POSSIBLE MECHANISM FOR THE CELLULAR EFFECTS OF MARIJUANA ON MALE REPRODUCTIVE FUNCTION*

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I. INTRODUCTION

In the years since the effects of marijuana on male reproductive functions were first reported, initial studies were focused on documenting these changes. More recently, attempts are being made to determine the endocrine and/or biochemical bases of these changes. Kolodny et al. (21,22) reported that acute and chronic administration of delta-9-tetrahydrocannabinol (THC) decreases plasma testosterone level, reduces sperm counts and impairs potency in humans. Later, these observations were confirmed by many other laboratories in different species (1,3,4,20). Similarly, Dixit et al. (8,9), Zimmerman et al. (36), and Dalterio et al. (5) noted that exposure of mice, rats and dogs to THC or marijuana extract decreases size of the testis and other reproductive organs, causes Leydig cell regression, arrests spermatogenesis and results in a decrease level of testosterone. Studies of Smith et al. (32) in rhesus monkey and observations of Symons et al. (33) in rodents implicated the hypothalamic-pituitary gonadal axis for these effects of THC on testicular integrity. In these studies, the observed decreases in testosterone were found to correlate with a reduction in luteinizing hormone (LH) and the follicle stimulating hormone levels. Beside these endocrine effects, argument for the indirect effect of THC on testicular androgen comes from studies of Harclerode et al. (13), which

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suggest an inhibition of cytochrome P-450 in the testis. Also, Schwartz et al. (30) noted that injection of either THC or cannabidiol (CBD) to rats not only causes a decrease in cytochrome P-450 from Leydig cells but also reduces gamma-glutamyl transpeptidase, a marker protein for Sartoli cells.

Other studies have also been reported which show a direct effect rather than a pituitary medicated effect of marijuana on the testis. Observations of Jakubovic and McGeer (18) indicated that different constituents of marijuana directly act on rat Leydig cells in vitro, whereby causing a significant decrease in the synthesis of nucleic acids and proteins in these cells. These effects were observed in non-stimulated Leydig cells as well as cells which were stimulated either with human chorionic gonadotropin or by dibutyryl c-AMP (19). Similarly, decapsulated mouse testis grown in culture and exposed to THC or cannabinol (CBN) were less able to release testosterone in the incubation medium than were controls (4). In another study, Huang et al. (15) reported that apart from changing the normal morphology of the sperm, THC also reduces the number of spermatozoa in marijuana smokers. Hembree et al. (14) examined the semen of 16 healthy chronic marijuana smokers under controlled research ward setting. They found no direct evidence for the involvement of gonadotropins inthis oligospermia and suggested this to be a direct effect of THC on the germinal epithelium of the testis.

The site of action and the mechanism by which THC or marijuana disrupts testicular morphology, metabolism and function remains unresolved. Various cellular processes including testosterone synthesis, nucleic acid synthesis, protein synthesis, spermatogenesis and sperm motility have been shown to be inhibited by THC and other cannabinoids. Since these cellular activities require energy for their successful completion, it was planned to examine the effects of THC on the utilization of energy rich substrates such as glucose, fructose and pyruvate in the rat testis. In addition, different experiments were conducted to delineate the site(s) at which THC may act to alter the metabolism of these substances in the testis.

II. MATERIALS AND METHODS

A. Animals and Chemicals

In all phases of this study, Sprague Dawley male rats (250 +/- 50 g) were used. Radiolabeled glucose and fructose were obtained from Amersham/Searle Corp., Arlington Heights, Il. (Specific activities 3/mCi/m mol each). New England Nuclear Corp., Boston, MA, supplied the radiolabeled pyruvate (spe-

cific activity 6.5 mCi/mmol) and 2-deoxy-D-glucose (specific activity 337 mCi/m mol). Scintillation cocktails (Instagel and Permaflour II), thixotropic gelling agent (Cab-o-Sil) and tissue solublizer (Soluene-350) were purchased from Packard Instrument Co., Downers Grove, IL. Other chemicals were of analytical grade. The National Institute on Drug Abuse kindly provided the THC, which was greater than 95% pure. Glycerol-3-phosphate dehydrogenase (EC 1.1.1.8), triosphosphate isomerase (EC 5.3.1.1.), aldolase (EC 4.1.2.13), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), phosphoglucose isomerase (EC 5.3.1.9), beta-NADH, beta-$NADP^+$, EDTA and triethanolamine were ordered from Sigma Chemical Co., St. Louis, Mo.

