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BLOCKADE OF CANNABINOID RECEPTORS BY SR141716 SELECTIVELY INCREASES FOS EXPRESSION IN RAT MESOCORTICOLIMBIC AREAS VIA REDUCED DOPAMINE D_2 FUNCTION

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Abstract—The present study investigated, in rats, whether blockade of cannabinoid CB_1 receptors may alter Fos protein expression in a manner comparable to that observed with antipsychotic drugs. Intraperitoneal administration of the selective CB_1 receptor antagonist, SR141716, dose-dependently (1.0, 3.0 and 10 mg/kg) increased Fos-like immunoreactivity in mesocorticolimbic areas (prefrontal cortex, ventrolateral septum, shell of the nucleus accumbens and dorsomedial caudate–putamen), while motor-related structures such as the core of the nucleus accumbens and the dorsolateral caudate–putamen were unaffected. In the ventrolateral septum, taken as a representative structure, the Fos-inducing effect of SR141716 (10 mg/kg) was maximal 2 h after injection and returned to near control levels by 4 h. Within the prefrontal cortex, SR141716 increased the number of Fos-positive cells predominantly in the infralimbic and prelimbic cortices, presumptive pyramidal cells being the major cell types in which Fos was induced.

The D_1-like receptor antagonist, SCH23390 (0.1 mg/kg), did not prevent the Fos-inducing effect of SR141716 in any brain region examined (prefrontal cortex, nucleus accumbens, ventrolateral septum and dorsomedial caudate–putamen), although SCH23390 significantly reduced Fos expression induced by cocaine (20 mg/kg) in all these regions. By contrast, the dopamine D_2-like agonist, quinpirole (0.25 mg/kg), counteracted SR141716-induced Fos-like immunoreactivity in the ventrolateral septum, the nucleus accumbens and the dorsomedial caudate–putamen, while no antagonism was observed in the prefrontal cortex. Microdialysis experiments in awake rats indicated that SR141716, at doses which increased Fos expression (3 and 10 mg/kg), did not alter dopamine release in the shell of the nucleus accumbens. Finally, SR141716 increased the levels of neurotensin-like immunoreactivity in the nucleus accumbens, but not in the caudate–putamen. Collectively, the present results show that blockade of cannabinoid receptors increases Fos- and neurotensin-like immunoreactivity with characteristics comparable to those reported for atypical neuroleptic drugs. © 1999 IBRO. Published by Elsevier Science Ltd.

Key words: SR141716, c-fos, quinpirole, SCH23390, neurotensin.

Marijuana (*Cannabis sativa*), the most commonly abused substance, and its major psychoactive constituent, Δ-9-tetrahydrocannabinol, produce characteristic psychotropic responses in humans. They also alter specific behaviors in animals, including nociception, thermal regulation and motor activity (see Ref. 28 for review). The recent identification and cloning of two G-protein-coupled cannabinoid receptors (CB_1 and CB_2),[36,44] the anatomical distribution of CB_1 receptors in the CNS,[26,35,67] and the discovery of putative endogenous cannabinoid ligands, anandamide and sn-2-arachidonylglycerol,[12,37,63] have led to the emergence of a new neurochemical system.

Although much is known about the central effects of exogenously applied cannabinoids, the function of endogenous cannabinoid systems remains largely to be elucidated. In this context, the discovery of the highly potent CB_1 receptor antagonist, SR141716, has opened new possibilities for the identification and characterization of cannabinoid-dependent neuronal regulations. SR141716 displays a high selectivity for CB_1 vs CB_2 receptors, and appears to antagonize most of the behavioral, electrophysiological and biochemical effects of cannabinomimetics.[8,49,50] Moreover, when administered alone, SR141716 altered several physiological or behavioral parameters, such as arousal, memory consolidation, sucrose intake, incentive learning and nociception.[1,7,46,48,57,65] This indicates that endogenous cannabinoid systems may be tonically active under certain conditions. Whether these endogenous systems are implicated in neuronal dysregulation leading to pathological conditions awaits further investigation.

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Abbreviations: EDTA, ethylenediaminetetra-acetate; MAP-2, microtubule-associated protein-2; PBS, phosphate-buffered saline.

Interestingly, Emrich *et al.*[14] have proposed a cannabinoid hypothesis for schizophrenia according to which some schizophrenic symptoms such as perceptual disturbance may be related to a dysregulation of endogenous cannabinoid systems. This proposal is supported by several clinical reports, which indicate the occurrence of psychodysleptic effects, including distorted sensory perceptions, impaired judgement and dissociation of ideas, following administration of cannabis.[6,24] These reports, and the observation that cannabinoid agonists activate dopaminergic systems in animals,[15,16,19,64] prompted us to examine whether the blockade of endogenous cannabinoid systems by SR141716 might alter neuronal functioning in a manner comparable to that observed with antipsychotic drugs.

