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Characteristics of Cocaine Interaction With Delta-9-Tetrahydrocannabinol on Glucose Metabolism in the Rat Testis

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HUSAIN, S. AND J. ANWER. *Characteristics of cocaine interaction with delta-9-tetrahydrocannabinol on glucose metabolism in the rat testis.* PHARMACOL BIOCHEM BEHAV **40**(3) 625–628, 1991.—In this study, the characteristics of cocaine (COC) interaction with delta-9-tetrahydrocannabinol (THC) on glucose metabolism in the rat testis were investigated at different time points. Rats receiving 20 mg/kg IP COC at 0830 h and sacrificed at 15 min, 30 min and 60 min posttreatment showed no change in glucose metabolism. Moreover, 5 mg/kg, IV, COC treatment at 0900 h to rats and sacrificed 60 min postexposure, also produced no change in glucose metabolism. However, at 1300 h, the same protocol with IV COC treatment resulted in a significant increase in glucose utilization by the testis. Furthermore, a THC dose of 10 mg/kg, PO to the rats at 0830 or at 1130 h, followed by sacrifice 90 min later caused a significant decrease in glucose utilization at both time points. Similarly, when rats were given both THC and COC (THC at 0830 h, COC 90 min later) and sacrificed 60 min post-COC, it resulted in a further decrease in testicular glucose metabolism as compared to the rats treated by THC alone. On the other hand, concurrent exposure of rats to these drugs at 1300 h with same protocol, ended up with no change in glucose utilization in the testis. These data suggest that not only the route of administration but also the time of exposure plays an important role in defining the nature of the effects of COC/THC interaction on rat testicular glucose metabolism.

Cocaine THC Interaction Glucose metabolism Rat testis

THE incidence of cocaine (COC) abuse has dramatically increased over the past decade (4,12). About 20 to 24 million Americans now abuse COC to varying degrees (19). According to the current data, treatment for COC abuse is escalating, street-prices are decreasing, and the age when first use occurs is dropping (3,5). Apart from other adverse effects, COC has been reported to cause dysfunction in the reproductive system (1,9). Similarly, cannabis popularly known as marihuana, has been in use as a recreational drug for some time. A good deal of research has been done to evaluate the effects of tetrahydrocannabinal (THC), the active constituent of marihuana, on different body systems. Studies in animals and humans have revealed that THC exposure causes many profound effects in reproductive system (1). Further, we have reported that THC causes these reproductive insults by inhibiting the metabolism of energy-rich substrate in the testis (11,12).

At present, there is a lack of information as to the interactions between COC and THC. Also, the effect of this combination on glucose metabolism following concurrent exposure has not been investigated. On the other hand, studies on energy metabolism in the rat testis indicate that spermatocytes and spermatids depend largely for their normal activity on glucose metabolism by Sertoli cells (10). This prompted us to evaluate the interactive effects of COC and THC on the utilization of glucose at different time points in the rat testis.

METHOD

Male Sprague Dawley rats weighing 250 ± 25 g were used in all phases of this investigation. The rats were housed in a light- and temperature-controlled room with laboratory chow and water available ad lib. All chemicals used in this study were of analytical grade. The glucose-6-^{14}C, with a specific activity of 3 mCi/mmol, was obtained from Amersham Corp., Arlington Heights, IL. THC and COC were kindly provided by National Institute on Drug Abuse (NIDA), Rockville, MD.

In this study, initially, three groups of rats were injected 20 mg/kg COC, IP at 0830 h and sacrificed 15, 30 and 60 min posttreatment. For intravenous treatment, the femoral veins of rats were cannulated under pentobarbital anesthesia (50 mg/kg, IP) and animals were allowed 24 h for recovery. The COC was dissolved in physiological saline and injected IV (5 mg/kg), at 0900 h. Another group received COC at 1300 h with the same IV dose. Animals were sacrificed one h after COC together with controls which were given equal volumes of saline. Similarly, in case of THC treatment, two groups of rats were given 10 mg/

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TABLE 1

EFFECTS OF INTRAPERITONEAL COCAINE (a.m.) ON GLUCOSE METABOLISM IN THE RAT TESTIS

Treatment*	Sacrifice Time	Glucose Metabolism† μmol CO_2/g Dry Tissue/100 Min	Percent Change From Control	Significance‡
Saline	15 min	3.8 ± 0.59 (9)		
COC	15 min	3.7 ± 0.64 (10)	−2.0	N.S
Saline	30 min	2.9 ± 0.26 (28)		
COC	30 min	2.8 ± 0.32 (29)	−2.0	N.S
Saline	60 min	4.3 ± 0.58 (42)		
COC	60 min	4.5 ± 0.54 (43)	+3.0	N.S

*Control and test rats were treated at 0830 h with 2 ml/kg, IP saline or 20 mg/kg, IP cocaine respectively. Animals were sacrificed at 15, 30 or 60 min postcocaine treatment and glucose metabolism in the testis was measured as described in the Method section.

