Cannabinoid-Precipitated Withdrawal: A Time-Course Study of the Behavioral Aspect and Its Correlation with Cannabinoid Receptors and G Protein Expression[1]

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ABSTRACT

To characterize the time course of the behavioral and biochemical aspects of the cannabinoid withdrawal syndrome, we injected the cannabinoid antagonist SR141716A (5 mg/kg i.p.) in rats made tolerant to CP-55,940 (0.4 mg/kg i.p., twice daily for 6.5 days), 1, 24 and 96 h after the last CP-55,940 injection. Because the CB1 receptor and G protein *alpha* subunit are involved in cannabinoid tolerance, we observed their changes throughout the brain during the withdrawal syndrome by use of *in situ* hybridization. In vehicle-pretreated rats SR141716A *per se* induced abnormal behavior significantly different from the vehicle group: wet dog shakes, forepaw fluttering and scratching. These signs remained significantly elevated even after the second and third antagonist doses. SR141716A significantly modified the mRNA levels of $G\alpha s$ and $G\alpha i$ subunits in some brain areas without affecting CB1 receptor and $G\alpha o$ expression. These findings led us to conclude that SR141716A may have intrinsic activity. Concerning cannabinoid withdrawal, the first SR141716A injection in tolerant rats resulted in behavioral signs different from those observed with the antagonist alone; this moderate withdrawal syndrome was characterized by turning, chewing and digging. Additional SR141716A doses 24 and 96 h later did not induce a significant abstinence syndrome. *In situ* hybridization after the first SR141716A injection showed that CB1 receptor and G protein *alpha* subunits, whose levels were low in tolerance, recovered their basal level of expression. Thus, the general desensitization of the cannabinoid receptor and of the transduction system in tolerance are recovered in abstinent rats and might be part of the molecular mechanisms underlying cannabinoid dependence.

During the past few years our knowledge of the development of tolerance to the behavioral effects of Δ^9-THC and synthetic compounds has increased, and biochemical data suggest that it results to some extent from agonist-induced receptor down-regulation (Oviedo *et al.*, 1993; Rodriguez De Fonseca *et al.*, 1994; Fan *et al.*,1996). As we recently demonstrated, alteration in G protein expression or level is another cellular event correlated to cannabinoid tolerance, which suggests that proximal events in the cannabinoid receptor machinery (receptor amount and down-regulation of expression) and more distal events through the balance of the signal transduction system affect G protein expression (Rubino *et al.*, 1997). Childers' group found a significant reduction (up to 70%) in WIN 55212–2-stimulated [^{35}S]GTPγS binding in most brain regions of rats chronically treated with Δ^9-THC, which suggests that cannabinoid-activated signal transduc-

tion mechanisms may be desensitized profoundly after this treatment (Sim *et al.*, 1996).

Our understanding of cannabinoid dependence, however, is still in its early stages. Dependence on cannabinoids is an important topic not only because cannabis is widely used recreationally, but also because we have a continuing interest in the therapeutic potential of natural and synthetic cannabinoids. Therefore, animal studies are important in assessing drug dependence, but studies with Δ^9-THC have not provided consistent evidence that dependence arises with the cannabinoids and the findings are questionable because of the robust dose regimens used (Leite and Carlini, 1974; Jones *et al.*, 1976; Kaymakcalan *et al.*, 1977; Dewey, 1986).

The development of the competitive cannabinoid receptor antagonist, SR141716A, by Rinaldi-Carmona *et al.* (1994) provided a unique opportunity to study and demonstrate dependence on cannabinoids in experimental animals. Three papers have reported that SR141716A precipitates a withdrawal syndrome in rats chronically exposed to Δ^9-THC (Tsou *et al.*, 1995; Aceto *et al.*, 1995, 1996). Throughout the

ABBREVIATION: SR141716A, N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide·HCl; Δ^9-THC, Δ^9-tetrahydrocannabinol; CP-55, 940, [1α,2β-(R)-5α]-(−)-5-(1,1-dimethylheptyl)-2-[5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]-phenol.

1-h observation period the animals presented a disorganized pattern of constantly changing brief sequences of motor behavior, with no autonomic signs. The motor aspect of the syndrome apparently is unique to cannabinoid withdrawal, because it is not characteristic of the acute effects of any known drugs.

Starting with these data and considering the lack of behavioral and biochemical information on the time course of cannabinoid withdrawal syndrome, we conducted a series of parallel behavioral and biochemical studies designed to assess the involvement of the cannabinoid receptor and G protein system during withdrawal and to relate the cellular changes to the appearance of behaviors associated with the abstinence syndrome.

