC. However, on increasing the Δ^9-THC concentration to 1 μg, the levels of cAMP rose to 59·1 pmol. But this stimulatory effect of Δ^9-THC was reversed on increasing the concentration to 10 μg, when cAMP levels decreased to 36·9 pmol.

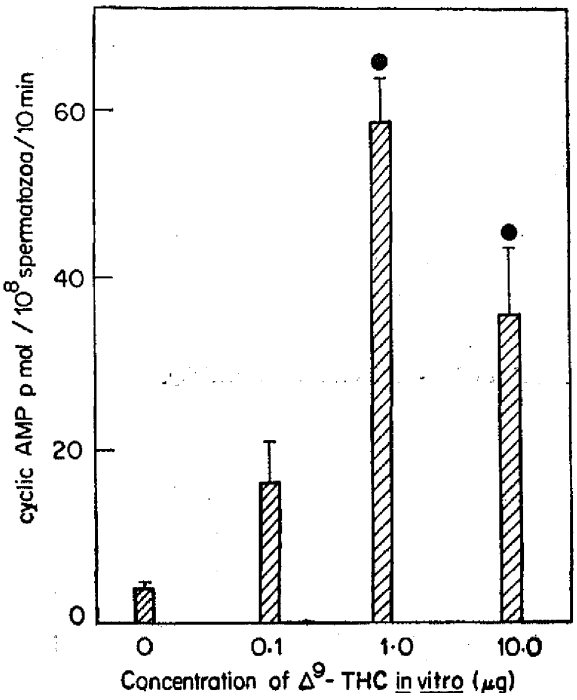

Figure 1. Effect of Δ^9-THC on adenyl cyclase activity.

Effect of Δ^9-THC on ATPase activity of spermatozoa

Figure 2 reveals the effect of Δ^9-THC on the ATPase activity of human spermatozoa at 0·1 μg, 1 μg and 10 μg dose. It is seen that activity of ATPase is increased by 12% over control at 0·1 μg Δ^9-THC. This increases further by 99% when incubated with 1 μg of Δ^9-THC; but this stimulation is reversed at 10 μg and the activity of ATPase is 8% over the control.

A comparison of ATPase activity at 0·1 μg Δ^9-THC, with 10 μg Δ^9-THC shows stimulation at 1 μg which then decreases to a value below that at 0·1 μg.

Effect of Δ^9-THC on phosphodiesterase activity of spermatozoa

Phosphodiesterase activity increased to 75% of the control value at 0·1 μg Δ^9-THC. On increasing the concentration of Δ^9-THC to 1 μg, the activity decreased to 140% of the control value. The value at 10 μg Δ^9-THC was 235% of the control value (figure 3).

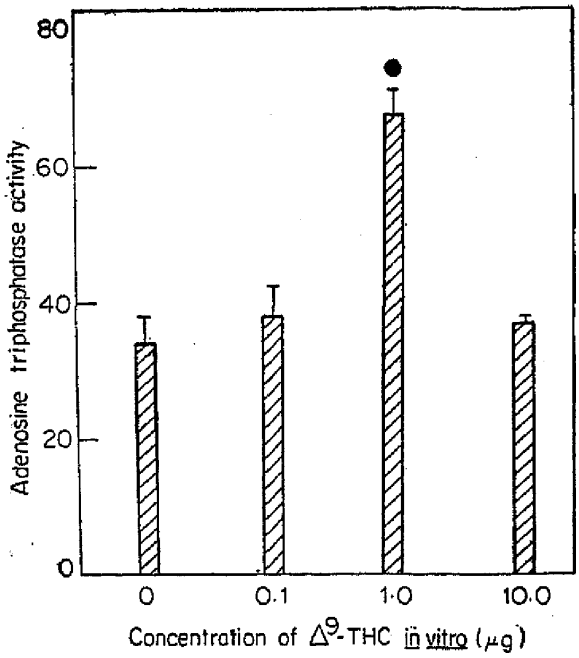

Figure 2. Effect of Δ^9-THC on ATPase activity.

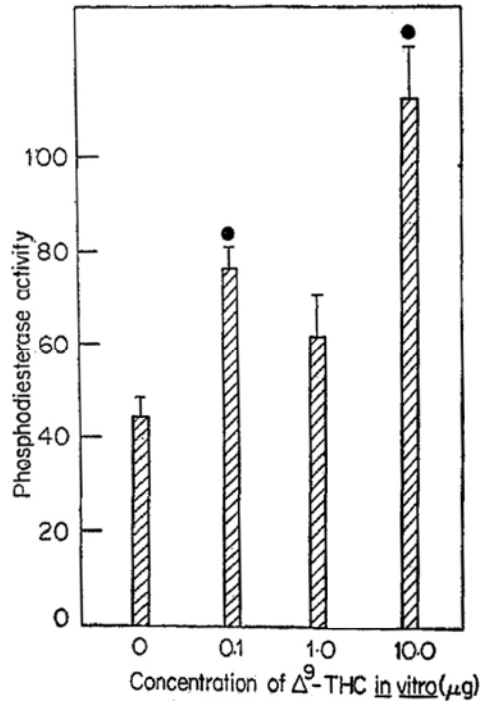

Figure 3. Effect of Δ^9-THC on phosphodiesterase activity.

Discussion

Cyclic AMP has been implicated in sperm energy metabolism and motility. Casillas and Hoskins (1971) reported that ejaculated sperms contain cyclic AMP-dependent protein kinase and is a regulator of sperm cell fructolysis and motility. Cyclic AMP level in spermatozoa, like other tissues, is balanced through the activity of adenyl cyclase and phosphodiesterase enzymes (Garbers *et al*., 1971). Thus, by regulating cyclic AMP levels, phosphodiesterase influences the sperm motility and metabolism (Schoenteld *et al*., 1973; Haesungchavern and Chulavatnatol, 1973). It has been reported that enhanced sperm motility results in increased ATP utilisation for the functioning of the contractile elements of the flagellum (Mann, 1964; Nelson, 1959).

From the present data, it is evident that 1·0 μg concentration of Δ^9-THC affects the metabolic pathway responsible for sperm motility and metabolism by increasing adenyl cyclase and ATPase activities and by decreasing phosphodiesterase activity. However, at 10 μg concentration, phosphodiesterase activity increases, thus lowering the cyclic AMP levels, which could adversely affect the energy metabolism of spermatozoa. Cyclic AMP, being the regulator of the energy metabolism of the spermatozoa, is considered as the most reliable indicator of the spermatozoal activity (Task and Mann, 1973).

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