†Glucose metabolism determined by radiorespirometric technique with 5.5 mM ^{14}C-glucose as the substrate. Values are mean ± S.E.M. for number of experiments in parentheses.

‡Significance determined by Student t-test, $p<0.5$.

kg, PO THC at 0830 and 1130 h respectively with THC dissolved in sesame oil (S.O.). Both groups were sacrificed 90 min after the THC treatment. To characterize the interactions between COC and THC, two groups of rats were exposed to 10 mg/kg, PO THC and 2 ml/kg, PO S.O. at 0830 h. This was followed 90 min by 1 ml/kg, IV saline in controls and 5 mg/kg, IV COC to THC-treated group. In addition, the same protocol of treatment was carried out in another set of rats, except THC was given at 1130 h and COC was administered at 1300 h. Controls received S.O. and saline respectively. In either set of experiments (a.m. and p.m.), all the animals were sacrificed one hour post-COC injection.

The testes from all rats were removed immediately upon sacrifice, decapsulated and sectioned into small pieces. These testicular tissues were divided into control and test groups and placed into Warburg flask containing 2 ml of Tris buffered medium with 5.5 mM glucose and 6-^{14}C glucose as the tracer. After 100 min of incubation at 37°C, the reaction was terminated by acidifying the medium with $HClO_4$ from the side arm of the flask. The catabolism of radiolabeled glucose produced $^{14}CO_2$ which was trapped for 1 h in KOH of the center well in the flask. The radioactivity in KOH samples was then counted in a Beckman LS 1701 scintillation counter. The rates of $^{14}CO_2$ production were calculated by converting CPMs into μmol of $^{14}CO_2$/g dry tissue/100 min incubation by the method described earlier (11). It was then used as an index of glucose metabolism in these testicular tissues. Finally, statistical comparisons were made between the mean $^{14}CO_2$ production from different treatment groups and their respective controls.

RESULTS

With the above treatment protocols, the effect of COC and THC alone and in combination on glucose utilization in the rat testis was evaluated. The results of IP COC (20 mg/kg) treatment at 0830 h (a.m.) are summarized in Table 1. The testes of rats sacrificed 15 min post-COC had no significant difference in glucose metabolism from their respective controls (3.8 ± 0.59 vs. 3.7 ± 0.64 μmol CO_2/g dry tissue/100 min). Similarly, the testicular tissues of rats after 30 min and 60 min IP COC treatment showed no significant change in glucose utilization from their respective controls (2.9 ± 0.26 vs. 2.8 ± 0.32 μmol/g dry tissue/100 min for 30 min group; 4.3 ± 0.58 vs. 4.5 ± 0.54 μmol/g dry tissue/100 min for 60 min group).

The effects of IV COC (5 mg/kg) at 0900 h (a.m. group) and 1300 h (p.m. group) on the energy substrate utilization in testis are presented in Table 2. In the a.m. group, animals sacri-

TABLE 2

EFFECTS OF INTRAVENOUS COCAINE (a.m. and p.m.) ON GLUCOSE METABOLISM IN THE RAT TESTIS

Treatment*	a.m./p.m.	Glucose Metabolism† μmol CO_2/g Dry Tissue/100 Min	Percent Change From Control	Significance‡
Saline	a.m.	4.1 ± 0.11 (45)		
COC	a.m.	4.0 ± 0.15 (22)	−3.0	N.S
Saline	p.m.	3.0 ± 0.15 (10)		
COC	p.m.	3.9 ± 0.14 (11)	+31.0	<0.0003

*In the a.m. group, control and test rats were treated at 0900 h with 1 ml/kg, IV saline or 5 mg/kg, IV COC respectively. Animals were sacrificed 60 min post-COC treatment and glucose metabolism in the testis was measured as described in the Method section. Similar protocol was followed in the p.m. group with animals receiving saline and COC at 1300 h and glucose metabolism in the testis measured 60 min post-COC administration.

†Glucose metabolism determined by radiorespirometric technique with 5.5 mM ^{14}C-glucose as the substrate. Values are mean ± S.E.M. for number of experiments in parentheses.

‡Significance determined by Student t-test, $p<0.5$.