B. Procedures

1. Radiorespirometric Experiments. Rats were decapitated, their testes rapidly removed, and each testis was stripped of its tunica and sectioned into several small pieces. These tissues were divided into control and experimental groups and incubated in separate Warburg flasks containing 2 ml of Tris-buffered medium of the following composition (mM): NaCl, 145.3; KCl, 4.8; KH_2PO_4, 1.2; $MgSO_4$, 0.7; H_2O, 1.33; $CaCl_2$, 0.2 H_2O, 1.22; and Tris 25.0. Depending upon the type of experiment, the medium also contained different concentrations ofglucose, fructose, or pyruvate as the substrate and 6-^{14}C glucose, U-^{14}C fructose, or 2-^{14}C pyruvate as the tracer. The center well and the side arm of the flasks contained 0.2 ml of 3.5 N KOH and 0.2 ml of 70% $HClO_4$ (w/v), respectively. The medium was aerated for 30 minutes with 100% O_2 and maintained at 37°C. THC was dissolved in ethanol and added to the experimental tissue medium prior to incubation in one of the following concentrations: 0.1 mM (3 mcl), 0.2 mM (6 mcl), 0.3 mM (9 mcl), or 0.4 mM (12 mcl). Control tissues received equal volumes of ethanol. Incubations were carried out for 100 minutes and the reaction was terminated by acidifying the medium with $HClO_4$ from the side arm of the Warburg flask. The $^{14}CO_2$ produced from glucose, fructose, or pyruvate in control and THC-exposed tissues were trapped for 60 minutes in KOH of the center well and counted in a Packard Model 3255 Scintillation Counter. Dry weights of the testicular tissues were determined, and the rates of $^{14}CO_2$ production in control and experimental tissues were expressed as mcMoles of CO_2 produced per g dry tissue/100 min incubation by the method of Husain and Paradise (16).

2. Determination of Glycolytic Intermediates.

a. Analysis of D-fructose-1,6-diphosphate (F-1,6-P_2), and dihydroxyacetone phosphate (DAP). Two rats were used for each control and experimental protocol. Testes were sectioned into small pieces and divided into two groups. Each group was washed with Tris-buffered, modified Krebs-Ringer's medium (described earlier) and placed in an Erlenmeyer flask containing 44.4 ml of the same medium kept at 37°C and aerated with 100% O_2 for 30 minutes. Before incubation, the experimental flasks received 0.3 mM THC dissolved in 0.2 ml of ethanol and the control flasks received equal volumes of ethanol. At the end of the 100 minute incubation, tissues were removed from flasks, rinsed several times with ice-cold medium, and freeze-dried in pre-cooled mortars. The control and THC-exposed tissues were then ground to a fine powder, weighed, transferred to separate tubes, and deproteinized with 5 ml $HClO_4$ (6%, w/v). All preparations were centrifuged for 15 min at 3000 x g. The supernatant and the washings of the precipitate, which were also spun, were transferred to calibrated conical tubes and the pH of both control and experimental samples were adjusted to 3.5 with 5 M K_2CO_3. After pH adjustment, both control and experimental supernatants were brought up to a final volume of 8 ml and allowed to stand for 15 minutes before spectrophotometric determination of the intermediates by the method of Michal and Beutler (25).

b. Analysis of D-glucose-6-phosphate (G-6-P). Determination of this glycolytic intermediate was done by the method of Lang and Michal (23). Incubation procedures and tissue preparations were similar to those performed for the analysis of F-1,6-P_2 and DAP.

C. Glucose Uptake Experiments with 2-Deoxy-D-Glucose

To study the effects of THC on glucose uptake, tissues were prepared similar to as in the respirometric experiments. The incubation medium was also identical except for substrate. In these studies, 5.5 mM 2-deoxy-D-glucose was used with ^{14}C-2-deoxy-D-glucose as the tracer. Tissues were transferred to Erlenmeyer flasks containing 2 ml of the aerated incubation medium. Prior to incubation, experimental flasks received 0.033 mM (1 mcl), 0.1 mM (3 mcl), 0.2 mM (6 mcl), 0.3 mM (9 mcl), or 0.4 mM (12 mcl) of THC. Control flasks received equal volumes of ethanol. After incubating for 100 minutes at 37°C, the tissues were washed three times with isotonic saline to remove any adhering radioactivity. Wet weights of the tissues were determined after blotting and each tissue (Con-

trol and THC-exposed) was digested in a scintillation vial for 4 hours at 55°C with 1.5 ml of toluene-350 tissue solubilizer. Afterwords, all samples were cooled and 10 ml of Instagel was added to each vial and counted. Efficiency constants for converting CPMs to DPMs were determined and the quantity of 2-deoxy-D-glucose in the control and THC-exposed tissue was calculated and expressed on a per gram tissue weight basis.

