Br. J. Pharmac. (1975), **54**, 406-408

THE γ-AMINOBUTYRIC ACID SYSTEM IN RAT CEREBELLUM DURING CANNABINOID-INDUCED CATALEPTOID STATE

H. EDERY

Israel Institute for Biological Research, Tel-Aviv University—Medical School, Ness-Ziona, Israel

ZEHAVA GOTTESFELD

Isotope Department, The Weizmann Institute of Science, Rehovot, Israel

Repeated, but not single, intraperitoneal injections of $\Delta^{1,6}$-tetrahydrocannabinol ($\Delta^{1,6}$-THC) 20 mg/kg to rats administered daily for two weeks, produced increased γ-aminobutyric acid (GABA) concentration and decreased glutamic acid decarboxylase (GAD) activity in the cerebellum, as well as enhancement of [^{3}H]-GABA uptake by cerebellar crude synaptosomes. It seems that the motor impairment elicited by $\Delta^{1,6}$-THC was not associated with the GABA system, but presumably might be related to changes in brain excitability.

Introduction Severe motor disturbances appeared in humans (Paton & Pertwee, 1973) and animals (Grunfeld & Edery, 1969) following administration of psychopharmacologically active cannabinoids. The mechanism(s) responsible for these motor impairments have not yet been elucidated. The cerebellum is considered to play an important role in motor control and maintenance of body equilibrium. The action of the cerebellum is mainly inhibitory, shown to be mediated by γ-aminobutyric acid (GABA) in interneurones and Purkinje cells (Kuriyama, Haber, Sisken & Roberts, 1966; Obata & Takeda, 1969).

The purpose of this work was to examine whether the GABA system, i.e., the concentration of GABA, its synthesis by glutamic acid decarboxylase (GAD), or the uptake of GABA by nerve terminals was affected during the cannabinoid-induced cataleptoid state in rats.

Part of the present results was communicated at the 32nd meeting of the Israel Physiological and Pharmacological Society, in February, 1975.

Methods Albino rats of either sex, 170-200 g, were injected intraperitoneally with 20 mg/kg of $\Delta^{1,6}$-tetrahydrocannabinol ($\Delta^{1,6}$-THC) dissolved in propylene glycol. Control animals were injected with the vehicle only. $\Delta^{1,6}$-THC was administered either as one single dose or as repeated single daily doses for 2 weeks. In all cases the maximum volume injected was 0.1 ml/100 g body weight. The rats were killed at the peak of motor disturbances elicited by $\Delta^{1,6}$-THC and the cerebellum was quickly removed. In some experiments brain regions associated with motor control such as the cerebellar flocculus and anterior vermis as well as the corpus striatum and parieto-temporal cerebral cortex were dissected. For GABA determination (Strasberg & Elliott, 1967) half of the cerebellum was immediately frozen in liquid N_2 and the remaining half was rapidly homogenized for subsequent GAD assay (Roberts & Simonsen, 1963). GABA uptake by crude cerebellar synaptosomes was determined by the method described by Iversen & Johnston (1971), with slight modifications. After 5 min incubation at 25° C, the [^{3}H]-GABA-containing particles were sedimented by high-speed centrifugation, rinsed with cold 0.9% w/v NaCl solution (saline) and recentrifuged. The pellet was dissolved with 0.3 ml of the tissue solubilizing agent NCS (Amersham) and the radioactivity was counted in toluene containing 0.5% w/v PPO (2,5-diphenyloxazole) and 0.03% w/v dimethyl POPOP [1,4-di-(2(5-phenyloxazole))-benzene]. The uptake was calculated as tissue : medium ratio and corrected for zero time, blank samples.

Results Following administration of 20 mg/kg of $\Delta^{1,6}$-THC severe cataleptoid reaction appeared in all animals within 30 min and normal behaviour returned 2 h later. The cataleptoid reaction was less severe towards the end of the period of repeated daily injections. In addition, there was a gradual decrease of body weight in the $\Delta^{1,6}$-THC treated group, but not in the control. The rats regained their normal weight by the end of the second week.

A single injection of $\Delta^{1,6}$-THC produced no changes in whole cerebellar GABA content or synthesis (Table 1). Similarly, no changes were found in discrete areas such as the flocculus and anterior vermis, as well as in the corpus striatum and cerebral cortex. In contrast, repeated daily treatment induced an increase in GABA concentration and a decrease in GAD activity. A small, but

Table 1 Effect of $\Delta^{1,6}$-tetrahydrocannabinol ($\Delta^{1,6}$-THC) on γ-aminobutyric acid (GABA) concentration, glutamic acid decarboxylase (GAD) activity and [^{3}H]-GABA uptake in rat cerebellum.

	Treatment	*GABA (μmol/g)*	*GAD (μmol/g^{-1} h^{-1})*	*[^{3}H]-GABA uptake Tissue: medium ratio* §
Single injection†	Control (propylene glycol)	1.49 ± 0.09	16.67 ± 0.50	
	$\Delta^{1,6}$-THC (20 mg/kg)	1.59 ± 0.06	16.21 ± 0.23	
Repeated single daily injections (2 weeks)†	Control (propylene glycol)	1.34 ± 0.07	17.66 ± 0.60	3.11 ± 0.25
	$\Delta^{1,6}$-THC (20 mg/kg)	2.21 ± 0.26*	13.31 ± 0.60**	4.98 ± 0.28**

§ Tissue : medium ratio expresses the amount of [^{3}H]-GABA taken up by the crude cerebellar synaptosomes compared to that in the medium (ct/min in lg of original tissue : ct/min in 1 ml of incubation medium)
† 0.1 ml/100 g body weight.
Results are mean ± s.e. of six rats. * $P < 0.05$; ** $P < 0.001$ (Student's *t*-test).

significant, drop in GAD activity was also found in the motor cortex of these animals (15.04 ± 0.39 μmol g^{-1}h^{-1}; 6 rats; $P < 0.05$) compared to control (17.99 ± 1.36; 6 rats). No change of GAD activity was observed in the corpus striatum.