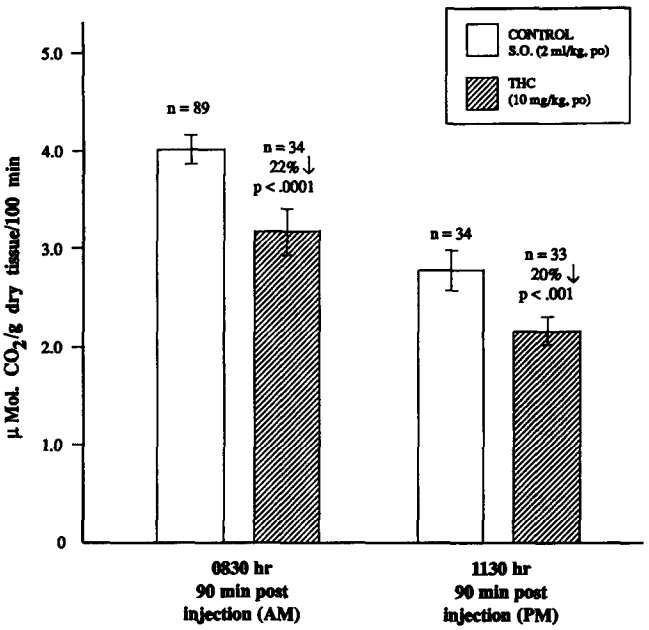

FIG. 1. Testicular tissues of rats sacrificed 90 min after 10 mg/kg, PO THC at 0830 and 1130 h were incubated in modified Tris buffer medium containing 5.5 mM of glucose with ^{14}C-glucose as the tracer. The open bars represent CO_2 production from controls whereas the hatch bars show CO_2 production from testicular tissues of rats exposed to THC. The CO_2 production was expressed as μmol CO_2/g dry tissue/100 min incubation.

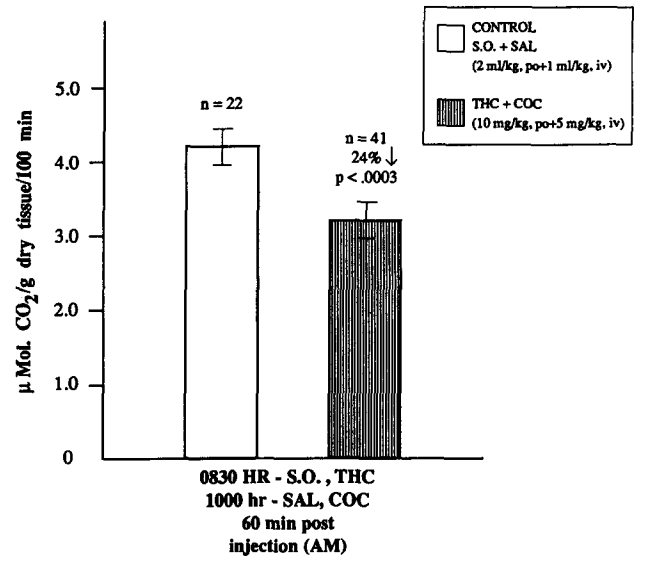

FIG. 2. Testicular tissues of rats sacrificed 60 min post-COC after 10 mg/kg, PO THC at 0830 h and 5 mg/kg, IV COC at 1000 were incubated in modified Tris buffer medium containing 5.5 mM glucose with ^{14}C-glucose as the tracer. The open bar represents CO_2 production from control whereas the hatch bar shows CO_2 production from testicular tissues of rats exposed to the drug combination. The CO_2 production was expressed as μmol CO_2/g dry tissue/100 min incubation.

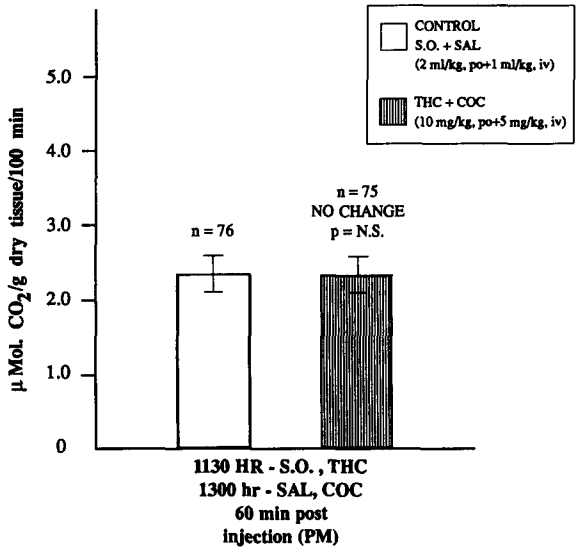

FIG. 3. Testicular tissues of rats sacrificed 60 min post-COC after 10 mg/kg, PO THC at 1130 h and 5 mg/kg, IV COC at 1300 h were incubated in modified Tris buffer medium containing 5.5 mM glucose with ^{14}C-glucose as the tracer. The open bar represents CO_2 production from controls whereas the hatch bar shows CO_2 production from testicular tissues of rats exposed to the drug combination. The CO_2 production was expressed as μmol CO_2/g dry tissue/100 min incubation.

ficed 60 min post-COC treatment showed no change in glucose metabolism when compared to controls (4.1 ± 0.11 vs. 4.0 ± 0.15 mol/g dry tissue/100 min). However, COC exposure at 1300 h (p.m. group) resulted in a significant increase (31%) in glucose metabolism from controls (3.0 ± 0.15 vs. 3.9 ± 0.14 μmol/g dry tissue/100 min).