Data from control and THC-exposed testicular tissues of all experiments was compared using Students' t-test. The level of significance for these comparisons was $p < 0.05$.

III. RESULTS

In initial radiorespirometric experiments, effects of different concentrations of THC on glucose metabolism in rat testicular tissue were studied. Tissue preparations were incubated in a radiolabeled medium containing 5.5 mM glucose. There was a 15% inhibition in the catabolism of radiolabeled glucose to $^{14}CO_2$ in those tissues which were exposed to 0.1 mM THC. Other tissues exposed to 0.2, 0.3 and 0.4 mM THC dissolved in 6, 9, 12 mcl ethanol also showed a significant reduction in $^{14}CO_2$ production when compared to controls. This inhibition was dose-related as it caused an 18, 20, and 25% inhibition respectively (Fig. 1).

TABLE I. Effects of Ethanol on CO_2 Production From Rat Testicular Tissue Incubated in a 5.5 mM Glucose Medium

Group	Control[a]	Ethanol Exposed[b]	Inhibition (%)	P
1	1.1 +/- 0.2[c] (10)	1.1 +/- 0.3[c] (10)	0	N.S.
2	7.8 +/- 0.2 (6)	7.4 +/- 0.5 (7)	5.1	N.S.
3	2.4 +/- 0.6 (6)	2.5 +/- 0.6 (8)	4.2	N.S.

[a]Control tissues received no ethanol in the incubation medium which contained 5.5 mM glucose with 6-^{14}C glucose as the tracer.

[b]Ethanol exposed tissues in groups 1,2 and 3 received 6.9 and 12 mcl of ethanol in the medium prior to incubation.

[c]Values are expressed as mean mcMoles CO_2/g dry tissue/100 min incubation +/- SEM for the number of experiment in parentheses.

Since ethanol served as a vehicle for THC, a separate study was conducted to determine the effects of different concentrations of ethanol on $^{14}CO_2$ production from rat testicular tissue incubated in 5.5 mM radiolabeled glucose medium. These experiments revealed no significant effects of 6, 9, or 12 mcl ethanol on glucose metabolism in the testis (Table I).

