A major metabolite of Δ^1-tetrahydrocannabinol reduces its cataleptic effect in mice[1]

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Summary. The results described here demonstrate that THC-induced catalepsy in mice can be substantially inhibited by the prior administration of Δ^1-THC-7-oic acid, the major metabolite of THC in most species including humans. This raises the possibility that the intensity and duration of action of THC may depend to a large degree on the levels of this metabolite at the sites of action.
Key words. Δ^1-Tetrahydrocannabinol; metabolites; antagonists.

The metabolism of the cannabinoids involves a series of oxidative transformations that ultimately lead to a group of carboxyl containing derivatives of the parent substances[2]. These acidic metabolites display none of the biological activities of their precursors and have been generally regarded as inactive metabolic end-products. Their close structural similarity to the primary cannabinoids suggest that they would be good candidates to test as antagonists to the active cannabinoids in a model system which we have developed recently[3]. Interestingly, although many drugs have been shown to interact synergistically with cannabinoids, few antagonists to their actions have been reported.

We are currently examining the changes in lipid metabolism in cell culture systems following exposure to Δ^1-THC. In particular, we found that the arachidonic acid cascade seems to be markedly stimulated by cannabinoids. This has led to a hypothesis on the mechanism of action of Δ^1-THC in which the prostaglandins are mediators of at least some of the effects of THC. The structure-activity relationships[4], the development of tolerance[5] and stereospecificity in the in vitro system[6] suggest that it is a mechanistically relevant model for THC action. Using this model, we tested a series of THC metabolites as potential antagonists for the stimulatory action of THC on prostaglandin E_2 (PGE_2) synthesis by WI-38 human lung fibroblasts in monolayer culture[7].

The monohydroxy metabolites of Δ^1-THC that were tested showed no inhibitory effects on PGE_2 synthesis when the cells were treated 30 min prior to THC exposure[7]. On the other hand, two of the carboxyl containing derivatives 3″,4″,5″-tris nor-Δ^1-THC-2″-oic acid and Δ^1-THC-7-oic acid were inhibitory and, in particular, the latter reduced the effect of Δ^1-THC at doses similar to that of THC. The inhibition appeared to be non-competitive and further investigation showed that the metabolite acted at the cyclooxygenase step in the biosynthetic pathway leading to PGE_2.

An obvious question then arose, namely, would Δ^1-THC-7-oic acid antagonize any of the in vivo actions of THC, especially those which may involve the central nervous system? The cataleptic response in the mouse has been developed by Pertwee[8] into a reliable assay for THC and may have relevance to the psychoactive effects of cannabis in humans. Briefly, the test consists of placing the mouse on a steel ring of specific dimensions with the front feet resting on one side of the ring and the hind paws at the opposite side. The fraction of time the mouse remains immobile over a five minute test period is taken as a measure of catalepsy. Pertwee referred to this method as the 'ring test' and has reported satisfactory dose response relationships for Δ^1-THC. Others have used this test for cannabinoid structure-activity studies[9] and for experiments related to mechanism of action[10].

Based on the above considerations, we decided to utilize the ring test to measure the possible in vivo inhibition of THC activity by Δ^1-THC-7-oic acid. Table 1 summarizes the results of these experiments. As predicted by the in vitro model, mice pretreated with the metabolite exhibited a greatly diminished cataleptic response, thus making the acid an effective antagonist of THC. Mice treated with the acid alone (40 mg/kg) showed essentially the same response (0.09 ± 0.02, $N = 3$) as vehicle treated animals. This is consistent with the reported lack of activity of this metabolite in humans[11]. The data also shows that we were able to obtain a large and significant increase in immobility when THC treated mice were compared to vehicle treated animals confirming what has been reported in the literature[8].

Table 1. The inhibition of Δ^1-THC induced catalepsy in mice by Δ^1-THC-7-oic acid. Female, CD-1 mice (Charles River) weighing 20–25 g were given Δ^1-THC (50 mg/kg) in sesame oil (10 µl) orally. One hour later the cataleptic effect was measured using the procedure described by Pertwee[8]. The control groups received only sesame oil and the pretreated group was given Δ^1-THC-7-oic acid (40 mg/kg) in oil (50 µl) orally one hour prior to the administration of Δ^1-THC. The values are expressed as the means of the fraction of time the mice remained immobile ± SD. Values in parentheses indicate the number of mice in each group. Asterisks denote a p value of < 0.01 as determined by a one-way analysis of variance ($F = 26.4$, $DF = 2,33$) and a post hoc Newman-Keuls Test

	Control	Treated	Pretreated
Experiment 1	0.05 ± 0.05 (4)	0.28 ± 0.02 (4)	0.11 ± 0.04 (5)
Experiment 2	0.09 ± 0.04 (3)	0.26 ± 0.03 (3)	0.19 ± 0.10 (5)
Experiment 3	0.08 ± 0.03 (4)	0.42 ± 0.01 (4)	0.19 ± 0.10 (4)
Mean	0.07 ± 0.02	0.32 ± 0.01*	0.16 ± 0.05*

Table 2. Prostaglandin mediation of Δ^1-THC induced catalepsy in mice. The cataleptic response was measured as described in table 1. The PGs were administered i.p. 20 min prior to the measurement; indomethacin was given i.p. 30 min before the THC