In another set of experiments, the effect of THC (10 mg/kg, PO) alone on glucose metabolism were determined at two different time points. These data are presented in Fig. 1. Rats receiving THC at 0830 h (a.m. group) and sacrificed 90 min postinjection showed a significant inhibition (22%) of energy substrate utilization in the rat testis (4.0 ± 0.08 vs. 3.1 ± 0.17 μmol CO_2/g dry tissue/100 min). Likewise, in the p.m. group, THC treatment at 1130 h caused a 20% decrease in glucose utilization which was significantly different from their respective controls (2.69 ± 0.64 vs. 2.15 ± 0.68 μmol CO_2/g dry tissue/ 100 min).

Figure 2 illustrates the effects of combined treatment (a.m.) of oral THC 10 mg/kg at 0830 followed 90 min by 5 mg/kg IV COC at 1000 h. Rats were sacrificed 60 min post-COC exposure. This combined treatment resulted in a significant inhibition (24%) in glucose utilization (4.2 ± 0.1 vs. 3.2 ± 0.2 μmol CO_2/g dry tissue/100 min). However, in contrast to the a.m. data, the concurrent exposure of rats to THC and COC at 1300 h (p.m. group) ended up with no change in energy substrate utilization by the rat testis (2.41 ± 0.090 vs. 2.40 ± 0.09 μmol CO_2/g dry tissue/ 100 min).

DISCUSSION

Currently, the abuse of COC in epidemic proportions is the most significant problem of our society. Emergency room admissions for COC-related cases has risen 800% in the past decade (17). Most abusers of COC do not restrict themselves to the use of only one type of drug (2, 6, 18) and street COC usually contains many adulterants (20). The likelihood of a deliber-

ate and/or accidental use of COC with marihuana (THC) is also real. This polydrug abuse has led to a number of clinical dilemmas, the prognosis of which has never been fully understood. These observations led us to investigate the effects of COC in combination with THC and COC and THC alone on glucose metabolism in the rat testis.

In this study, irrespective of the route of its administration, COC (20 mg/kg, IP and 5 mg/kg, IV) caused no effects on rat testicular substrate utilization when given in the morning (Table 1). However, the p.m. administration of COC by IV route significantly increased glucose metabolism in the testis (Table 2). Previous reports in literature indicate that route of administration plays a major role in manifestation of COC effects (8,15). It is apparent from this study that apart from route of administration, the time of exposure also exerts an influence on COC effects in the testis. This characteristic effect of COC is much different from THC. With THC, a 10 mg/kg oral dose produced a significant inhibition in testicular substrate metabolism which was independent of the time THC was administered to the rats (Fig. 1). The differential characteristic effects of COC and THC also caused an interesting interaction when both drugs were concurrently administered to rats at different time points. Combined treatment of rats with oral THC and IV COC in the morning caused a significant somewhat greater decrease in testicular substrate utilization (Fig. 2). However, this decrease seems mostly to be a reflection of THC effects at this time point. On the other hand, the p.m. administration of this combination of oral THC and IV COC showed no effects in glucose metabolism in the testis (Fig. 3). This interaction is probably the consequence of the opposing effects of THC and COC at this time point. This conclusion is based on the observation that p.m. administration of THC (10 mg/kg, PO) to rats had produced a 20% significant decrease, whereas COC (5 mg/kg, IV) at this time point caused a 31% increase in rat testicular glucose metabolism (Fig. 1 and Table 2). The nature of the opposing effects of COC and THC is in agreement with the previously reported observations in which COC was shown to reverse the THC-induced depression of behavioral activity in rabbits (7). Similarly, others have found that COC antagonizes the THC depression of photocell activity and rotarod performance (16).

The results of this study, therefore, suggest that time and route of exposure play an important role in defining the characteristics of COC/THC interactions on energy substrate utilization in the rat testis. Furthermore, it has been reported that THC causes stimulation of insulin secretion from the beta cells of the Islet of Langerhans in the pancreas (14). Therefore, it will be interesting to determine if the effects of THC on insulin secretion are responsible for the modulation of glucose metabolism in the testis at different times during the 24-hour period.

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