EFFECT OF Δ^9 -TETRAHYDROCANNABINOL ON THE MEMBRANE PERMEABILITY OF BULL SPERMATOZOA TO POTASSIUM

E. GIBERMANN, S. GOTHILF, A. SHAHAR AND T. BINO

Israel Institute for Biological Research, Ness-Ziona, Israel

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Previous investigations in our laboratory have indicated that Δ^9 -tetrahydrocannabinol (THC), the main psychoactive component of cannabis (hashish), adversely affected bull spermatozoa *in vitro*. The effect consisted of a decrease in respiration and ATP content, changes in motility, and morphological abnormalities, such as swelling of the mitochondrial helix region (Shahar & Bino, 1973; Shahar, Bino, Kalay & Homonnai, 1973). At the same time, THC has been found to decrease ATPase in rat and human erythrocytes, protecting these cells against hypotonic haemolysis and inhibiting cation uptake (Chari-Bitron, 1971; Raz, Schurr & Livne, 1972; Gibermann, Chari-Bitron, Millo & Gothilf, 1974). We therefore decided to investigate the possible changes in the membrane permeability of bull sperm cells to potassium following exposure to THC.

Freshly collected bull spermatozoa (Artificial Insemination Service, Beit Dagan, Israel) were washed and suspended in modified Ringer, pH 7.3, to give a sperm concentration of 10^8 cells/ml (Shahar & Bino, 1973); 50- μ l aliquots of ^{42}K solution (10 mM-KCl, 140 mM-NaCl at pH 7.3, 500 $\mu\text{Ci/ml}$) were added to four 1-ml samples of sperm suspension at the beginning of the experiment. The THC was dissolved in ethanol and added to the test tubes at the start of the experiment (time 0) or 60 min later, to a final concentration of 0.1 mM. Sperm suspensions to which 1% ethanol was added without THC acted as controls. The radioactive uptake of the sperm cells was measured after various intervals by separating the cells from the fluid, employing the differential flotation method described previously (Gibermann *et al.*, 1974). The density of the separating fluid was 1.024 g/cm³.

Text-figure 1 shows a linear influx of ^{42}K from the start of the experiment until the addition of THC 60 min later. Within 1 to 2 min following the addition of THC, the intracellular ^{42}K concentration dropped considerably. When THC was added to the cell suspension at time 0, no accumulation of ^{42}K occurred. Similar results were obtained when the buffer in the suspension was replaced by a mixture of buffer and seminal plasma (10:1, v/v). A negligible effect of THC on K^+ transport was noted when the THC concentration was lowered to 0.02 mM. No change in the potassium uptake kinetics resulted from the addition of 1% ethanol.

Swelling of the flagellar region has been detected after exposure to THC

TEXT-FIG. 1. Kinetics of ^{42}K uptake by bull spermatozoa. The radioactive potassium was added to the suspension at time 0. The arrow indicates the addition of THC.

(Shahar & Bino, 1973). The drastic change in the cation permeability of the sperm cells demonstrated in the present work may be attributed to the damage of the membrane at this region.

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REFERENCES

- CHARI-BITRON, A. (1971) Stabilization of rat erythrocyte membrane by Δ^1 -tetrahydrocannabinol. *Life Sci.* **10**, 1273–1279.
- GIBERMANN, E., CHARI-BITRON, A., MILLO, S. & GOTHILF, S. (1974) Effect of Δ^1 -tetrahydrocannabinol on K^+ influx in rat erythrocytes. *Experientia*, **30**, 68–69.
- RAZ, A., SCHURR, A. & LIVNE, A. (1972) The interaction of hashish components with human erythrocytes. *Biochim. biophys. Acta*, **274**, 269–271.
- SHAHAR, A. & BINO, T. (1973) *In vitro* effect of Δ^9 -tetrahydrocannabinol (THC) on bull sperm. *Biochem. Pharmacol.* **23**, 1341–1342.
- SHAHAR, A., BINO, T., KALAY, D. & HOMONNAI, T. Z. (1973) Effect of Δ^9 -tetrahydrocannabinol (THC) on the kinetic morphology of spermatozoa. In *The Functional Anatomy of the Spermatozoon*, pp. 189–193. Ed. B. Afzelius. Pergamon Press, New York.