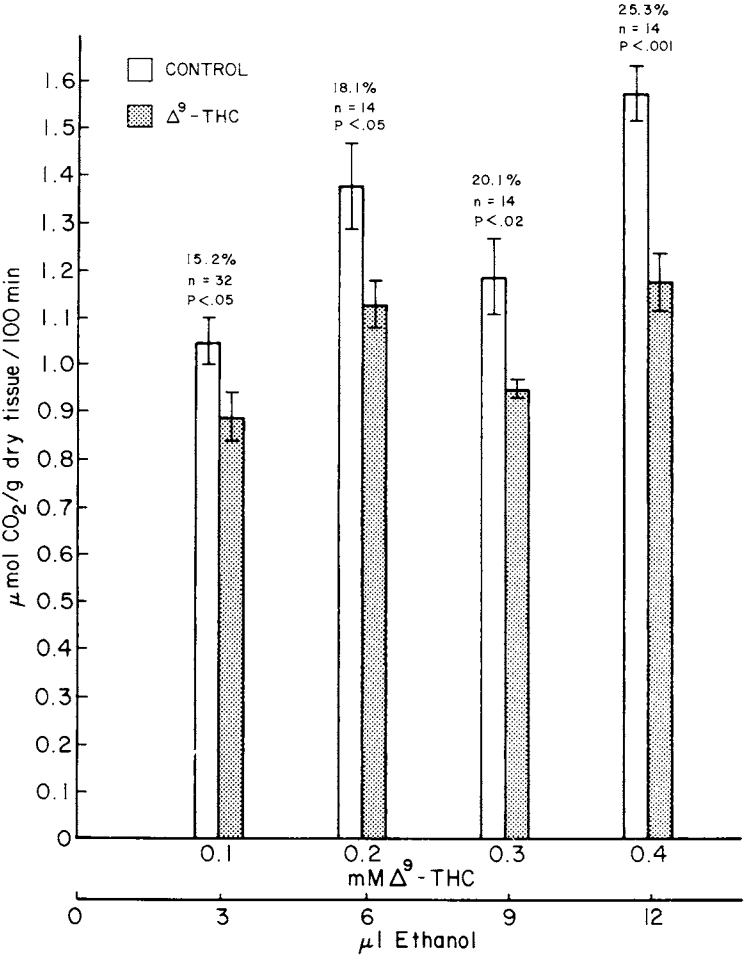

FIGURE 1. Dose-response effects of THC on CO_2 production from rat testicular tissue incubated with 5.5 mM glucose and 6-^{14}C glucose as the tracer. The CO_2 production at each concentration of THC (shaded bars) is the mean of 14 experiments +/- SEM, except at 0.1 mM THC which represents a mean of 32 experiments +/- SEM. Controls = open bars.

In the initial experiments, our observations indicated that above 0.3 mM, THC tends to form a fine suspension and begins to precipitate in the incubation medium. Therefore, in all subsequent experiments, 0.3 mM conc. was chosen to investigate the effects of THC on rat testicular glycolysis. With 1, 3, 5.5 and 10 mM glucose in the medium, exposure of testicular preparations to 0.3 mM THC decreased the output of $^{14}CO_2$. The magnitude of this decrease was 10, 24, 20, and 8%, respectively (Fig. 2). The reductions in $^{14}CO_2$ output with 3.0 and 5.5 mM glucose in the medium were statistically significant.

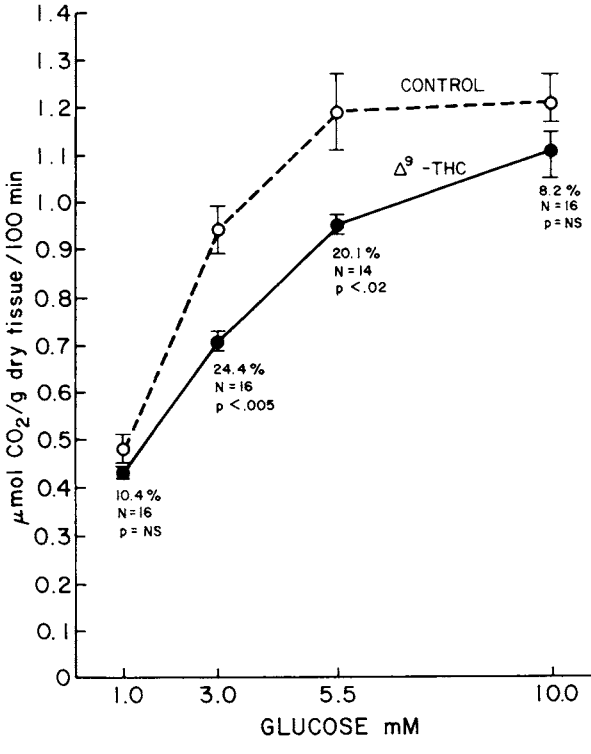

FIGURE 2. Effects of THC on CO_2 production from rat testicular tissue incubated in different concentrations of glucose with 6-^{14}C glucose as the tracer. The CO_2 production is plotted as a function of the concentration of glucose in the presence of 0.3 mM THC in the medium. Each point is a mean of 14 or 16 experiments +/- SEM.

TABLE II. Effects of Delta-9-Tetrahydrocannabinol on CO_2 Production From Rat Testicular Tissue Incubated in Different Concentrations of Pyruvate[a]

Pyruvate (mM)	Control[b]	THC	Inhibition (%)	P
1.0	3.5 +/- 0.2 (8)[c]	3.2 +/- 0.2 (8)	8.6	N.S.
2.5	3.0 +/- 0.1 (8)	2.9 +/- 0.3 (8)	3.3	N.S.
5.5	6.9 +/- 0.4 (8)	6.3 +/- 0.3 (8)	8.7	N.S.
11.0	6.9 +/- 0.5 (8)	7.1 +/- 0.3 (8)	-2.9	N.S

[a]Rat testicular tissues were exposed in vitro to 0.3 mM THC with different concentrations of pyruvate and 2-^{14}C-pyruvate (6.5 mCi/mmol) as a tracer in the medium.

[b]Controls received 9 mcl of 95% ethanol per 2 ml of incubation medium.

