Δ⁹-Tetrahydrocannabinol Increases Activity of Tyrosine Hydroxylase in Cultured Fetal Mesencephalic Neurons**

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Received October 14, 1996; Accepted December 11, 1996

Abstract

The exposure of pregnant rats to Δ⁹-tetrahydrocannabinol (Δ⁹-THC), the main psychoactive constituent of Cannabis sativa, during gestation and lactation, affects the gene expression and the activity of tyrosine hydroxylase (TH) in the brain of their offspring, measured at fetal and early postnatal ages, when the expression of this enzyme plays an important role in neural development. In the present article, we have examined whether Δ⁹-THC is able to affect TH activity in cultured mesencephalic neurons obtained from fetuses at gestational d 14. Thus, TH activity increased approximately twofold in cells obtained from naive fetuses when exposed for 24 h to medium containing Δ⁹-THC. In addition, TH activity was also approx twofold higher in cells obtained from fetuses exposed daily to Δ⁹-THC from d 5 of gestation than in cells obtained from control fetuses, when both were exposed to basal media. This effect of Δ⁹-THC on TH activity seems to be produced via the activation of cannabinoid receptors, in particular the CB₁ subtype, which would presumably be located in these cells. This is because the exposure to medium containing both Δ⁹-THC and SR141716A, a specific antagonist for CB₁ receptors, abolished the effect observed with Δ⁹-THC alone. SR141716A alone was without effect on TH activity. Collectively, our results support the notion that Δ⁹-THC increased TH activity in cultured mesencephalic neurons, as previously observed in vivo, and that this effect was produced by activation of CB₁ receptors, which seem to be operative at these early ages. All this points to a role for the endogenous cannabimimetic system in brain development.

Index Entries: Δ⁹-tetrahydrocannabinol; marijuana; cannabinoid receptors; brain development; tyrosine hydroxylase; tyrosine hydroxylase-containing neurons; fetal mesencephalic neurons.

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**Part of this work has been previously presented in abstract form at the 24th Meeting of the Federation of European Biochemical Societies, Barcelona, Spain, July, 1996.
Introduction

In a set of related studies, we have progressively reported that the development of tyrosine hydroxylase (TH)-containing neurons is markedly affected by perinatal exposure to Δ⁹-tetrahydrocannabinol (Δ⁹-THC), the major psychoactive compound of marijuana (for review, see Fernández-Ruiz et al., 1992, 1994, 1996). Among the most affected TH-containing neuronal systems are the midbrain pathways, in particular those neurons whose fibers reach the striatum and constitute the nigrostriatal dopaminergic system (for review, see Fernández-Ruiz et al., 1992, 1994, 1996). Thus, we have found a persistent and marked decrease in TH activity during peri pubertal period (15–40 d after birth) in the striatum of males perinatally exposed to cannabinoids (Rodríguez de Fonseca et al., 1991), which paralleled a slight but significant decrease in motor activity (Navarro et al., 1994). A similar decrease in the activity of this enzyme at immature ages was also found by Walters and Carr (1986). Accompanying this modification in TH activity, we have also found decreases in the immunoreactivity for TH in the substantia nigra of immature male rats (Suárez, Fernández-Ruiz, and Ramos, unpublished results) and changes in the gene expression of this enzyme in the midbrain (Bonnin et al., 1994), the area in which cell bodies of nigrostriatal neurons, and also of other TH-containing neurons, are clustered, although these effects appeared to be subsequent to the changes in the enzymatic activity. All these effects were exclusively observed in males and tended to normalize after the drug withdrawal occurring at weaning and, particularly, in adulthood (Navarro et al., 1994, 1996), probably because of compensatory mechanisms during the course of further development.

All these studies were conducted at peri pubertal ages, although animals had been drug-exposed from d 5 of gestation, hence, the effects probably reflected more the last consequences of perinatal cannabinoid exposure rather than the etiology of Δ⁹-THC-induced TH disturbances. Recently, we have presented evidence that Δ⁹-THC is also able to affect TH gene expression and activity during fetal and early postnatal periods (Bonnin et al., 1995, 1996; Fernández-Ruiz et al., 1996). Because TH enzyme is present in the growing axons before their contact with their target neurons, and play an important role in the formation of connections (axonal guidance, neuronal recognition, and synaptogenesis) of TH-containing neurons with other neurons (for review, see Fernández-Ruiz et al., 1992; Insel, 1995), it is reasonable to assume that the effects observed at peri pubertal ages should be the consequence of the effects caused by Δ⁹-THC during fetal and neonatal development (Bonnin et al., 1995, 1996; Fernández-Ruiz et al., 1996). Thus, our studies demonstrated that the prenatal Δ⁹-THC exposure caused a marked rise in the expression of TH gene in the brain of fetuses at gestational d 14, in parallel to a pronounced increase in the activity of this enzyme (Bonnin et al., 1996). We do not know whether this increase reflects an accelerated maturation of TH-containing neurons in terms of increased number of these neurons or in terms of enhanced capacity of each cell to express the TH gene, but it appears that TH-containing neurons are particularly sensitive to Δ⁹-THC at this fetal age, since the subsequent effects observed were never significant and sometimes tended to disappear (Bonnin et al., 1996).

The present study has been designed to extend the above findings. Concretely, we have tried to elucidate whether the Δ⁹-THC-induced increases in the gene expression and activity of TH observed in the fetal brain in vivo after the prenatal exposure to this cannabinoid (Bonnin et al., 1996) might be also elicited in vitro by using cultured mesencephalic neurons obtained from fetuses at gestational d 14. In that case, these cultures would be a valuable tool for studying the molecular mechanisms that underlie these effects. To this end, three different experiments were carried out. In experiment I, cultured mesencephalic neurons obtained from naïve fetuses were exposed to medium containing Δ⁹-THC and the activity of TH was measured 24 h later. In experiment II, the activity of TH was analyzed in cultured mesencephalic neurons obtained from fetuses exposed daily to Δ⁹-THC or to vehicle from d 5 of gestation. In experiment III, cultured mesencephalic neurons obtained from naïve fetuses were exposed to medium containing Δ⁹-THC in the absence or presence of a specific antagonist for CB₁ receptors, SR141716 (Rinaldi-Carmona et al., 1994) and the activity of TH was measured 24 h later. This experiment should clarify