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Attenuation of Δ^9-Tetrahydrocannabinol-Induced Withdrawal-like Behaviour by Δ^9-Tetrahydrocannabinol

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Abstract. Rats chronically treated with increasing daily doses of Δ^9-tetrahydrocannabinol (Δ^9-THC) for up to 5 or 10 days exhibit a withdrawal-like behavioural response when challenged with clomipramine, a potent inhibitor of serotonin (5-hydroxytryptamine) reuptake, at the time when the next injection of Δ^9-THC was due. To determine whether this response is indeed a withdrawal syndrome, different groups of rats were injected IV twice daily for up to 5 days with either Δ^9-THC, in doses increasing from 2–6 mg/kg, or polyvinylpyrrolidone (PVP), the vehicle in which Δ^9-THC was suspended. On day 6, an acute dose of 6 mg/kg Δ^9-THC was given 30 min before IP clomipramine (15 mg/kg). The total behavioural response, which included writhing, backward kicking, jumping, head shaking, and paw tremor, was attenuated in rats chronically treated with Δ^9-THC, but not in rats which received an acute dose of PVP. These results lend further evidence to our hypothesis that chronically administered Δ^9-THC produces a state of physical dependence in the rat.

Key words: Δ^9-Tetrahydrocannabinol – Clomipramine – Withdrawal-like behaviour

Reports from a number of laboratories currently assessing the pharmacological effects of chronic administration of Δ^9-tetrahydrocannabinol (Δ^9-THC) have been concerned with neurochemical alterations following administration of Δ^9-THC (Mazurkiewicz-Kwilecki and Filczewski 1973; Ho et al. 1974; Hattendorf et al. 1977; Sofia et al. 1977; Aulakh et al. 1980; Miczek and Dixit 1980). We have shown that Δ^9-THC, given acutely, modifies the metabolism of 5-hydroxytryptamine (5-HT) and that tolerance develops to these alterations following chronic administration (Taylor and Fennessy 1978a). Concurrent studies also showed that Δ^9-THC-induced hypothermia was inhibited by clomipramine (Fennessy and Taylor 1978) and by another inhibitor of 5-HT reuptake, fluoxetine (unpublished observations). These observations were interpreted as supportive evidence of involvement of central tryptaminergic mechanisms, at least in the effects of Δ^9-THC on thermoregulation.

Clomipramine and other amine reuptake inhibitors induce a characteristic pattern of behaviour, tentatively termed a 'withdrawal' syndrome, in rats chronically treated with Δ^9-THC (Taylor and Fennessy 1978b; Verberne et al. 1980). However, before these observations can be submitted as evidence for development of physical dependence on Δ^9-THC, Δ^9-THC should be shown to attenuate the intensity of these behavioural changes as do drugs which cause physical dependence. Consequently, modification of behaviour in rats chronically treated with Δ^9-THC was observed and measured when acute doses of Δ^9-THC were injected 30 min before induction of a clomipramine-precipitated withdrawal syndrome.

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Materials and Methods

Male albino Wistar rats (220–300 g) were prepared for IV Δ^9-THC as described by Fennessy and Taylor (1977). Rats were individually caged and kept in an animal room (21 ± 2° C ambient temperature, 12-h light-dark cycle) with food and water ad libitum. Animals were allowed 48 h to recover from surgery before being randomly assigned into two groups which received twice-daily IV injections of either Δ^9-THC or the vehicle, PVP, for 5 days. Doses of Δ^9-THC, which increased from 2 mg/kg on day 1 to 6 mg/kg on day 5, were administered according to a previously described dosage schedule (Taylor and Fennessy 1978b). On day 6 both the Δ^9-THC- and PVP-treated rats were divided into a further two groups each. This resulted in four groups of six rats: two Δ^9-THC-treated and two PVP-treated groups. One Δ^9-THC-treated group and one PVP-treated group were injected IV with Δ^9-THC (6 mg/kg), and, in addition, one Δ^9-THC-treated group and one PVP-treated group were injected IV with PVP (120 mg/kg). These drugs were administered 30 min before the challenging injection of clomipramine HCl (15 mg/kg IP). This was the time at which the next chronic injection of Δ^9-THC was due. The behaviour of the rats was observed for 30 min following clomipramine administration as previously described (Verberne et al. 1980). The number of writhes, backward kicks of the hind legs, jumps, head shakes and front paw tremors were counted by experienced observers who were unaware which treatment each rat had received chronically and which drug was being administered acutely.

