Biochemical Pharmacology, Vol. 26, pp. 859–862. Pergamon Press, 1977. Printed in Great Britain.

EFFECT OF CANNABIS EXTRACT ON UTERINE GLYCOGEN METABOLISM IN PREPUBERTAL RATS UNDER NORMAL AND ESTRADIOL-TREATED CONDITIONS

INDIRA CHAKRAVARTY and J. J. GHOSH

Department of Biochemistry, University College of Science, 35, Ballygunge Circular Road, Calcutta-19, India

(*Received* 15 *January* 1976; *accepted* 18 *March* 1976)

Abstract—Repeated administration of cannabis extract on two uterine glycogen metabolising enzymes, glycogen phosphorylase and glycogen synthetase in prepubertal rats, treated with or without estradiol benzoate reduces the glycogen content of uterus by increasing phosphorylase activity (both total and form *a*) and by decreasing glycogen synthetase activity. In estradiol treated rats, however, cannabis extract has been found to inhibit the estradiol-induced rise in type *a* phosphorylase activity, glycogen synthetase activity being inhibited as in the previous case. Hence reduction of glycogen content in uterus by cannabis extract in estradiol-treated rats appears to be primarily due to decreased synthesis. The results indicate the antiestrogenic effect of this drug at the level of uterine glycogen metabolizing enzymes.

Several studies done in the past by other workers [1–3] as well by our laboratory [4–8] have indicated that both cannabis extract and its active component delta-9-tetrahydrocannabinol (Δ^9-THC) might have an antiestrogenic type of action in the female rats. Earlier work in this laboratory [4] has shown that the drug prolongs the estrous cycle particularly at the Di-estrous–Met-estrous phase. Borgen *et al.* [1] further showed that Δ^9-THC decreases lactation. Observations made by Nir *et al.* [3] as well as by us [5, 6] indicate that the drug suppresses serum luteinizing hormone and prolactin concentrations and also blocks ovulation. Further confirmation of the work came from Freudenthal *et al.* [2] who showed that radioactive Δ^9-THC, when injected to female mice, is localized in high concentration in the corpora lutea of ovary.

Working with prepubertal rats it was further indicated from our laboratory that cannabis extract increases uterine monoamine oxidase activity [8] and decreases uterine glycogen and water content [7, 9] significantly, even in the presence of extraneous estradiol.

In the present paper we have tried to ascertain the mode of action by which cannabis reduces the glycogen content of uterus in normal as well as in estradiol-treated prepubertal rat uterus. For this study two most important glycogen metabolizing enzymes of the organ have been estimated, namely glycogen phosphorylase (α-1,4-glucan orthophosphate-α-glucosyl transferase) and glycogen synthetase (UDP glucose-glycogen glucosyl transferase).

Address correspondence to: Prof. J. J. Ghosh, Ph.D., D.Sc., Centenary Professor & Head of the Department of Biochemistry, University College of Science, 35, Ballygunge Circular Road, Calcutta-19, India.

MATERIALS AND METHODS

Animals and treatment. Eleven-days-old female rats of Charles Foster strain (10–12 g) were used in this study. The rats used were from an average litter size of 4 or 5 per mother and were maintained under standard conditions of light for 14 hr/day (5.00–19.00 hr). The animals were divided into six groups, each group having at least ten rats.

(I) Control groups treated with saline–6% Tween 80 vehicle using the same volume as in treated animals.

(II) Animals treated daily with cannabis extract starting on day 11 after birth to 21 days of age, with 11 injections.

(III) Animals treated with a single dose of estradiol 14 hr prior to sacrifice.

(IV) Animals treated with cannabis extract as in Group II plus treatment with estradiol 14 hr before sacrifice.

(V) Animals treated with estradiol 40 hr prior to sacrifice.

(VI) Animals treated with cannabis extract as in Group II plus treatment with estradiol 40 hr prior to sacrifice.

Injection was given between 10.00 hr to 11.00 hr daily and all the rats were sacrificed by decapitation at 22 days after birth, about 24 hr after the last injection of cannabis. Estradiol was administered at two time-intervals *viz.*, at 20.00 hr on day 21 and at 18.00 hr on day 20 before sacrifice. The uterine tissues were rapidly removed, trimmed, weighed and a small piece was used for glycogen estimation. The rest was then homogenized in ice cold saline using a Potter–Elvehjem homogenizer. The homogenate was centrifuged at a low speed of 3000 *g* for 30 min in an International Refrigerated Centrifuge and the supernatant was used as the enzyme source.