Methods

***In vivo* drug treatments.** Male Sprague-Dawley rats (initial weight, 150 g) were housed three per cage in standard conditions. Tolerance was induced by injecting CP-55,940 (generous gift from Dr. Casilli, Pfizer Italiana, Roma, Italy) twice a day (between 9:00 and 10:00 A.M. and 5:00 and 6:00 P.M.) for 6 days at a dose of 0.4 mg/kg i.p. dissolved in a vehicle consisting of Emulphor/ethanol/saline, 1:1:18. Control animals received the vehicle at the same times. On days 1, 4 and 6 the analgesic effect of CP-55,940 was evaluated by the tail-flick test to monitor the development of tolerance. The results were expressed as total area under the time-response curve (AUC).

On day 7, rats received only the morning injection and 1 h later were treated with the cannabinoid antagonist SR141716A (5 mg/kg i.p., generous gift from Sanofi Recherche, Montpellier, France, dissolved in Tween 80/dimethyl sulfoxide/distilled water, 1:2:7) or its vehicle. Therefore this schedule consists of four treatments: chronic CP-55,940 + SR141716A; chronic CP-55,940 + SR vehicle; chronic CP vehicle + SR141716A; and chronic CP vehicle + SR vehicle. To follow the time course of the withdrawal syndrome, the SR141716A dose was repeated in the same rats 24 and 96 h after the initial dose. The same treatment paradigm was followed for the behavioral and biochemical experiments.

Behavioral assessment of cannabinoid withdrawal. The severity and the time course of cannabinoid withdrawal were assessed for 1 h after each injection of SR141716A. The behavioral signs included wet dog shakes, forepaw fluttering, full turn left and right, chewing, teeth chatter, stretching, digging and scratching. These signs were counted and presented as number of events per hour, except for scratching which was recorded as time (seconds) spent scratching by the animals during the whole observation period.

***In situ* hybridization histochemistry.** One hour after each injection of SR141716A the rats were sacrificed by decapitation and their brains were rapidly removed and frozen in liquid nitrogen. Coronal sections (12 μm) were cut at -18°C and processed for *in situ* hybridization histochemistry as described previously (Parolaro *et al.*, 1993). Sections were hybridized with ^{35}S-labeled oligonucleotide probes (3×10^5 dpm per section), washed, air dried and exposed to X-ray film (βmax, Amersham, Milan, Italy) for 5 to 14 days. The intensity of the hybridization signal was assessed by measuring the grey levels of the autoradiographic films with an image analysis system consisting of a video camera (Hamamatsu, Tokyo, Japan) connected to an Apple Macintosh II personal computer. The public domain Image 1.47 software was used (National Institutes of Health, Bethesda, MD). Each cerebral area was traced with the mouse cursor control and the light transmittance was determined as the grey level. The grey level of densitometric measurements calculated after subtraction of the film background density was established within the linear range determined with radioactive ^{35}S standards prepared in the laboratory. The mean grey level of every cerebral area was related to its control in the same autoradiogram and expressed as a percentage of the control.

The probes (all supplied by Du Pont, Milan, Italy) consisted of a 39-mer oligonucleotide directed against bases 580–618 of the rat Gαs mRNA sequence; a 39-mer directed against bases 384–422 of the rat Gαo mRNA sequence; a 39-mer directed against bases 514–552 of the rat Gαi mRNA sequence; a mix of three oligonucleotides complementary to bases 4–51, 349–396 and 952–999 of rat cannabinoid receptor cDNA mixed in a 1:1:1 ratio. The specificity of the signal with these probes has been confirmed elsewhere (Parolaro *et al.*, 1993; Rubino *et al.*, 1994).

Statistical analysis. One-way analysis of variance was done by collapsing data across all groups. This was followed by Newman-Keuls *post hoc* test (Zar, 1974).

Results

Behavioral studies. The effect of repeated CP-55,940 injections (0.4 mg/kg i.p.) on analgesia is reported in figure 1. The first injection produced significant analgesia ($F = 230.01$; P < .0001) that diminished during treatment, and a significant degree of tolerance developed within 6 days, as demonstrated by the reduction of the area under the analgesic curve (75%). This dosing regimen also produced complete tolerance to hypomotility induced by CP-55,940 (data not shown).

