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Chronic L-DOPA treatment increases striatal cannabinoid CB1 receptor mRNA expression in 6-hydroxydopamine-lesioned rats

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Abstract

The effect of a unilateral 6-hydroxydopamine (6-OHDA) lesion of the left medial forebrain bundle and 3 weeks treatment with L-DOPA of normal and 6-OHDA lesioned rats on CB1r mRNA expression was investigated by in situ hybridization. A 6-OHDA lesion of nigrostriatal pathway alone, confirmed by the loss of nigral tyrosine hydroxylase mRNA, did not alter CB1r mRNA levels in the dopamine depleted striatum. Similarly, chronic L-DOPA treatment of normal rats had no effect on striatal CB1r mRNA expression. In contrast, chronic L-DOPA treatment of 6-OHDA-lesioned rats significantly increased CB1r mRNA expression in the denervated striatum. These results suggest that the CB1r activity may be altered by L-DOPA's action and this may be related to the treatment of Parkinson's disease.

Keywords: Cannabinoid CB1 receptor mRNA; L-DOPA; 6-Hydroxydopamine; Striatum; Subthalamic nucleus

The major psychoactive component of marijuana (*Cannabis sativa*), △-9-tetrahydro-cannabinol (THC), has numerous effects on brain including alterations of motor function [1]. The receptors that mediate the central and peripheral effects of cannabinoids have been identified as cannabinoid receptor-1 (CB1r) and cannabinoid receptor-2 (CB2r), respectively, although CB1r also mediate some peripheral effect of cannabinoids [13,14]. A high density of CB1r are found in brain areas that participate in motor control and in particular in basal ganglia [8,11]. Basal ganglia CB1r may mediate the motor effects of cannabinoids since high densities of CB1r are found in the outflow nuclei of rat basal ganglia notably substantia nigra pars reticulata (SNpr), globus pallidus (GP) and entopeduncular nucleus (EP) [8,11]. Thus, the cannabinoid agonist CP-55,940 decreased locomotor activity of normal rats and this was accompanied by a down-regulation of striatal CB1r mRNA expression [16] and unilateral nigral injection of CP-55,940 induced contralateral rotation [17].

Although high levels of CB1r are located in the basal ganglia, the function of CB1r in the basal ganglia diseases, such as Parkinson's disease, is not well understood. In 6-hydroxydopamine (6-OHDA) lesioned rats, CB1r binding density in the striatum was not altered following nigrostriatal degeneration suggesting that they are not regulated by dopaminergic tone [8]. However, in another study 6-OHDA lesion increased striatal CB1r mRNA expression suggesting exactly the opposite [12]. To clarify the effect of nigrostriatal destruction on striatal CB1r expression, we have studied both the effects of 6-OHDA lesioning of rat nigrostriatal pathway and the effects of chronic L-DOPA administration to normal or 6-OHDA lesioned rats on CB1r mRNA expression in the striatum using in situ hybridization.

Male Wistar rats (180–200 g, Bantin & Kingman, UK) were anaesthetized with halothane and positioned in a Kopf stereotaxic frame. Unilateral lesions of the nigrostriatal pathway were produced by stereotaxic injection of 6-OHDA hydrochloride (Sigma Chemical Co., UK) into the left medial forebrain bundle (MFB, AP −2.2, L +1.5, V −8.0) [15]. 6-OHDA (8 μg in 4 μl of saline with 0.01% ascorbic acid) was infused at a rate of 1 μl/min. Sham-lesioned rats were infused with 4 μl of saline containing 0.01% ascorbic acid into the left MFB and served as controls. The 6-OHDA-lesioned rats were challenged with apomorphine hydrochloride (0.5 mg/kg, s.c., Sigma Chemical Co., UK) 2 weeks after surgery. Only those rats showing

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>6 contralateral rotations per minute, corresponding to a dopamine loss of >95% in the lesioned substantia nigra [7], were selected for further study.