[c]Values are expressed as mean mcMoles CO_2/gram dry tissue/100 min incubation +/-SEM. The number of experiments is placed in parentheses.

The effects of THC on the ability of testicular tissue to utilize pyruvate as a substrate are presented in Table II. Unlike glucose, testicular tissues incubated with different concentrations of pyruvate and exposed to 0.3 mM THC failed to show any significant differences in $^{14}CO_2$ production when compared to controls.

THC produced a significant decrease in fructose metabolism with all concentrations of this substrate in the medium (Fig. 3). Testicular preparations incubated with 1.0 mM fructose and exposed to 0.3 mM THC exhibited 50% inhibition in $^{14}CO_2$ output compared to controls. Likewise, 2.5, 5.5, and 11.0 mM fructose in the medium showed a decrease in $^{14}CO_2$ production of 43, 39, and 31%, respectively.

The results of the effects of 0.3 mM THC on the concentration of DAP in testicular preparations incubated with 5.5 mM glucose reflected a statistically significant decrease of 23%. In these experiments, the mean control DAP was 163.6 +/- 4.4 nmoles/g frozen tissue/100 min of incubation, whereas the THC-exposed tissue had a mean DAP of 126.5 +/- 3.9 nmoles (Table III). In this experiment, THC also produced a significant reduction in the concentration of F-1,6-P_2 from the control value of 173.0 +/- 8.2 to 120.1 +/- 7.7 nmoles/g frozen tissue/100 min of incubation. This represents a 31% decrease in F-1,6-P_2 in relation to controls ($p < 0.05$). Similarly, tissue concentrations of G-6-P decreased by 29% in the THC-

exposed testicular preparations. The mean control value observed for G-6-P was 41.9 +/- 1.5 nmoles, whereas G-6-P in THC-treated tissue showed a mean concentration of 29.6 +/- 1.0 nmoles/g frozen tissue/100 min of incubation.

The uptake of 2-deoxy-D-glucose by testicular tissue incubated in the medium containing 5.5 mM 2-deoxy-D-glucose and

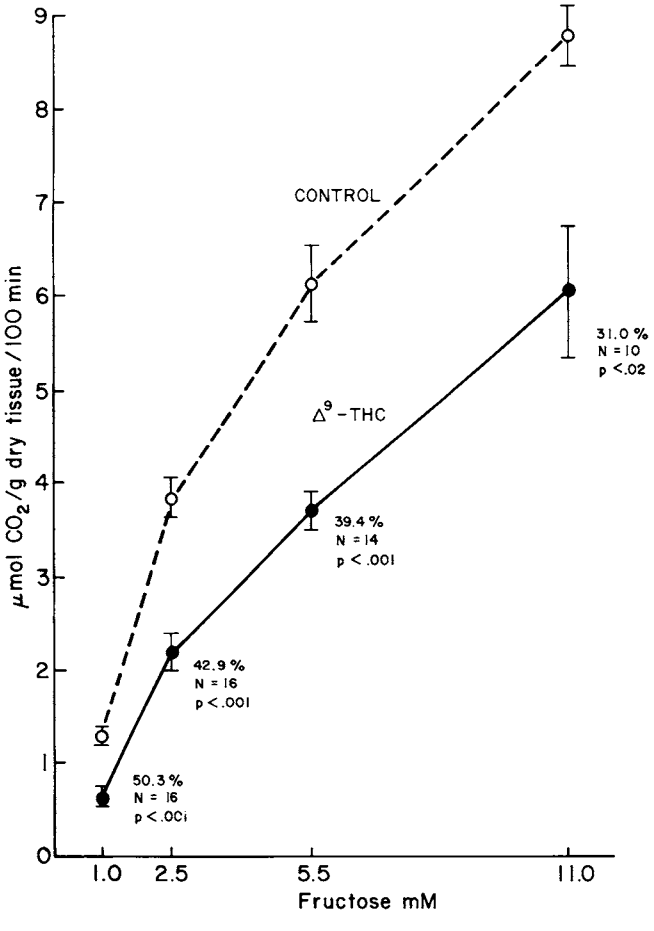

FIGURE 3. Effects of THC on CO_2 production from rat testicular tissue incubated in different concentrations of fructose with U-^{14}C fructose as the tracer. The CO_2 production is plotted as a function of the concentration of fructose in the presence of 0.3 mM THC in the medium. Each point is a mean of 14 or 16 experiments +/- SEM, except for the point with 11.0 mM fructose, which is the mean of 10 experiments +/- SEM.