On day 7 the cannabinoid antagonist SR141716A (5 mg/kg i.p.) or its vehicle was injected to the chronically treated rats and behavioral signs were evaluated for 1 h. As already reported, in vehicle-pretreated rats SR141716A induced abnormal behaviors significantly different from the vehicle group (CP vehicle + SR vehicle) (fig. 2). SR141716A alone significantly increased the number of wet dog shakes ($F = 8.472$; P < .0002) and forepaw fluttering ($F = 5.734$; P < .002) and the time spent in scratching behavior ($F = 32.625$; P < .00001), whereas other signs such as chewing, teeth chattering and digging were not affected. When the same SR141716A dose was repeated 24 and 96 h later in the same rats, all these signs were still present and significantly different from the vehicle group. The wet dog shakes and forepaw fluttering were significantly higher than vehicle, as after the first injection, whereas the time spent scratching was less than after the first injection but still significantly different from the vehicle. In view of these findings, the behavioral signs induced by SR141716A in chronically CP-55,940-
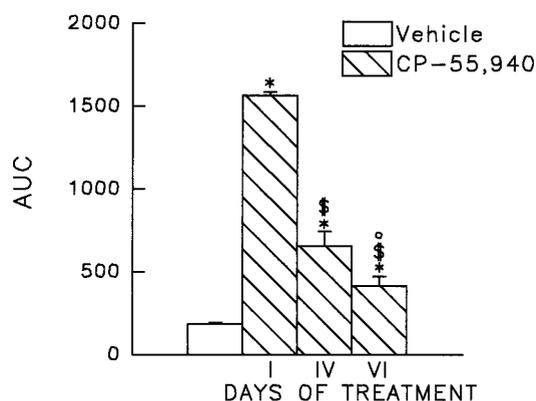
Fig. 1. Effect of CP-55,940 (0.4 mg/kg i.p. twice daily for 6.5 days) on analgesia expressed as area under the analgesic curve (AUC). Mean ± S.E. of at least 30 animals. *P < .001; *vs.* vehicle; $P < .001 *vs.* first day; °P > .001 *vs.* fourth day.
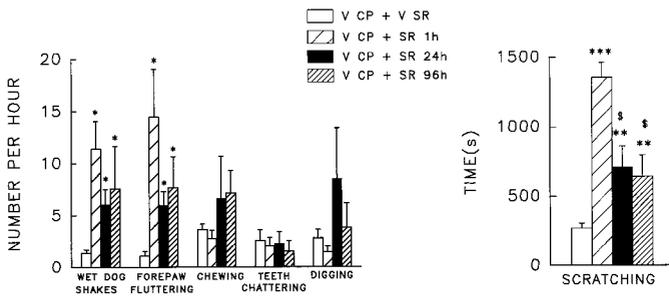
Fig. 2. Time course of the behavioral signs induced by repeated doses of SR141716A (5 mg/kg i.p.) in rats observed for 1 h. Mean ± S.E. of at least 10 animals. V, vehicle; SR, SR141716A; CP, CP-55,940. *P < .05; **P < .01; ***P < .001 *vs.* vehicle + vehicle; $P < .001 *vs.* first injection.

treated rats were compared with those in the group receiving the antagonist alone, referred to as the control group.

The first injection of SR141716A in animals chronically treated with CP-55,940 induced different behavioral signs besides the ones already observed with the antagonist alone. The rats showed turning to the left or right ($F = 6.63$; P < .014), chewing ($F = 6.47$; P < .015), digging ($F = 7.447$; P < .0103) and, curiously, a significant reduction in the time spent in scratching behavior ($F = 17.97$; P < .0003) compared with the control group (fig. 3). Therefore only these could be considered cannabinoid withdrawal signs because of their appearance solely in the abstinent group. When the antagonist was repeated 24 h after the first injection the only significant difference between the abstinent and SR141716A animals was the shorter time spent scratching ($F = 4.53$; P < .045) (fig. 4A); after the 96-h dose all the withdrawal signs had disappeared (fig. 4B).

In our study, therefore, a noticeable behavioral withdrawal syndrome was seen mostly after the first dose of the cannabinoid antagonist, and after the 96-h dose behavior was no different than after SR141716A alone, which thus indicates fast recovery.

Biochemical studies. At the end of each behavioral session the rats were sacrificed and *in situ* hybridization was done to check whether the levels of cannabinoid receptor and G protein mRNA, which are low in cannabinoid-tolerant rats (Rubino *et al.*, 1997), were affected during the abstinence syndrome.