Four groups of randomly selected animals were used. (1) Sham-operated animals received carbidopa (12.5 mg/kg per day, i.p.; Merck, Sharp and Dohme; $n = 6$). (2) Sham-operated animals received L-DOPA (50 mg/kg per day, i.p.; Roche Products Ltd., UK) plus carbidopa (12.5 mg/kg per day, i.p.; $n = 6$). (3) 6-OHDA-lesioned rats received carbidopa (12.5 mg/kg per day, i.p.; $n = 6$). (4) 6-OHDA-lesioned rats received L-DOPA (50 mg/kg per day; i.p.) plus carbidopa (12.5 mg/kg per day; $n = 7$).

At the end of 3 weeks drug administration, 3 h after last dose of treatment, animals were killed by decapitation and the brains quickly removed and flash-frozen in isopentane at −70°C. Coronal sections (20 μm) were cut using a Bright cryostat at −20°C at the levels of striatum (AP 0.2 mm from bregma) and substantia nigra (AP −5.6 mm from bregma) [15] and processed for in situ hybridization histochemistry as previously described [20]. Briefly, sections were hybridized with ^{35}S-labelled oligonucleotide probes ($5–7 \times 10^6$ dpm/ml) at 37°C overnight, washed, air dried and exposed to X-ray film (βmax, Amersham) for 3 weeks. The autoradiograms were analyzed by computerized densitometry (MCID, Imaging Research Inc.). Results were analyzed by two-way ANOVA followed by post-hoc Newman–Keuls test. Oligonucleotide probes complementary to bases 4–51 and 349–396 of rat CB1r cDNA [13] and to bases 1223–1252 of rat tyrosine hydroxylase (TH) cDNA [6] were used in the studies.

In the intact striatum of the sham lesioned control animals, high levels of CB1r mRNA labelling were present in the lateral striatum with moderate to low levels in the medial striatum. Sham lesioning and treatment of sham lesioned animals with L-DOPA plus carbidopa for 3 weeks had no effect on CB1r mRNA expression (Fig. 1).

There was no significant difference in CB1r mRNA labelling in the striatum ipsilateral to 6-OHDA-lesioned side compared with intact side or to control animals (Figs. 1 and 2A). In contrast, L-DOPA treatment of 6-OHDA lesioned animals significantly increased CB1r mRNA labelling in the denervated striatum compared with intact side and to control animals (+ 27%, *$P < 0.01$; Figs. 1 and 2B).

Nigral TH mRNA levels were not altered by sham lesioning compared with the intact side (Fig. 2C). In the 6-OHDA-lesioned rats there was an almost complete loss of TH mRNA labelling in the 6-OHDA-lesioned substantia nigra compared with the intact side (Fig. 2D). L-DOPA had no effect on TH mRNA expression in the SN of sham or 6-OHDA lesioned animals (data not shown).

The results of the present study confirm the localization of CB1r mRNA to intrinsic cell bodies in the rat striatum rather than on the terminals of mesencephalic DAergic neurones [8]. The high levels of CB1r mRNA found in the lateral striatum and the low levels in the medial striatum in the present study are in agreement with previous reports of