TABLE III. Levels of Glycolytic Intermediates in Rat Testicular Tissue Exposed to 0.3 mM THC

Glycolytic Intermediates	Control[a]	THC	Inhibition (%)	P
Dihydroxyacetone Phosphate	163.6 +/- 4.4 (6)[b]	126.5 +/- 3.9 (6)	22.7	< 0.001
Fructose-1,6-Diphosphate	173.0 +/- 8.2 (6)	12.01 +/- 7.7 (6)	30.6	< 0.001
Glucose-6-Phosphate	41.9 +/- 1.5 (6)	29.6 +/- 1.0 (6)	29.4	< 0.001

[a]Control tissues received 200 mcl of ethanol (95%) of 44.4 ml of incubation medium which contained 5.5 mM glucose.

[b]Values are mean nmol/gram frozen tissue/100 min incubation +/- SEM for the number of experiments in parentheses.

exposed to THC concentrations ranging from 0.1 through 0.4 mM was significantly reduced. This decrease in uptake ranged from 26 to 46% (Table IV). It was observed that 0.1 mM THC reduced the 2-deoxy-D-glucose tissue concentration from 8.6 +/- 0.4 nmoles to 6.4 +/- 0.2 nmoles/g wet tissue/100 min of incubation. Similarly, 0.2, 0.3, and 0.4 mM THC reduced the concentrations of 2-deoxy-D-glucose in testicular tissue from 7.99 +/- 0.3 to 4.5 +/- 0.1 nmoles, from 8.0 +/- 0.2 mcMoles/g wet tissue/100 min of incubation, respectively. A smaller dose of THC (0.033 mM) had no significant effect on the uptake of 2-deoxy-D-glucose into testicular tissue.

IV. DISCUSSION

Although there are numerous accounts of morphological, metabolic, and functional disruptions of gonadal function by THC or marijuana, the mechanism or mechanisms by which THC produces these effects remain elusive. Data obtained from the experiments reported here and our previous observations that THC inhibits glucose metabolism in rat testicular tissue (17), suggest possible mechanism of THC action. These studies also suggest some sites at which THC may act to produce these gonadal effects. In the initial dose-response experiments, THC showed a definite effect on the evolution of $^{14}CO_2$ from a fixed substrate concentration of 5.5 mM glucose. Two explanations for this action of THC seemed likely. First, THC may be

TABLE IV. Effects of Delta-9-Tetrahydrocannabinol on the Membrane Uptake of 2-Deoxy-D-Glucose By Rat Testicular Tissues[a]

THC (mM)	Control[b]	THC	Inhibition (%)	P
0.033	8.0 +/- 0.3 (8)[c]	7.8 +/- 0.3 (8)	2.5	N.S.
0.1	8.6 +/- 0.4 (8)	6.4 +/- 0.2 (8)	25.6	< 0.001
0.2	7.9 +/- 0.3 (8)	4.5 +/- 0.1 (8)	43.0	< 0.001
0.3	8.0 +/- 0.2(13)	4.6 +/- 0.2(13)	42.5	< 0.001
0.4	8.1 +/- 0.4 (8)	4.4 +/- 0.2 (8)	45.7	< 0.001

[a]The incubation medium contained 5.5 mM 2-deoxy-D-glucose with ^{14}C-2-deoxy-D-glucose (specific activity, 337 mCi/mmol) as a tracer.

[b]Controls received, in each case, an equivalent amount of 95% ethanol used to introduce THC into test flasks. For 0.033, 0.1, 0.2, 0.3 and 0.4 mM doses of THC, the equivalent amounts of ethanol were 1,3,6, 9, and 12 mcl, resp., for 2 ml of incubation medium.

[c]Values are expressed as mean mcMoles 2-deoxy-D-glucose/gram wet tissue/100 min incubation +/- SEM. The number of experiments is placed in parentheses.

inhibiting the uptake of carbohydrate into cellular catabolic cycles by alteration of mitochondrial and/or cytoplasmic membranes. Secondly, it may be inhibiting the enzymes responsible for the breakdown of six carbon sugars, such as enzymes of Embden-Meyerhof pathway, pyruvate dehydrogenase complex, or the citric acid cycle. With these observations, experiments were designed to determine the role of THC in diminishing the supply of energy to the testis by inhibition of carbohydrate catabolism.