First, we checked the biochemical effect of SR141716A alone because it was behaviorally active (table 1). After the first injection the abundance of αi mRNA was reduced significantly in the cerebral cortex, thalamus, hypothalamus
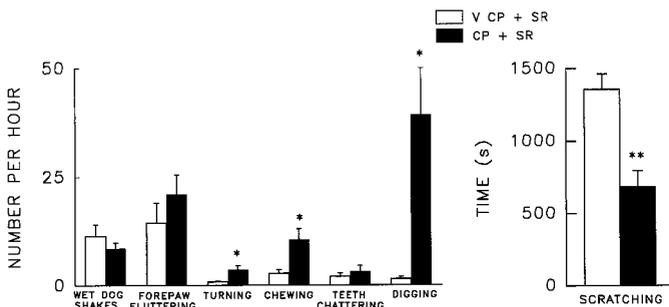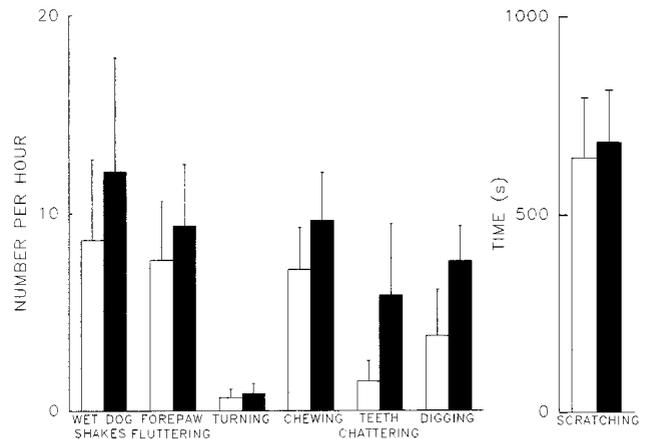

Fig. 3. Behavioral withdrawal signs in rats chronically treated with CP-55,940 and challenged with SR141716A (5 mg/kg i.p.) 1 h after the last CP-55,940 injection. The animals were observed for 1 h. Mean ± S.E. of at least 10 animals. V, vehicle; SR, SR141716A; CP, CP-55,940. *P < .01; **P < .001 *vs.* controls (vehicle CP + SR141716A).
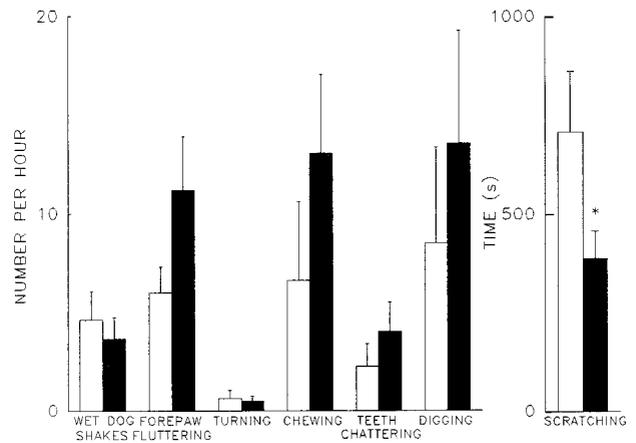
Fig. 4. Time course of the behavioral signs of withdrawal after repeated SR141716A injections in rats made tolerant to CP-55,940. (A) results after the second SR141716A injection; B, results after the third SR141716A injection. The animals were observed for 1 h after the SR141716A challenge. Mean ± S.E. of at least ten animals. V, vehicle; SR, SR141716A; CP, CP-55,940. *P < .05 *vs.* controls (vehicle CP + SR141716A).

and mesencephalon, whereas αs mRNA was increased only in the thalamic area. The slight alterations in αo and CB1 receptor mRNAs in the brain regions containing the basal message did not reach statistical significance.

When SR141716A was repeated at 24 and 96 h this pattern of alterations changed again. The second dose caused an increase in the αs message in most of the brain areas analyzed. In contrast, the αo and CB1 receptor messages were unmodified, and the decrease in mRNA coding for αi observed after the first dose had recovered completely. After the third SR141716A injection the αs message in the thalamus/hypothalamus remained significantly elevated and αi mRNA in the thalamus/hypothalamus showed some increase (20%), but did not reach statistical significance (table 1).

