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SHORT COMMUNICATIONS

In vitro effects of Δ^9tetrahydrocannabinol (THC) on bull sperm

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Δ^9TETRAHYDROCANNABINOL (THC) is the main psychoactive component of cannabis.[1–4] The high lipid solubility of this drug suggests that it interacts with lipoprotein fractions.[5] Recent work in this laboratory,[6] has indicated that THC had drastic effects on isolated rat liver mitochondria, detectable in the form of damage to cristae and outer membranes, as well as by changes in respiration and ATPase activity.

In order to study the effect of THC on mitochondria in the living cell *in vitro*, a highly specialized cell with unique features - the spermatozoa—was chosen. The mitochondria in spermatozoa are located in the middle piece of the flagellum to form the mitochondrial helix.[7] The mitochondrial sheath is considered to be the "motor" of sperm motility. If therefore THC affects all mitochondria, one would expect to find changes in the sperm mitochondria, as well as in motility and respiration.

In this study we employed fresh bull sperm (Artificial Insemination Service, Beit Dagan). Measurements of respiration were performed as follows: the semen was diluted four-fold in modified Ringer's buffer solution (125 mM NaCl; 7 mM KCl; 2 mM $MgCl_2$; 10 mM phosphate buffer, and 10 mM Tris buffer at pH = 7·3), and purified by two cycles of centrifugation (at 600 g for 6 min). In the final step the sperms were resuspended in buffer to give 3–4 × 10^8 cells/ml. Oxygen uptake by such suspensions was measured in a Warburg manometer.[8] The flasks contained 1 × 10^8 cells/ml; 0·03–0·3 mM THC dissolved in ethanol and buffer to a final volume of 3 ml. The volume of ethanol added did not exceed 0·03 ml. The control suspensions contained equivalent amounts of ethanol without THC. After 10 min of equilibration, respiration measurements were made for 60 min.

ATP was determined using luciferase according to Stanley and Williams.[9] Following exposure to THC as previously described, the sperm was first diluted with an equal volume of 0·01 M EDTA and 0·7 N H_2SO_4 and then disintegrated by repeated freezing and thawing.

Sperm motility was observed in a Zeiss microkine-camera at 37° using Nomarski interference contrast optics. For scanning electron microscopy, the sperm was fixed in 2·5% glutaraldehyde, dried on coverslips coated with carbon and gold and observed in the Jeol Sem U3 microscope.

As demonstrated in Table 1, THC caused a decrease in oxygen uptake and ATP content of bull sperm. These effects were not significant at concentrations below 0·03 mM. Above this concentration of THC, sperm respiration and ATP content gradually decreased, reaching minimal values at the concentration of 0·3 mM. Almost the same effects were obtained in endogenic and exogenic respiration with fructose and glucose as substrates.

Concomitantly with these biochemical changes, effects of THC on sperm motility and sperm morphology were observed. In the presence of 0·03 mM THC, most of the sperms maintained their normal motility for at least 1 h. On scanning electron microscopy no morphological changes were observed. At doses

TABLE 1. OXYGEN UPTAKE AND ATP CONTENT OF WASHED SAMPLES FROM DIFFERENT EJACULATES OF BULL SPERM, ± THE S.E. OF THE MEAN, AFTER 1 HR EXPOSURE TO Δ^9TETRAHYDROCANNABINOL AT 37°

THC (mM)	Oxygen uptake (μl/10^8 cells/hr)			ATP content (μg/10^8 cells)
	No substrate	Fructose	Glucose	
—	8·60 ± 0·43 (5)	13·10 ± 1·10 (5)	11·25 ± 1·11 (4)	9·38 ± 1·38 (4)
0·03	7·67 ± 0·33 (3)	11·67 ± 1·20 (3)	9·67 ± 0·67 (3)	7·63 ± 0·55 (4)
0·10	4·13 ± 0·43 (4)	6·50 ± 0·74 (4)	5·33 ± 0·67 (3)	4·13 ± 0·31 (4)
0·20	1·17 ± 0·17 (3)	2·50 ± 0·65 (4)	1·17 ± 0·17 (3)	1·00 ± 0·20 (4)
0·30	0·03 ± 0·03 (4)	0·33 ± 0·33 (3)	0·00 ± 0·00 (3)	0·03 ± 0·03 (4)

The number of experiments is given in parentheses.

of THC approaching 0·2 mM most sperm cells ceased to move: individual sperm, however, were still observed in circular or twisting motion. A striking morphological change was visible in scanning electron microscopy; it consisted of a remarkable swelling of the mitochondrial helix in most of the sperm which has been exposed to 0·2 and 0·3 mM of THC (Fig. 1). No such swellings, however, were observed in the examined samples of untreated sperms.

These findings indicate that THC has a pronounced effect on fresh bull sperm. This effect consisted of damage to the mitochondrial helix, decrease of respiration and ATP content, and changes in sperm motility.

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Lysis of rat liver lysosomes *in vitro* by Δ^9-tetrahydrocannabinol*

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THE MAJOR psychoactive component of cannabis is Δ^9-tetrahydrocannabinol (THC; atoms numbered as in dibenzopyran); the metabolism and properties of this substance have been extensively investigated in recent years.[1–3] In rats the liver is a major site of accumulation of THC and its metabolites,[4] and these are associated with the particulate subcellular fractions.[5] The THC molecule is lipophilic and appears to have an affinity for biological membranes; it stabilizes erythrocyte membranes[6] but disrupts mitochondrial membranes by a detergent-like action.[7–9] The present study describes the lytic effect of THC on rat liver lysosomes which results in the loss of structural integrity and the release of acid hydrolases associated with this subcellular particle.

Male Wistar rats weighing 100–150 g were decapitated and their livers homogenised in 0·25 M sucrose–0·001 M EDTA (pH 7·4). The homogenisation procedure, and the isolation of a lysosomal mitochondrial pellet have been described previously.[10] The lysosomal–mitochondrial pellet was suspended in 0·15 M sucrose–0·001 M EDTA (pH 7·4) and the suspension divided into 1·35-ml aliquots. A solution of Δ^9-tetrahydrocannabinol in ethanol was diluted with dimethylsulfoxide and 0·15 ml was added to each aliquot of lysosomal–mitochondrial suspension. Replicate groups of six aliquots were used for the determination of each point in Fig. 1. Equivalent solutions of dimethylsulfoxide and of ethanol were added to control groups. Another control group contained 0·1 per cent Triton X-100 in place of the drug, to facilitate lysis

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