In experiments utilizing different concentrations of glucose in the medium, THC caused a significant effect on $^{14}CO_2$ production from 3 and 5.5 mM glucose. A similar inhibition of sugar catabolism was also observed with 1 and 10 mM glucose; however, this decrease in $^{14}CO_2$ production was not significant. Free has reported that glucose uptake in the testis appears to be directly related to the concentration of glucose in the surrounding medium (11). Therefore, if THC inhibited the uptake of glucose in our experiments, it is possible that reduction of $^{14}CO_2$ production with 0.3 mM THC could be overcome by increasing the concentration gradient of glucose across the membrane. Jakubovic and McGeer also observed no

significant effects of THC (0.1 mM) on CO_2 production from rat testicular tissue incubated in a Kreb's-Ringer phosphate medium containing 10 mM glucose (19). On the other hand, in case of tissue preparations incubated with 1 mM glucose, the control $^{14}CO_2$ output was only 0.48 micromole/g dry tissue/100 min incubation. It is likely that due to an already low output of $^{14}CO_2$ with 1 mM glucose, THC was unable to depress it any further.

After noticing that THC produces a significant inhibition in glucose utilization, attempts were made to localize the sites of this inhibition. Figure 4 depicts a simplified schematic representation of glycolysis and shows entry points at which different substrates enter this pathway. Thus far, 0.3 mM THC significantly inhibited glucose conversion to $^{14}CO_2$. Pyruvate formation is an intermediate step in this conversion; however, THC failed to inhibit testicular tissue respiration supported by a medium containing different concentrations of pyruvate. This meaningful observation led us to conclude that THC inhibits glucose metabolism at steps above pyruvate entry into glycolytic scheme. Therefore, sites of THC inhibition could be the uptake step and/or the enzymes preceding pyruvate kinase in the pathway. However, if step A is involved, only uptake of glucose and fructose was affected, whereas pyruvate, due to its structural difference was exempt from such a block.

Data obtained from different concentrations of fructose support our observations with glucose since all concentrations of fructose tested had significantly lower $^{14}CO_2$ production from testicular tissues exposed to THC. These results also exclude another step in the glycolytic sequence as a possible site of THC inhibition. Fructose, when taken up by the cell, is phosphorylated by hexokinase to fructose-6-phosphate (Fig. 4) and then further metabolized by subsequent steps in the Embden-Meyerhof pathway. Since fructose-6-phosphate was not derived from glucose, it skipped the phosphoglucose isomerase (PGI) step; however, we still observed inhibition by THC when we used fructose as substrate. Therefore, THC appears not to affect step C in glucose metabolism.

Formation of fructose in the male accessory gland is testosterone-dependent (24); however, testosterone production in itself is inhibited by THC (3,6,21,22,33). Thus, it is possible that THC could presumably affect fructose concentrations in the testis. Fujimoto et al. (12) reported earlier that adult rats, treated chronically with crude marijuana extract, contained a smaller number of sperm in epididymis as well as had a decreased fructose content. Also, in anotherstudy, glucose was found to be a necessary substrate for in vitro capacitation and acrosomal reaction of hamster sperm (10). Our studies showed that THC depressed the energy yielding catabolism of glucose and fructose in the testis. In other

words, these combined effects of THC could decrease the energy supply of the testis to the point where it could no longer function normally.

Analysis of the glycolytic intermediates further narrowed the possible sites of THC inhibition. Since concentrations of dihydroxyacetone phosphate (DAP) decreased rather than increased in tissues exposed to THC, it can be concluded that THC inhibition must take place at steps preceding formation of this intermediate. Thus, aside from the Step A, Step E, Step D, and/or Step B were implicated in this inhibition (Fig. 4). However, fructose-1,6-diphosphate (F-1,6-P_2) concentrations of tissue incubated with 0.3 mM THC were also reduced significantly. This observation ruled out the possibility of Step E being a site of THC inhibition since aldolase inhibition should increase F-1,6-P_2 and not decrease it. On the other