Figure 5 shows the autoradiograms of rat brain coronal sections and quantitative assessment of the cannabinoid receptor mRNA hybridization signal. In tolerant rats, as demonstrated previously, cannabinoid receptor mRNA decreased significantly in the caudate-putamen; the first SR141716A

TABLE 1

Biochemical effects of repeated SR141716A (SR) injections in vehicle and tolerant rats

"SR alone" means CP vehicle + SR; "abstinent" is the group of rats tolerant to CP-55,940 and treated with SR141716A. The values in tolerant rats are reported for comparison. The values are percentages of the vehicle + vehicle group.

| mRNA | Cerebral Areas | Tolerant | SR141716A Injection | | | | | |
| | | | First | | Second | | Third | |
			SR alone	abstinent	SR alone	abstinent	SR alone	abstinent
CB1	cortex	=[a]	=	=	=	=	=	=
receptor	caudate-putamen	30 ↓ *	=	=	=	=	=	=
αs	cortex	20 ↓ *	=	=	=	=	=	=
	thalamus	=	24 ↑ *	=	29 ↑ *	25 ↑ *	25 ↑ *	26 ↑ *
	hypothalamus	=		=	15.5 ↑ *	15 ↑ *	17.5 ↑ *	16.5 ↑ *
	mesencephalon	25 ↓ *	=	24 ↓ *	15 ↑ *	22 ↑ *	=	=
αo	cortex	=	=	=	=	=	=	=
	caudate-putamen	19 ↓ *	=	=	=	=	=	=
	olfactory area	22 ↓ *	=	=	=	=	=	=
αi	cortex	22 ↓ *	20 ↓ *	=	=	=	=	=
	thalamus/hypothalamus	38 ↓ **	25 ↓ *	=	=	=	20 ↑	21 ↑
	mesencephalon	43 ↓ **	40 ↓ **	30 ↓ *	=	=	=	=

[a] = alterations within 15% of vehicle; ↓ lower than vehicle; ↑ higher than vehicle; * P < .05; ** P < .01 *vs.* vehicle + vehicle.
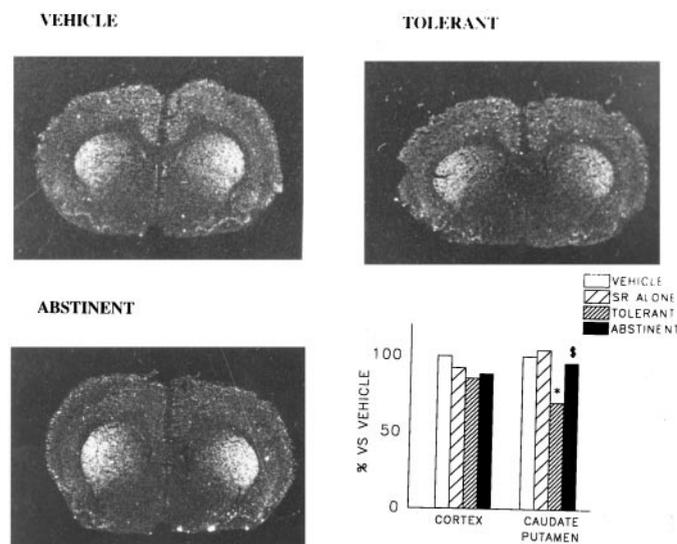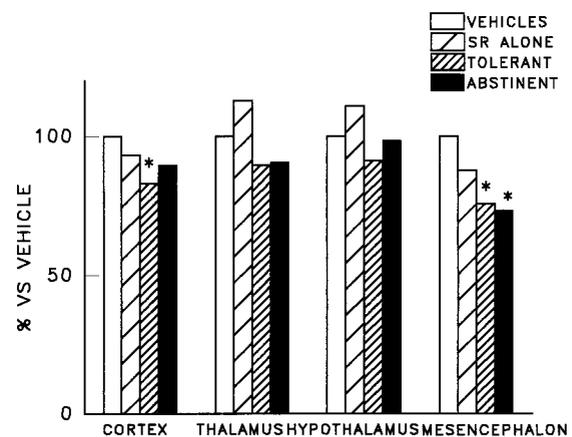