(–)-Trans-Δ^9-THC (batch N780401A, NIDA, Bethesda, USA) was suspended in normal saline with the use of PVP (Kollidon 25, BASF, New York, USA), following the method of Fenimore and Loy (1971), and injected in a volume of 1 ml/kg body weight. Clomipramine HCl (Anafranil, Ciba-Geigy, Crow's Nest, Australia) was dissolved in normal saline.

The behavioural results were analysed by the χ^2-test.

Results and Discussion

The results of this study are summarised in Table 1. When PVP was administered acutely 30 min before the clomipramine challenge, the latter induced a significantly greater number of writhes, backward kicks, jumps and paw tremors in rats chronically treated with Δ^9-THC compared with the behaviour exhibited by rats chronically treated with PVP ($P < 0.05$). These results confirm previous observations

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Table 1. Clomipramine-precipitated behavioural signs 30 min following acute IV injections of PVP (120 mg/kg) or Δ^9-THC (6 mg/kg) in rats chronically treated with either PVP or Δ^9-THC for 5 days. The doses of Δ^9-THC increased from 2–6 mg/kg. Six animals were in each group

Behavioural signs	Acute PVP		Acute Δ^9-THC	
	PVP (chronic)	Δ^9-THC (chronic)	PVP (chronic)	Δ^9-THC (chronic)
Writhes	27	114*	20	61*,**
Backward kicks	0	15*	3	4
Jumps	0	24*	21	2*,**
Head shakes	1	8	1	0**
Paw tremor	2	73*	0	12*,**
Total behavioural signs	30	234*	45	79*,**

* $P < 0.05$ compared to PVP control
** $P < 0.05$ compared to Δ^9-THC control

(Verberne et al. 1980). On the other hand, when Δ^9-THC was given acutely to the other two groups 30 min before clomipramine challenge, a significantly greater number of only writhes and paw tremors ($P < 0.05$) were obtained in rats chronically treated with Δ^9-THC when compared to those of the corresponding PVP-treated group. In these latter groups, the number of backward kicks and jumps induced by the acute dose of Δ^9-THC was no longer significant ($P > 0.05$).

When the two chronic PVP-treated groups are compared, none of the behavioural parameters differed, except for an increase in the jumping response ($P < 0.05$) in the group treated acutely with Δ^9-THC. Such a result is not unexpected because it is well-documented that Δ^9-THC, injected acutely, induces spontaneous jumping in naive rats (Schildkraut and Efron 1971; Taylor and Fennessy 1978a). In addition, tolerance has been shown to develop rapidly to this phenomenon, even though it is one of the behavioural signs precipitated by clomipramine in rats chronically treated with Δ^9-THC (Verberne et al. 1980). Clomipramine does not inhibit jumping in rats acutely injected with Δ^9-THC, as shown in the present results and in those reported by Fennessy and Taylor (1978).

Comparison of the two chronic Δ^9-THC-treated groups shows that Δ^9-THC, given acutely 30 min before clomipramine administration, significantly reduces ($P < 0.05$) the number of writhes, jumps, head shakes and paw tremors. Such an attenuation of the behavioural syndrome by an acute dose of Δ^9-THC tends to support our earlier contention that these clomipramine-precipitated behavioural changes may be manifestations of a withdrawal-like syndrome from chronic administration of Δ^9-THC.

When the total behavioural signs are calculated for each group (Table 1), it is apparent that there was no significant difference between the two chronic PVP-treated groups ($P > 0.05$). On the other hand, there was a significant difference in total behavioural counts between the two chronic Δ^9-THC-treated groups ($P < 0.05$). In these two groups, injection with an acute dose of Δ^9-THC, administered prior to clomipramine, had significantly reduced the total number of behavioural signs compared to those injected acutely with PVP.

The mode of action of clomipramine in inducing this withdrawal-like behaviour is uncertain. We have previously suggested that it may act through inhibition of 5-HT reuptake, because fluoxetine, a potent inhibitor of serotonin (5-HT) reuptake, induces withdrawal-like behaviour in rats chronically treated with Δ^9-THC (Verberne et al. 1980). Nevertheless, clomipramine has other pharmacological properties which may account for some of the observed behaviour, for example, it is also a histamine H_2-receptor antagonist (Kanof and Greengard 1978), a weak muscarinic receptor antagonist and a weak α-adrenoreceptor antagonist (Rand and McCulloch 1977). Consequently, other putative neurotransmitter systems may be involved in the expression of the syndrome. Pharmacological interference with more specific agents may be more useful in gaining a greater understanding of this response.

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