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The cannabinoid CB_1 receptor antagonist SR141716A increases norepinephrine outflow in the rat anterior hypothalamus

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Abstract

The effects of the selective cannabinoid CB_1 receptor antagonist *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide-hydrochloride (SR141716A) on extracellular concentrations of norepinephrine and 5-hydroxytryptamine (5-HT) were assessed by in vivo microdialysis in the anterior hypothalamus of freely moving rats. SR14716A (0.3, 1, 3 mg/kg, i.p.) dose-dependently increased norepinephrine efflux to about 300% of baseline, without affecting 5-HT levels. This increase in norepinephrine outflow could play an important role in the pharmacological and potentially therapeutic actions of SR141716A. © 2001 Published by Elsevier Science B.V.

Keywords: Microdialysis; Norepinephrine; SR141716A

Cannabinoid research has greatly benefited from the synthesis of *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide-hydrochloride (SR141716A), a selective inhibitor of the central cannabinoid (CB_1) receptors (Rinaldi-Carmona et al., 1994). SR141716A has mostly been used to delineate the neuroanatomical and molecular substrates mediating the acute and chronic actions of exogenous cannabinoids. Thus, SR141716A potently blocks behavioral and biochemical effects induced by cannabimimetics (see, e.g. Chaperon and Thiebot, 1999) and precipitates a withdrawal syndrome in animals chronically treated with cannabinoid agonists (Tzavara et al., 2000).

More importantly, SR141716A, when given alone, induces conditioned place preference, reduces food intake and enhances arousal and memory (reviewed in Chaperon and Thiebot, 1999). Although SR141716A has been reported to display inverse agonist properties in vitro and in ex vivo preparations, such effects are far from being clearly defined for in vivo systems. The aforementioned pharmacological effects of SR141716A are most likely due to disruption of an inhibitory tone exerted by endogenous cannabinoids.

Nevertheless, the biochemical events underlying the behaviors elicited by SR141716A have not been elucidated. At doses similar to those being effective in behavioral tests, it has been shown that SR141716A increases acetylcholine efflux in hippocampal microdialysates (Gessa et al., 1998) and Fos expression in mesocorticolimbic areas (Alonso et al., 1999). It has also been shown that SR141716A increases glutamate mediated (Kathmann et al., 1999) but not electrically evoked (Gifford et al., 1997) norepinephrine release from hippocampal slices. These scarce reports suggest an excitatory action of SR141716A on regional neurotransmitter release and neuronal activity. Along these lines, by using in vivo microdialysis, we studied the effects of SR141716A on extracellular concentrations of norepinephrine, 5-hydroxytryptamine (5-HT), and monoamine metabolites in the anterior hypothalamus, a limbic structure with a possible role in the behaviors affected by this drug.

In vivo microdialysis experiments were performed in compliance to the European Community guidelines for the use of experimental animals (approved by the Animal Care and Use Committee of Eli Lilly), and in accordance to the methods described by Perry and Fuller (1997) with minor modifications. Specifically, male Wistar rats (250–300 g) were implanted with commercially available, concentric

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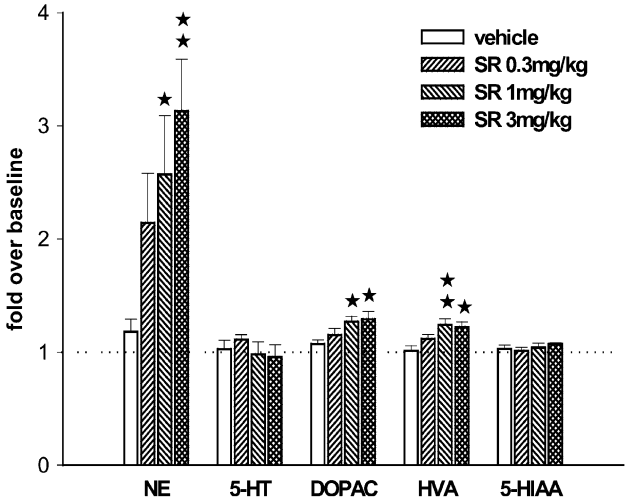

Fig. 1. Effects of SR141716A (0.3, 1, 3 mg/kg, i.p.; $n = 4$ in each group) or vehicle ($n = 4$) on extracellular concentrations of norepinephrine, 5-hydroxytryptamine (5-HT), and the monoaminergic metabolites DOPAC, HVA and 5-HIAA from the anterior hypothalamus of the rat. Data are expressed as average multifold changes (mean ± S.E.M.) from baseline over the 4-h post-injection period. Basal values (mean ± S.E.M., $n = 16$) of norepinephrine, 5-HT, DOPAC, HVA and 5-HIAA are 0.23 ± 0.05, 0.23 ± 0.04, 22.8 ± 2.4, 11.5 ± 1.9 and 334 ± 18 pmol/ml, respectively. Data are statistically evaluated with one-way (treatment) analysis of variance and Duncan's test for multiple comparisons. $^{*}: p < 0.05$, $^{**}: p < 0.01$ vs. vehicle.

microdialysis probes (BAS, BR-2) in the anterior hypothalamus. Twenty-four-hour postimplantation, a modified Ringer's solution was perfused at a rate of 1 μl/min and samples were collected every 30 min. Five hours after the beginning of the experiment, vehicle or SR141716A (0.3, 1, 3 mg/kg, dissolved in a solution that consisted of 2% dimethylsulfoxide, 2% cremophor EL in 0.9% NaCl) was injected intraperitoneally at a volume of 3 ml/kg. Microdialysate samples were collected for an additional period of 4 h. All samples were analyzed with high-pressure liquid chromatography coupled to electrochemical detection on the same day of the experiments. Data are expressed as percent change from baseline, which is the average of the three basal values before injection of vehicle or SR14716A. The data in Fig. 1 are depicted as multifold average percent changes from baseline for the duration of the experiment post-injection (overall effects).

SR141716A induced a dose-dependent augmentation in norepinephrine efflux ($F(3,12) = 4.2$, $P < 0.05$) that was detectable even at the lowest dose (0.3 mg/kg) used. With the highest dose (3 mg/kg), the increase was very pronounced reaching 300% in half an hour after the injection and persisting throughout the experiment. On the contrary, no change was seen in 5-HT. In accordance, the levels of the 5-HT metabolite 5-hydroxyindoloacetic acid (5-HIAA) were not altered. We did observe a dose-dependent increase of about 30% in the levels of the metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) ($F(3,12) = 3.4$, $P = 0.05$) and homovanillic acid (HVA) ($F(3,12) = 4.8$, $P < 0.05$). Whether this increase in DOPAC and HVA reflects changes in dopamine or in norepinephrine metabolism is not certain, since we were not able to measure dopamine in the anterior hypothalamus due to an interfering peak. It is noteworthy that SR141716A at the dose of 3 mg/kg induced a similar increase in norepinephrine efflux (250%) in the hippocampus ($n = 3$, data not shown).

These data provide direct in vivo evidence that the CB_1 receptor antagonist SR141716A increases extracellular concentrations of norepinephrine in distinct regions of the brain, an effect that may be relevant for its reported behavioral actions. Norepinephrine neurotransmission in these limbic brain regions appears to be under a tonic inhibitory control through CB_1 receptors. Consequently, CB_1 receptor antagonists may facilitate norepinephrine neurotransmission via a disinhibitory action. Considering the role of norepinephrine in endocrine, affective and cognitive functions, it is tempting to speculate that the central norepinephrine system might be an important mediator of the pharmacological actions of SR141716A.

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