Fig. 5. Autoradiograms of representative coronal sections of the rostral portion of the rat brain showing the alteration in CB1 mRNA expression in the caudate-putamen of tolerant and abstinent rats. The bottom right-hand panel shows the quantitation of CB1 mRNA in the cerebral areas presented in the autoradiograms. Mean ± S.E. of at least six animals. SR, SR141716A; *P < .05 *vs.* vehicles; $P < .05 *vs.* tolerant.
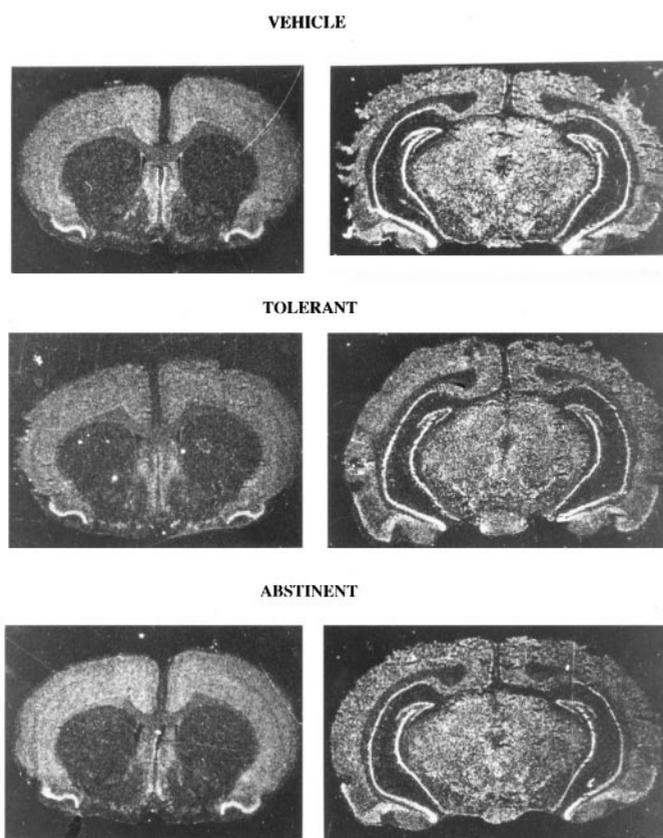

Fig. 6. Quantitation of the Gαs mRNA hybridization signal in cerebral areas containing the basal message of tolerant and abstinent rats. SR, SR141716A; *P < .05; **P < .01 *vs.* vehicle.

injection further modified the message in this region, and in the abstinent animals the cannabinoid receptor gene expression significantly increased (25% *vs.* tolerant rats) thus attaining the control level (fig. 5), without any further change after the second and third antagonist doses (table 1).

The effect of the first SR141716A injection on Gαs mRNA in cannabinoid-tolerant rats is reported in figure 6. All the brain areas containing Gαs mRNA showed a lower hybridization signal in tolerant rats, reaching statistical significance only in the cortex (20%) and mesencephalon (25%). The antagonist had a dual effect: in some areas such as the cortex there was a tendency to return to basal expression whereas in other areas, such as the mesencephalon, the hybridization signal remained lower. Representative autoradiograms are shown in figure 7.

When the antagonist was administered 24 and 96 h after the first injection the level of αs message was modified further (table 1). It was increased in the thalamus, hypothala-

mus and mesencephalon, although these changes were the same as in animals treated with SR141716A alone, which suggests they are related to the antagonist *per se* and are not linked to the cannabinoid withdrawal. This would be in line with the finding that withdrawal behavioral signs were seen only after the first dose of SR141716A.

The effect of SR141716A on Gαi mRNA in tolerant rats resembles the one for Gαs expression. Chronic CP-55,940, as demonstrated previously, reduced αi expression in all the brain areas containing the basal message; the first antagonist dose had a double effect, with a tendency to normal in the cortex and in the thalamic/hypothalamic area, the significant decrease persisting in the mesencephalon (fig. 8). SR141716A alone affected Gαi expression in the cortex, thalamus/hypothalamus and mesencephalon in the same way as in tolerant rats (fig. 8). After the second SR141716A dose in abstinent rats, αi mRNA returned to the basal level; however the third injection induced an increase in the thalamus/hypothalamus area, but because it was also seen in the SR141716A alone group, it presumably was caused by the drug *per se* (table 1).

Figure 9 shows the autoradiograms of rat brain coronal sections and quantitative assessment of the αo mRNA hybridization signal. In tolerant animals the level of mRNA

Fig. 7. Autoradiograms of representative coronal sections of the rostral portion of the brain and mesencephalic area showing the altered Gαs mRNA level in tolerant and abstinent rats.
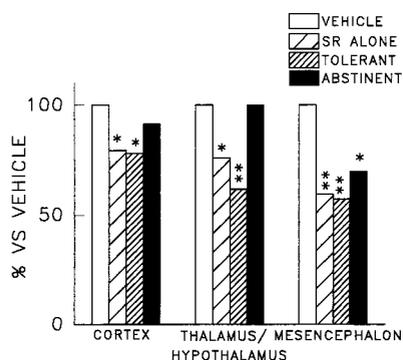

Fig. 8. Quantitation of the Gαi mRNA hybridization signal in cerebral areas containing the basal message of tolerant and abstinent rats. SR, SR141716A; *P < .05; **P < .01 *vs.* vehicle.

coding for αo decreased significantly in the forebrain and reached the control value after the first SR141716A dose. This pattern of expression was not modified by the second and third antagonist injections (table 1).