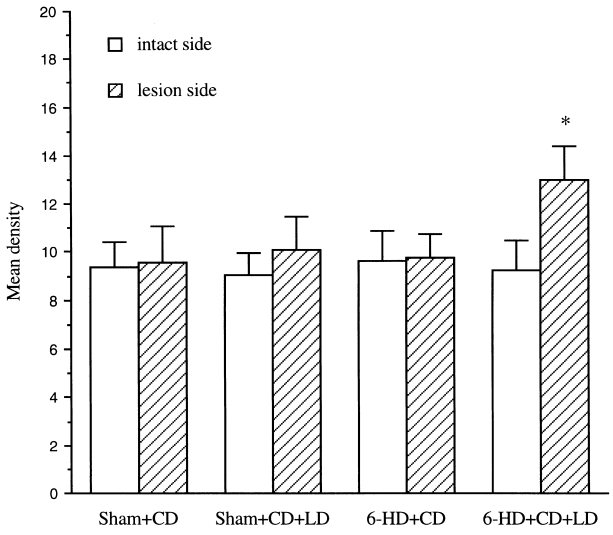

Fig. 1. Effect of 6-OHDA lesioning and subsequently chronic L-DOPA administration on striatal cannabinoid CB1 mRNA expression in rats. Unilateral 6-OHDA-lesioned rats were treated for 3 weeks with L-DOPA (50 mg/kg per day; i.p. plus 12.5 mg/kg per day carbidopa) and killed after last drug injection. The values shown represent the means ± SEM for six or seven animals per group. Results were analyzed by two-way ANOVA followed by post-hoc Newman–Keuls test. Sham, sham lesion; CD, carbidopa; LD, L-DOPA; 6-HD, 6-OHDA lesioned. *$P < 0.01$ vs. intact side and control groups.

striatal CB1r mRNA expression in the rat striatum [11]. This is also consistent with CB1r protein expression as reported by autoradiographic binding and immunohistochemistry studies [8,19]. Thus, numerous CB1r immuno-positive neurones are present in the rat striatum and immuno-positive nerve fibres are found in the EP, SNpr and GP [19].

Mailleux and Vanderhaeghen [12] reported that 6-OHDA lesioning of nigrostriatal pathway increased CB1r mRNA expression in DA denervated striatum. However, in the present study, we found that nigrostriatal degeneration had no effect on striatal CB1r mRNA expression. This is supported by a previous finding that no difference was found in CB1r binding density between the lesioned and intact striatum in 6-OHDA lesioned rats [8]. The high density of CB1r present in the striatal output targets: notably SNpr, GP and EP suggests that they reside on both the indirect strio-GP and direct strio-EP/SNpr output pathways. The former co-expresses D2 receptors, enkephalin and the latter co-expresses D1 receptors, substance P and dynorphin [3,5]. Following 6-OHDA lesioning, the indirect pathway becomes overactive as reflected by the upregulation of D2 and enkephalin mRNA expression while the direct pathway becomes underactive as indicated by the downregulation of D1 and substance P mRNA expression [3]. Since CB1r are located on the cell bodies of both pathways, it is conceivable that there would be no overall alteration in CB1r expression following nigrostriatal lesioning if both upregulation and downregulation balanced each other, but this remains to