Treatment	Cataleptic response ± SD (N)
Tris buffer (0.05M, pH 8)	0.10 ± 0.04 (4)
PGE_2 (4 µg/kg)	0.12 ± 0.02 (3)
PGE_2 (400 µg/kg)	0.26 ± 0.09 (3)
PGI_2 (400 µg/kg)	0.33 ± 0.11 (6)
Saline (0.154 M)	0.06 ± 0.05 (3)
Sesame oil (50 µl)	0.07 ± 0.04 (11)
Δ^1-THC (50 mg/kg)	0.32 ± 0.09 (11)
Indomethacin (15 mg/kg) + Δ^1-THC (50 mg/kg)	0.03 ± 0.03 (3)

A strong inference from the results in table 1 is that products of the arachidonic acid cascade may be mediators of THC-induced catalepsy in mice. Earlier, Fairbairn and Pickens[10] had suggested this based on analogous experiments in which THC-induced catalepsy could be inhibited by a variety of cyclooxygenase inhibitors such as aspirin, indomethacin, etc. and could be restored by the subsequent administration of PGE_2. We have confirmed some of their findings and extended them to prostacyclin (PGI_2) as seen by the data in table 2. Both PGE_2 and PGI_2 produced a cataleptic effect when compared to vehicle and the values suggest that PGI_2 may be more effective. Indomethacin, a potent inhibitor of cyclooxygenase, was also very effective in preventing THC-induced catalepsy.

The above experiments were carried out by administering the Δ^1-THC-7-oic acid orally. It has been reported that acidic metabolites of THC appear in the brain following systemic administration of the drug[9,12]. It is not certain, however, what fraction of these metabolites is formed intracerebrally and how much is derived from outside the brain. We have administered tritium labelled Δ^1-THC-7-oic acid systemically to mice and measured the distribution into brain and liver. One hour after injection only about 0.2% of the dose was found in a total brain homo-

genate while more than 13% was present in liver. The small proportion of Δ^1-THC-7-oic acid reaching the brain suggests the possibility that it may exert most of its inhibitory action at sites not in the central nervous system. If this is the case, it follows that the major primary site of cataleptic action of Δ^1-THC could also be located outside of the brain. This is supported by the data in table 2 showing that the i.p. injection of PGE_2 and PGI_2 gave a THC-like response in the ring test.

The finding reported here that a major metabolite of Δ^1-THC has in vivo biological activity that antagonizes the effects of the parent drug should be considered in any studies with THC where metabolism can occur. For example, pharmacokinetic studies have yielded data which suggest that the plasma levels of THC do not correlate well with the intensity of its effects[13]. Such a self-generated antagonism as we have described for THC could provide a basis for a lack of correlation except in the special situation where THC and its antagonist are always present in the same ratio at the site of action.

In terms of mechanism of action of THC, this report extends our previous findings based on an in vitro model[7]. A role for prostaglandins in the pharmacodynamics of THC, thus, seems to be a reasonable hypothesis. It is interesting to note that throughout its long history cannabis has been considered to have anti-inflammatory properties[14]. These reports can now be better explained on the basis of the cycloxygenase inhibitory action of Δ^1-THC-7-oic acid[6] rather than Δ^1-THC itself.

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Development of high blood pressure in spontaneously hypertensive rats is delayed by treatment with cyclosporin at an early age

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Summary. In spontaneously hypertensive rats the effect of the T-cell inhibitor cyclosporin was studied at different ages. If treatment was started at the age of 2 weeks the development of hypertension was delayed, but the ultimate level of blood pressure was not affected. These results indicate the involvement of immune mechanisms in the early development of hypertension in spontaneously hypertensive rats.

Key words. SHR; cyclosporin; immune mechanisms; hypertension.

In recent years immunological abnormalities have been reported to be present in a strain of spontaneously hypertensive rats (SHR) originally developed by Okamoto and Aoki[1] by selective inbreeding of Wistar Kyoto rats (WKY).

Cell-mediated immune responses, including mitogenic responses, are reduced in SHR. This suggests a selective suppression of T-lymphocyte function in SHR, the cause of which may be a natural cytotoxic autoantibody against T cells[2–5]. This autoantibody occurs from the age of about one month onward, its titer varying widely among individuals[4,5].

The role of the immunological depression in the development of hypertension in SHR is as yet unclear, but restoration of the immune responsiveness by transplantation of histocompatible thymus tissues into neonatal SHR suppressed the development of hypertension[6]. Administration of thymosin (fraction 5) to young and adult SHR resulted in restoration of T cell functions and lowering of blood pressure[7]. Since anti-rat-thymocyte serum and implantation of WKY thymuses also proved capable of reducing blood pressure in SHR, it is possible that hypertension in SHR has an immunological basis[8–10]. Results of cyclophosphamide treatment of SHR are as yet inconclusive, since the observed decrease in blood pressure was accompanied by a reduced growth rate[11,12].

We report the effect of treatment with cyclosporin on the development of hypertension in SHR. Cyclosporin is a nonpolar cyclic oligopeptide immunosuppressive agent of fungal origin, which appears to act specifically at the level of the T helper cell and reversibly inhibits the production of lymphokines[13–15].

Materials and methods. Male rats of strains SHR and WKY were obtained from Central Breeding Laboratories TNO, Zeist, The Netherlands. Adult animals (12 weeks old) weighed 180–200 g. Young animals (4 weeks) were used after weaning, weighing 50–60 g, and before weaning (2 weeks old), weighing 20–25 g. Systolic blood pressure was assessed in trained conscious rats by the indirect method of tail sphygmography[16].

Cyclosporin was administered by intragastric gavage, dissolved in a mixture of 96% ethanol, Tween 80 and normal tap water[17,18]. The animals received a dose of 10 mg/kg/day, controls