As summarized in table 1, after the first SR141716A injection in abstinent rats there was a tendency for CB1, Gαs, Gαi and Gαo to return to their basal levels of expression, thus cancelling out the decrease induced by tolerance.

Our data thus indicate that the cannabinoid abstinence syndrome is characterized by a general recovery of the basal level of expression of all the mRNA considered, which parallels the appearance of the behavioral signs. Nevertheless in some areas, such as the mesencephalon, the message for αs and αi was still reduced after the first hour and recovered
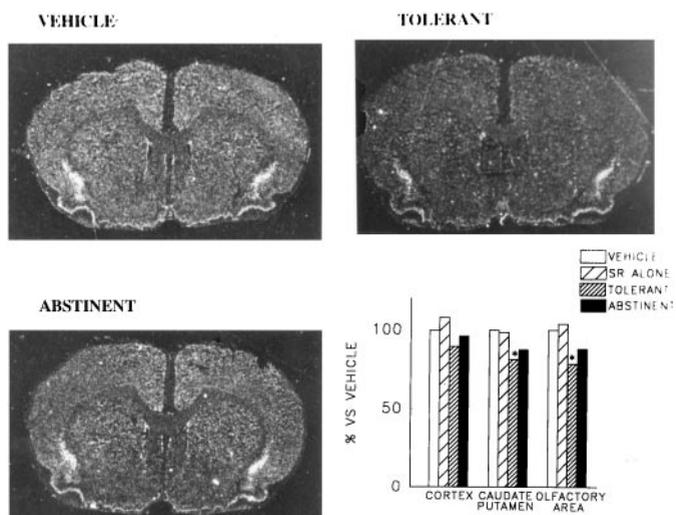
Fig. 9. Autoradiograms of representative coronal sections of the rostral portion of the rat brain showing the alteration in Gαo mRNA expression in the caudate-putamen and the olfactory area of tolerant and abstinent rats. The bottom right-hand panel shows the quantitation of Gαo mRNA in the cerebral areas presented in the autoradiograms. Mean ± S.E. of at least six animals. SR, SR141716A; *P < .05 *vs.* vehicles.

respectively after 96 and 24 hours. The second and third SR141716A doses in CP-55,940 pretreated rats further modified the biochemical parameters for αs expression. There was a tendency to increase in the thalamus, hypothalamus and mesencephalon which resembled that in SR141716A-treated rats, so it might well have been caused by the antagonist. The same can be proposed for αi expression in the thalamic/hypothalamic area after 96 h.

Discussion

The present study casts fresh light on the biochemical mechanisms underlying the cannabinoid withdrawal syndrome but also clearly shows that the cannabinoid antagonist SR141716A has behavioral and biochemical activities. An i.p. dose of 5 mg/kg, the challenge dose usually used in other works to precipitate the withdrawal syndrome (Tsou *et al.*, 1995; Aceto *et al.*, 1995), resulted in some behaviors (scratching, wet dog shakes and forepaw fluttering) significantly different from those in vehicle-treated rats. The signs induced by SR141716A seem to belong to a pattern of stimulatory motor behaviors and our data are therefore in line with other reports that the cannabinoid antagonist produces locomotor stimulation and hyperalgesic effects in mice (Compton *et al.*, 1996; Durnett Richardson *et al.*, 1997).

In agreement with other suggestions, two proposals can be put forward. First, SR141716A may have intrinsic activity on the cannabinoid receptor, producing pharmacological effects opposite to those of cannabimimetic agents. Thus the effects we describe may represent some novel direct actions of this drug. In addition, slight tolerance to SR141716A seems to appear after repeated injections; after the second and third doses the behavioral signs were still present but were less intense (*e.g.*, scratching after the third injection was about half that after the first). This further supports the concept of a pharmacological action of the antagonist *per se*.

However, it cannot be excluded that the effects of SR141716A are mediated by antagonism of the actions of an

endogenous cannabinoid system. Endogenous and exogenous cannabinoids exert an inhibitory role on the extrapyramidal system that modulates some of the behaviors observed (*e.g.*, scratching) (Navarro *et al.*, 1993). Thus we can assume that SR141716A reverses the inhibitory effects of the endogenous cannabinoid system, which results in the appearance of stimulatory motor behavior. As yet we have no basis for deciding between these two possibilities, but the data reported here, together with other findings, may help explore further the action of SR141716A and could lead to new therapeutic approaches in the field of neurodegenerative movement disorders.