Table 1. Effect of cannabis extract on uterine glycogen content and phosphorylase activity of prepubertal rats

Group	Uterine wt (mg; mean ± S.E.M.)	t-value	Glycogen content (μg present per uterus; mean ± S.E.M.)	t-value	Sp. act of phosphorylase: Total activity (mean ± S.E.M.)	t-value	Phos-a activity mean ± S.E.M.	t-value	Activity* ratio
Control	20.60 ± 1.21	1.28	51.73 ± 2.67	18.21*	151.9 ± 7.21	21.40*	9.92 ± 1.52	22.10*	0.064
Cannabis	20.00 ± 2.21		39.18 ± 1.36		282.8 ± 6.23		23.22 ± 2.28		0.082
Estradiol^{14}	30.60 ± 4.12	8.21	224.78 ± 6.10	88.62*	284.8 ± 12.67	2.26	64.48 ± 3.15	19.21*	0.226
Cannabis^{+} Estradiol^{14}	28.40 ± 3.16		57.74 ± 3.76		323.3 ± 22.12		45.59 ± 3.21		0.141
Estradiol^{40}	75.60 ± 3.12	14.28*	626.40 ± 9.01	53.54*	166.6 ± 4.23	1.11	6.22 ± 0.86	6.10*	0.037
Cannabis^{+} Estradiol^{40}	60.50 ± 1.12		166.11 ± 9.59		175.0 ± 10.68		2.71 ± 0.33		0.015

Superscript in group column indicates hr of estradiol treatment.
t-Values calculated on the basis of data obtained from ten rats in each group; students *t*-test.
* Highly significant $P > 0.005$.
Specific activity of phosphorylase expressed as μg phosphate liberated/mg protein/hr.
** Activity ratio = (Phosphorylase *a* activity)/(Total phosphorylase activity)

Dosage of Δ^9-THC. Standard samples of cannabis obtained from United Nations Narcotics Laboratory, Geneva (Sample No. UNC-266, containing 10.6% Δ^9-THC) was semi-purified in the laboratory [10] and used for the experiments. The cannabis extract sample containing 8.0% Δ^9-THC, 0.8% cannabidiol, 2.2% cannabinol and other undetermined cannabinoids, was administered at a dose of 10 mg Δ^9-THC/kg body wt subcutaneously (s.c.). Dosages were prepared by diluting the extract to a concentration of 1 mg Δ^9-THC/ml with normal saline containing 6% Tween 80 [11]; the suspension was administered in a volume of 0.1 ml/10 g body wt to each animal for eleven consecutive days, beginning when the rats were 11 days old till they reached 21 days of age. Control animals received equivalent volumes of saline Tween 80 vehicle for a similar period.

Dosage of estradiol. Estradiol benzoate (Ovocycline, Ciba) in olive oil suspension was administered s.c. at a single dose of 1 μg in 0.1 cm^3/rat either 14 hr or 40 hr prior to sacrifice of animals on 22 days of age (body wt 25–30 g).

Estimations. Glycogen content: Glycogen content of fresh tissue was estimated according to the method of Seifter *et al.* [12] by the anthrone colour reaction.

Glycogen phosphorylase activity: Glycogen phosphorylase activity was estimated according to the method of Freedland *et al.* [13], both in the presence of 6 mM AMP (total activity) and in the absence of AMP (active phosphorylase *a*), respectively. Liberated phosphate was measured by using the method of Lowry *et al.* [14].

Glycogen synthetase activity: Glycogen synthetase activity was estimated according to the method of Rogers *et al.* [15], by using the phosphoenol pyruvate kinase coupled reaction method for UDP of Leloir and Goldenberg [16]. Specific activity has been expressed as change in O.D. 540 μm/mg protein/hr.

Protein content: Protein was estimated according to the method of Lowry *et al.* [17].

RESULTS

Results presented in Table 1 indicate that cannabis extract reduces glycogen content of normal rat uterus significantly and also very prominently inhibits estradiol-triggered accumulation of this component in the uterus [7, 9]. This observation receives support from the work of El-sourogy *et al.* [18] who showed that Δ^9-THC depletes liver glycogen content and increased blood glucose level at the same time.

Regarding the results for total phosphorylase activity (in the presence of 6 mM AMP), Table 1 further indicates that the total phosphorylase activity is significantly increased by cannabis extract in normal rat uterus, whereas in the estradiol-treated rat uterus, this increase is not so significant ($P < 0.005$). Individually both cannabis extract and estradiol increase total phosphorylase activity but when given together an additive effect is not seen. In case of the active phosphorylase *a*, it is seen from Table 1 that, in normal rats, cannabis extract increases the enzyme activity significantly. Estradiol also increases type *a* formation. But when cannabis extract and estradiol are