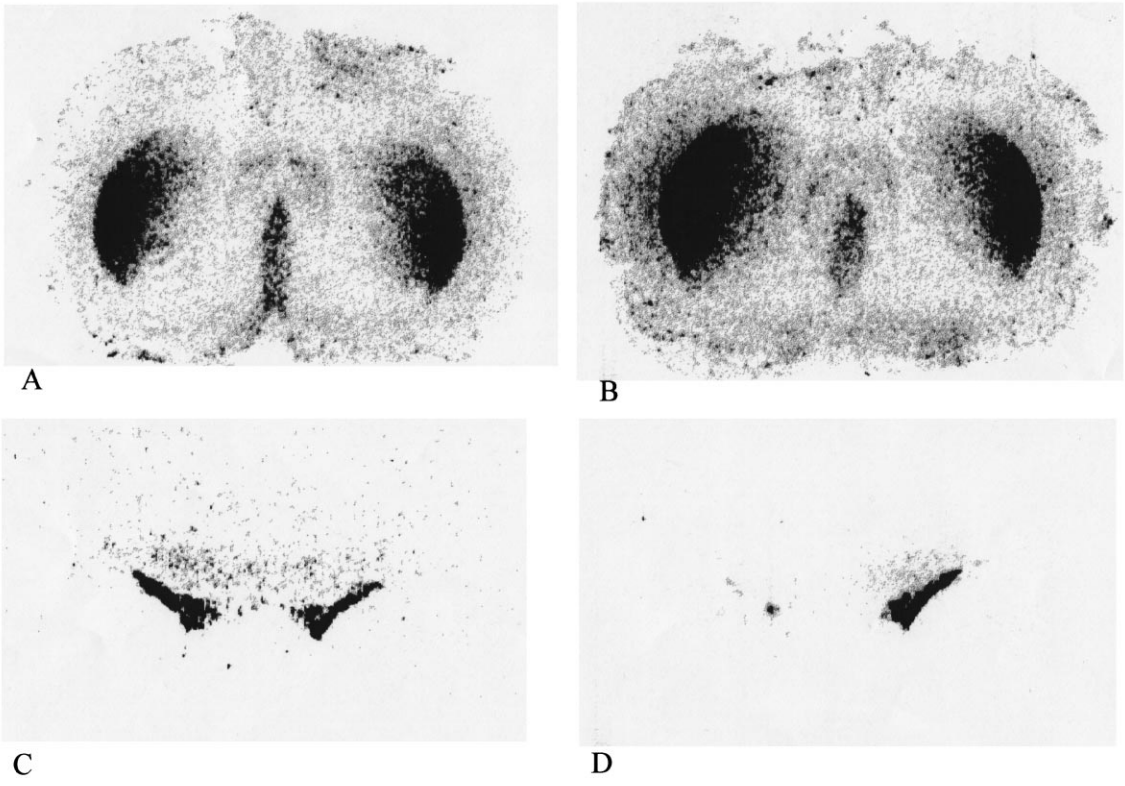

Fig. 2. Autoradiographic images showing in situ hybridization for CB1r and TH mRNAs in unilateral 6-OHDA-lesioned rats (left of each figure). (A) No difference of CB1r labelling present between lesioned side and intact side of the striatum. (B) Increased CB1r mRNA labelling in the dopamine depleted striatum of the animals treated with L-DOPA. (C) Normal distribution of nigral TH mRNA in the control animal. (D) The loss of TH mRNA labelling in the substantia nigro ipsilateral to the 6-OHDA lesion.

be explored. However, the current results most likely suggest that the regulation of CB1r is not under positive DAergic control.

Chronic L-DOPA treatment had no effect on striatal CB1r mRNA expression in the sham lesioned animals. This is surprising since both the DA D1 receptor antagonist SCH-23390 and DA D2 receptor antagonist sulpiride increase striatal CB1r mRNA expression in the normal rat [12]. One explanation might be that in the normal rats physiological DA release results in maximal suppression of CB1r mRNA and that no further decrease can be produced by L-DOPA whereas DA antagonists suppress the effect of DA so elevating the expression of CB1r mRNA. However, the failure of 6-OHDA lesioning to increase in CB1r mRNA would argue against this hypothesis.

In contrast to 6-OHDA lesioned rats chronic L-DOPA treatment increased CB1r mRNA expression in the striatum. This was unexpected because of the ability of DA antagonists to increase CB1r mRNA in normal rats and the previous reports (not confirmed by this study) that 6-OHDA lesioning similarly increased CB1r mRNA.

L-DOPA's actions appears paradoxically to be in the same direction as those produced by diminishing DAergic transmission. An analogous situation occurs with respect to L-DOPA's actions on striatal enkephalin mRNA expression in the indirect pathway. Thus, while lesioning of the nigrostriatal pathway by 6-OHDA or MPTP in rats or monkeys elevates enkephalin mRNA levels and this is reversed by DA agonists but not by L-DOPA [9,18,20]. In normal monkeys, chronic L-DOPA administration increases enkephalin mRNA expression suggesting that both lesioning of the nigrostriatal pathway and L-DOPA's action result in the same effect [21]. However, L-DOPA has actions other than increasing brain DA levels. In particular, it can act as a neuromodulator to increase glutamate release [4] and this may be of importance since removal of the cortical-striatal glutamate pathway results in a decrease in striatal enkephalin mRNA expression [10]. So, CB1r may be under both DA and glutamate influences and the development of postsynaptic DA receptor sensitivity following 6-OHDA lesioning coupled to the release of glutamate by L-DOPA may lead to CB1r mRNA upregulation.

In conclusion, the present study shows that chronic L-DOPA treatment increases striatal CB1r mRNA in 6-OHDA-lesioned rat. Recently co-administration of cannabinoid receptor agonist nabilone with L-DOPA was shown to reduce dyskinesia in MPTP treated marmoset without a loss of antiparkinsonian activity [2]. So striatal cannabinoid CB1r may be involved in motor complications resulting from the chronic L-DOPA treatment in Parkinson's disease.

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