The uptake of [^{3}H]-GABA by cerebellar crude synaptosomes was increased after 2 weeks of daily injections of $\Delta^{1,6}$-THC. However, no such effect was observed when the rats were killed 3 days after the last injection (tissue : medium, 2.98 : 3.12; 2 rats).

Discussion The present results indicate that motor disturbances in rats produced by either single or repeated injections of $\Delta^{1,6}$-THC could not be ascribed to changes in GABA concentration or synthesis in discrete brain regions. Leonard (1971) found a slight decrease (9-12%) in GABA in whole brain of rats during severe cataleptoid state produced by single doses (50 mg/kg and 100 mg/kg) of $\Delta^{1,6}$-THC. Comparison of the results is difficult since Leonard used much higher doses than those used in the present work and there was no indication whether precautions were taken to prevent post-mortem changes in GABA content (Norberg & Siesjö, 1975).

The uptake of GABA into nerve terminals was found to be decreased following ATPase inhibition (Iversen & Neal, 1968). On the contrary, increased ATPase activity caused by cannabinoids (Jain, Curtis & Bakutis, 1974) could be responsible for the enhancement of GABA uptake observed in this work. The last phenomenon could in turn result firstly in increased GABA concentration, and secondly in decreased GAD activity. The latter has been shown to be regulated by a feedback mechanism involving repression of the enzyme activity *pari passu* with GABA accumulation in nerve terminals (Haber, Sze, Kuriyama & Roberts, 1970).

The observed changes in the cerebellar GABA system were not related to the motor disturbances. On the other hand, they might be associated with the electrophysiological changes such as high voltage waves intermingled with epileptiform bursts elicited by cannabinoids in the cerebellum (Martinez, Stadnicki & Schaeppi, 1972), presumably reflecting alterations of neuronal activity. In this regard, Elliott (1965) postulated that free but not stored GABA could affect neuronal function.

The able technical assistance of Mr N. Oz is gratefully acknowledged. Special thanks to Mrs Malka Gottesfeld for her valuable assistance. Please send reprint requests to Z.G.

References

ELLIOTT, K.A.C. (1965). γ-aminobutyric acid and other inhibitory substances. *Br. Med. Bull.*, **21**, 70-75.

GRUNFELD, Y. & EDERY, H. (1969). Psychopharmacological activity of the active constituents of hashish and some related cannabinoids. *Psychopharmacologia (Berl.)*, **14**, 200-210.

HABER, B., SZE, P.W., KURIYAMA, K. & ROBERTS, E. (1970). GABA as a repressor of L-glutamic decarboxylase (GAD) in developing chick embryo optic lobes. *Brain Research*, **18**, 545-547.

IVERSEN, L.L. & JOHNSTON, G.A.R. (1971). GABA uptake in rat central nervous system: comparison of

uptake in slices and homogenates and the effects of some inhibitors. *J. Neurochem.*, **18**, 1939-1950.

IVERSEN, L.L. & NEAL, M.J. (1968). The uptake of [^{3}H]-GABA by slices of rat cerebral cortex. *J. Neurochem.*, **15**, 1141-1149.

JAIN, M.L., CURTIS, B.M. & BAKUTIS, E.V. (1974). *In vivo* effect of LSD, morphine, ethanol and Δ^9-tetrahydrocannabinol on mouse brain adenosine triphosphatase activity. *Res. Comm. Chem. Pathol. Pharmac.*, 7, 229-232.

KURIYAMA, K., HABER, B., SISKEN, B. & ROBERTS, E. (1966). The γ-aminobutyric acid system in rabbit cerebellum. *Proc. Natl. Acad. Sci. U.S.*, **55**, 846-852.

LEONARD, B.E. (1971). The effect of $\Delta^{1,6}$-tetrahydrocannabinol on biogenic amines and their amino acid precursors in the rat brain. *Pharmac. Res. Comm.*, **3**, 139-145.

MARTINEZ, J.L., STADNICKI, S.W. & SCHAEPPI, U.H. (1972). Δ^9-tetrahydrocannabinol: Effects on EEG and behaviour of rhesus monkeys. *Life Sci.*, **11**, 643-651.

NORBERG, K. & SIESJÖ, B.K. (1975). Cerebral metabolism in hypoxic hypoxia. II. Citric acid cycle intermediates and associated amino acids. *Brain Research*, **86**, 45-54.

OBATA, K. & TAKEDA, K. (1969). Release of γ-aminobutyric acid into the fourth ventricle induced by the stimulation of the cat's cerebellum. *J. Neurochem.*, **16**, 1043-1047.

PATON, W.B.M. & PERTWEE, R.G. (1973). The action of cannabis in man. In *Marijuana*, ed. MECHOULAM, R. pp. 287-333. New York: Academic Press.

ROBERTS, E. & SIMONSEN, D.G. (1963). Some properties of L-glutamic decarboxylase in mouse brain. *Biochem Pharmac.*, **12**, 113-134.

STRASBERG, P. & ELLIOTT, K.A.C. (1967). Further studies on the binding of γ-aminobutyric acid by brain. *Can. J. Biochem.*, **45**, 1795-1807.

(Received April 18, 1975)