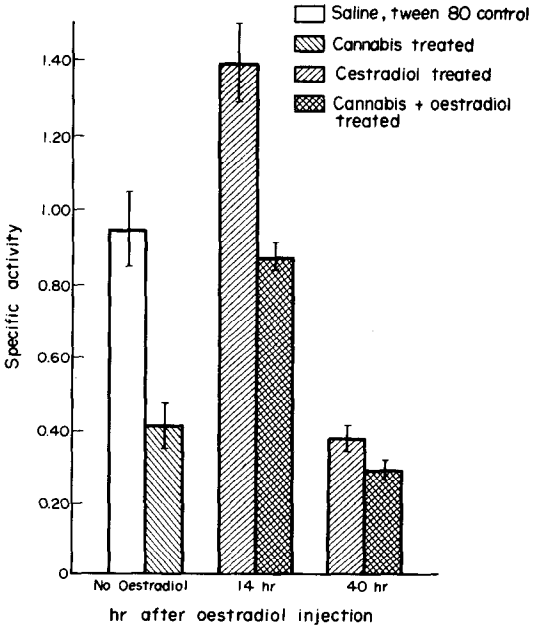

Fig. 1. Effect of cannabis extract on uterine glycogen synthetase activity.

given together, their combined effects are less than additive, i.e. the enzyme activity becomes lower than its respective hormonal control. Activity ratio (which is indicated by [Phosphorylase *a* activity]/[Total phosphorylase activity]) values also show similar trend. Both estradiol and cannabis increase the activity ratio, but when given together the drug inhibits the estradiol-induced increase significantly.

Figure 1 indicates that total uterine glycogen synthetase activity (in presence of 50 μM glucose-6-phosphate) is significantly decreased by cannabis extract, both in normal as well as in estradiol-treated conditions. Estradiol by itself is observed to increase the enzyme activity, but when cannabis is given simultaneously, this estradiol-induced increase is very prominently counteracted.

DISCUSSION

The two enzymes studied in the present text are glycogen phosphorylase and glycogen synthetase. Glycogen phosphorylase exists in two forms in almost all tissue systems including uterus [19, 20]. The active form of the enzyme is known as phosphorylase *a*, which is active even in absence of 5′ AMP while the inactive form, called phosphorylase *b* requires 5′ AMP for activation. Both types *a* and *b* are interconvertible by separate regulatory enzymes—phosphorylase *b* kinase favoring the formation of the *a* form while phosphorylase phosphatase favours the *b* form of the enzyme [21]. Glycogen synthetase acting at the rate-limiting step in the conversion of glucose to glycogen [22] also exists in two forms [23]. Interconversion of type I (active form) to type D (inactive form) proceeds by a phosphorylation reaction, similar to that described for the conversion of type *b* phosphorylase (inactive form) to type *a* (active form). Synthetase D requires glucose-6-phosphate for activation. Type I to Type D conversion is catalysed by the enzyme phosphoprotein kinase, which is very similar to the phosphorylase *b* kinase. In fact, there is now good evidence that phosphorylase kinase and synthetase I kinase are the same enzyme [24].

Cannabis when given to normal rats is found to increase total phosphorylase activity (with increased type *a* formation), and to decrease total synthetase activity. Hence in normal, prepubertal rats reduction of glycogen content by this drug may be due to both increased breakdown and decreased synthesis. Since there is evidence that the same kinase may be responsible for both of these processes, it is possible that cannabis might in some way enhance this enzyme activity so that more type *a* phosphorylase (active form) and more type D synthetase (inactive form) are synthesised. Adrenaline may also be involved in these reactions, since on one hand the sensitivity of kinase to adrenaline is well-known [25, 26], while on the other hand, the adrenal activation by cannabis has been observed by several workers [27].

In the case of estradiol-treated rats, the picture is quite different. Though total phosphorylase activity goes up to some extent, although not significantly ($P < 0.005$), it is seen that type *a* (active form) formation is being prominently inhibited. This provides further evidence for the anti estrogenic nature of the action of Δ^9-THC. Glycogen synthetase, on the other hand, is always inhibited by the drug in our experiments, while estradiol activates it. Hence in estradiol-treated uterus decreased synthesis appears to be the major mechanism by which cannabis extract reduces the glycogen content of the organ.

The inhibition by cannabis of this delicate, well-controlled process appears to be at a very basic level because more than one of the principal enzymes responsible seem to be affected. The lipophilic nature of the drug may play some important role regarding this. Previous studies made by Chari-Bitron and Bino [28], Mahoney and Harris [29] and Sarkar and Ghosh [30] showed Δ^9-THC to have a great affinity for membrane phospholipids and it is therefore possible that the drug may exert its anti estrogenic effect by inhibiting the binding of estradiol with some components at specific sites on the case membrane. Δ^9-THC-induced lowering of estradiol level in man has already been reported by Jones [31], while Dingell *et al.* [32] reported the inhibitory effect of Δ^9-THC on its conjugation.

All the data obtained so far indicate that cannabis causes drastic changes in the hormonal balance of the body. But the main problem which still remains to be solved is the mechanism by which these changes are mediated—are they central or are they peripheral?

Acknowledgements—Our best thanks are due to Dr. Olav J. Braenden, and Ms. Esme Lumsden, Narcotics Division, United Nations, Geneva, for the supply of authentic samples of cannabis used in this study. Our thanks are also to Kothari Scientific and Industrial Research, Calcutta, for their assistance and interest in this work.

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