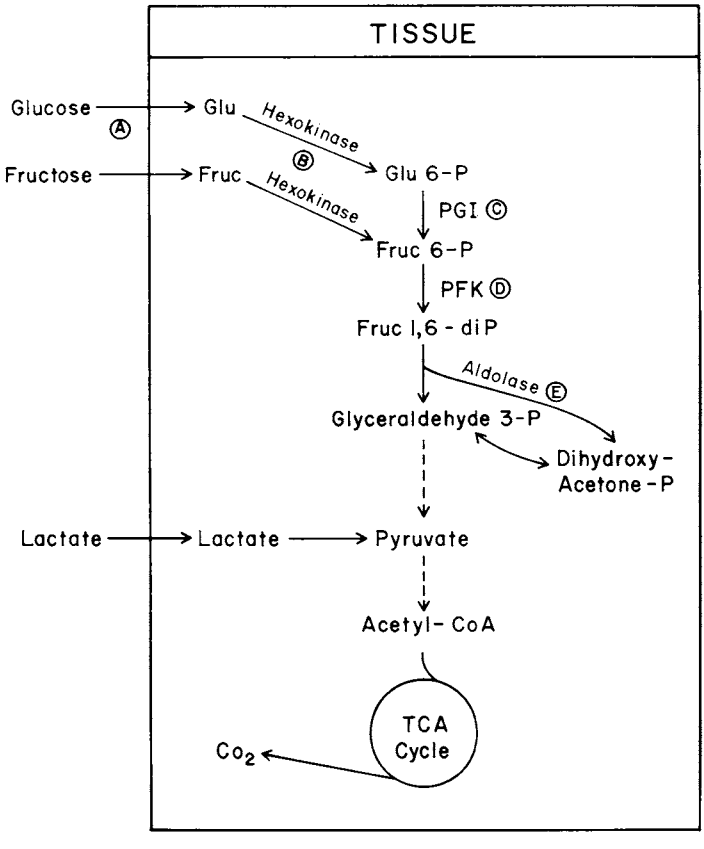

FIGURE 4. A simplified schematic representation of glycolysis in tissue showing entry points at which different substrates enter this pathway.

hand, phosphofructokinase (PFK) inhibition could result in reduction of F-1,6-P_2. Thus, it was anticipated that another glycolytic intermediate, fructose-6-phosphate (F-6-P), would increase; however, its concentration could not be detected by the analytical procedure used in this study.

The last glycolytic intermediate studied was glucose-6-phosphate (G-6-P). The cellular concentration of G-6-P decreased in the presence of 0.3 mM THC, thereby providing support to the data obtained from radiorespirometric experiments using fructose as a substrate. The latter experiments eliminated the possibility that PGI (Step C) as a site of THC action.

The results of the tissue concentrations of glycolytic intermediates suggest that the most likely sites of THC inhibition are the membrane uptake step, hexokinase, and/or possibly the PFK steps. This led to the analysis of the effects of THC on membrane uptake of glucose using uniformly labeled ^{14}C-2-deoxy-D-glucose as the substrate. The biochemical properties of 2-deoxy-D-glucose make it ideal for such studies since it is transported into the cell be the same carrier that transports glucose. However, after phosphorylation, no further metabolism of the compound takes place and it accumulates inside the cell. Results obtained from the 2-deoxy-D-glucose experiments indicated that THC in concentrations ranging from 0.1 to 0.4 mM significantly inhibited uptake of 2-deoxy-D-glucose. These results provide reasonable evidence that in testicular tissue some facets of membrane transport involving six carbon sugars are inhibited by THC. Several earlier studies reported that THC can alter membrane-bound ATPases (2,28,35). Therefore, if sugar transports (glucose and fructose) into the testicular cell is an active process supported by membrane-bound ATPase, inhibition of ATPase by THC could explain reduction in glucose and fructose uptake observed in this study.

THC is a highly lipophilic drug and is sequestered in gonadal fat (29). It is also reported to alter the configurational integrity of cellular membranes (7,31). Membrane active drugs (27) and drugs that interfere with cellular metabolism (34) are known inhibitors of sperm motility. Our in vitro experiments indicate that THC interferes with membrane uptake of glucose in rat testis and inhibits the metabolism of glucose and fructose as well. Observations of Perez et al. (26) with THC in human, rhesus monkey and rabbit sperm, and studies of Dravland and Meizel (4) with hamster sperm lend strong credence to our hypothesis. It is therefore concluded that, in addition to the highly specific hypothalamic-pituitary gonadal effect (32), THC also has an equally important direct effect on the metabolism of energy-yielding substrates in the rat testis. Since marijuana contains a variety of

cannabinoids, its effects on gonadal function could be exerted by a single constituent, or could be the result of several constituents. Cannabidiol (CBD) is a cannabinoid which has been shown to exert no effects on gonadotropins (36). Studies with this compound will help to fortify and confirm our hypothesis.

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