Besides the behavioral parameters, SR141716A also caused some biochemical alterations. The main effect was a decrease of Gαi expression after the first injection, followed by an increase in the expression of the stimulatory G protein, αs, after the second and third injections. At the cellular level this pattern of alteration might lead to enhancement of adenylyl cyclase activity and/or a regulation of ion channels which, by modifying neuronal activity, could lead to altered behavior. The alterations in Gαs/Gαi expression were evident in brain regions with few cannabinoid receptors (thalamus, hypothalamus, mesencephalon), which suggests that SR141716A alone may prime a series of events not only at the cannabinoid receptor but also as a result of the CB1 receptor cross-talking with other neurotransmitter systems, ultimately modulating Gα expression within cerebral regions not strictly related to the presence of the cannabinoid receptor itself.

In the light of the behavioral results with SR141716A alone, other biochemical parameters, not examined in this work, need to be investigated to better clarify the molecular mechanism of this drug's effect. However the aim of the present study was to characterize the cannabinoid withdrawal syndrome in comparison with the biochemical situation in tolerance, and therefore our observations on the antagonist effects are only a preliminary approach.

These considerations apart, it is clear that the selective cannabinoid antagonist is capable of precipitating a withdrawal syndrome in CP-55,940-tolerant rats. Besides the behavioral signs with the antagonist alone, we detected other behaviors such as chewing, digging and turning, which only appeared in the abstinent group and after the first SR141716A dose. There seem to be some differences between the withdrawal behaviors observed by other groups and ours (Tsou *et al.*, 1995; Aceto *et al.*, 1995, 1996). The wet dog shakes, forepaw fluttering, facial rubbing and grooming/scratching observed by other authors were not significantly different in our study from those seen in rats treated with SR141716A alone, so they cannot be ascribed to the withdrawal syndrome. In addition, the scratching behavior was significantly less in withdrawn rats than in antagonist-treated animals. We can assume only that after chronic treatment some CP-55,940 remains on the cannabinoid receptor, thus inhibiting the scratching behavior induced by SR141716A.

The differences between the behavioral sequelae observed by other authors and by us, of course, might reflect the different treatment regimens for producing dependence, or the drug used (Δ^9-THC *vs.* CP-55,940). In any case cannabinoid physical dependence was less marked in our study than

with other drugs of abuse (opioids) and appeared to be resolved completely within 24 h.

Another important finding is that cannabinoid withdrawal modified cannabinoid receptor and G protein expression in the central nervous system. Both the CB1 receptor and G protein mRNA, whose levels drop during tolerance, changed further during withdrawal. The brain areas showing intracellular adaptations are the ones that are positive in tolerance (Rubino *et al.*, 1997). A general recovery of the basal level of expression, parallel to the appearance of the behavioral signs, was observed except in the mesencephalon where both αs and αi messages were still low after the first hour and only recovered after 96 and 24 h. The changes in αs and αi expression in the thalamus and hypothalamus after the second and third SR141716A doses in tolerant rats overlapped those in SR141716A-alone treated rats, which suggests they are caused by the antagonist *per se*. This pattern of biochemical change strikingly parallels the time course of the behavioral signs at 24 and 96 h that appear to be caused by the antagonist *per se*, as already mentioned.

The precise cellular mechanism of the development of tolerance and dependence to cannabinoids is still not clear. One of the most widely accepted hypotheses in tolerant animals is that the CB1 receptor and expression are down-regulated and there is a desensitization of the cannabinoid-activated signal transduction mechanism, although there are reports of opposite results (Adams and Martin, 1996). Therefore we can suggest that the receptor desensitization may trigger an alteration in the cellular machinery, resulting in an altered balance of the most common signal transduction mechanisms linked to the CB1 receptor (cAMP, calcium levels, phosphokinase A activity, mitogen-activated protein kinase activity) that finally affects G protein expression. In withdrawn rats SR141716A, reversing the inhibitory influence of CP-55,940, might lead to a recovery of the altered cellular transduction machinery, with retrieval of the basal gene expression parallel to the appearance of the behavioral signs.

To conclude, our data confirm that the cannabinoid withdrawal syndrome is precipitated by the cannabinoid antagonist SR141716A in rats tolerant to CP-55,940 and show that the general desensitization of the cannabinoid receptor and of the transduction system in tolerance are overcome in abstinent rats and might be part of the molecular mechanisms underlying cannabinoid dependence. However, new, well-designed approaches are needed, using both *in vitro* (cell culture, transfected cell lines) and *in vivo* models to map the intracellular pathways (in terms of second messengers, protein kinases, transcription factors and target genes) activated by chronic cannabinoids during the development of tolerance and dependence

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