Typical and atypical neuroleptic drugs present the remarkable ability to increase the expression of immediate early genes such as c-fos in various brain regions, including the dorsolateral and dorsomedial caudate–putamen, the nucleus accumbens, the ventrolateral septum and the prefrontal cortex.[10,11,23,34,40,51,53,58,59,68] According to pharmacological and retrograde labeling experiments within the nucleus accumbens and the caudate–putamen, taken as reference structures, neuroleptic drugs increased Fos expression in dopamine D_2-like* receptor-bearing neurons through the removal of a tonic inhibition exerted by dopamine via D_2-like receptors.[23,40,52,54] Accordingly, the Fos-inducing effects of neuroleptic drugs are unaffected by D_1-like receptor blockade, but highly sensitive to D_2-like receptor stimulation.[10,23,39,40,42,54]

The first objective of the present study was thus to examine, in rats, whether SR141716 stimulates c-fos expression, assessed by Fos immunohistochemistry, with a regional pattern of distribution similar to that observed for neuroleptic drugs. Secondly, a pharmacological approach, based on the blockade of dopamine D_1-like vs stimulation of D_2-like receptors, was used to determine whether SR141716 could act, like neuroleptic drugs, through the selective disinhibition of neurons expressing D_2-like receptors. Thirdly, since cannabinoid agonists were shown to increase dopamine released in mesolimbic structures,[64] the hypothesis that SR141716 could disinhibit D_2-like receptor-bearing neurons through a reduction of dopamine release was addressed using dopamine microdialysis experiments in awake rats. Finally, the blockade of D_2-like receptors was found to increase the levels of neurotensin mRNA and neurotensin-like immunoreactivity in the nucleus accumbens and the caudate–putamen.[29,33,38,39,60] Therefore, we also examined whether SR141716 could increase the level of neurotensin-like immunoreactivity in these brain regions.

*According to dopamine receptor nomenclature,[61] the terms D_1-like and D_2-like receptors employed in this paper refer to the D_1 (D_1 and D_5) and D_2 (D_2, D_3 and D_4) receptor subfamilies, respectively.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley rats (250–300 g; Charles River, France) were used in all the experiments. Animals were housed two per cage in a temperature-controlled environment (21 ± 1°C) with a 12-h light/12-h dark cycle, and were given free access to standard laboratory chow and water. All experimental procedures were approved by the "Comité d'Expérimentation Animale" (Animal Care and Use Committee) of Sanofi Recherche and were carried out in accordance with French legislation (décret no. 87-848, 19 octobre 1987; and arrêtés, 19 avril 1988), which implemented the European directive 86/609/EEC.

Drugs and treatments

SR141716 was synthesized at Sanofi Recherche (Montpellier, France). SCH23390, quinpirole and haloperidol were purchased from RBI (Natick, MA, U.S.A.). Cocaine was from the "Coopérative Pharmaceutique Française" (Melun, France, lot no. D2804113). SR141716 and haloperidol were suspended in Tween 80 (0.01%) in distilled water, while SCH23390, quinpirole and cocaine were solubilized with saline. Rats received injections of SR141716 (1.0, 3.0 or 10 mg/kg, i.p.) or vehicle, and were killed 2 h later for the immunohistochemical detection of Fos, as described below. In another series of experiments examining the time dependence of Fos expression, rats were injected with SR141716 (10 mg/kg, i.p.) and were killed immediately or 1, 2 or 4 h after the injection. To examine the effects of dopamine D_1-like receptor blockade or D_2-like receptor stimulation on SR141716-induced Fos-like immunoreactivity, SCH23390 (0.1 mg/kg, i.p.) and quinpirole (0.25 mg/kg, i.p.) were injected 30 and 15 min, respectively, before the administration of SR141716 (10 mg/kg). For comparison, we also examined the effects of SCH23390 (0.1 mg/kg) on Fos expression stimulated by cocaine (20 mg/kg). Rats were killed 2 h after SR141716 or cocaine administration for Fos detection.

When the effects of SR141716 on neurotensin-like immunoreactivity were investigated, rats received i.p. injections of SR 141716 (1.25, 2.5 and 5 mg/kg), haloperidol (1 mg/kg) or vehicle, and were decapitated 18 h after the drug or vehicle injection. This postinjection time is necessary for the optimal detection of enhanced levels of neurotensin-like immunoreactivity.[33] Consequently, as SR141716 was reported to decrease food consumption in the rat,[1,7] the doses of SR141716 were lowered slightly compared with those used for Fos detection in order to minimize the possible effects of a prolonged reduction of food consumption on the regulation of neurotensinergic systems. Brains were cut into 1-mm-thick coronal slices, 2 mm rostral to the optic chiasma, using a coronal steel matrix. The nucleus accumbens and caudate–putamen were isolated by free-hand dissection on a cold plate according to Horn *et al.*[27] Brain structures were rapidly frozen on dry ice and stored at −80°C until extraction.

Immunohistochemistry

Rats were anesthetized with sodium pentobarbital (80 mg/kg, i.p.) and perfused transcardially with saline, followed by 4% (w/v) paraformaldehyde in 0.1 M phosphate-buffered saline (PBS; pH 7.4). Brains were removed and allowed to postfix overnight in 4% paraformaldehyde. Fifty-micrometer coronal sections were cut from each brain using a Vibratome. Immunohistochemistry was performed on free-floating tissue sections according to a standard

avidin–biotin–peroxidase procedure using an anti-Fos sheep polyclonal antibody (Genosys Biotechnologies, Cambridge, U.K.; CRB OA-11-824) directed against residues 2–16 of the N-terminal region of the Fos protein. Sections were rinsed with 0.02 M PBS and then pretreated for 10 min at room temperature with the same buffer containing 0.3% hydrogen peroxide. Next, they were rinsed three times in PBS, incubated with PBS containing 5% normal rabbit serum (Vector Laboratories, Burlingame, CA, U.S.A.) for 1 h at room temperature and placed for 72 h at 4°C in the Fos primary antiserum (diluted 1:8000) solution, in PBS containing 1% normal rabbit serum and 0.3% Triton X-100. Thereafter, the sections were incubated successively with a biotinylated rabbit anti-sheep secondary antibody (Vector Laboratories; diluted 1:300) for 2 h at 37°C and with avidin–biotinylated horseradish peroxidase complex (Vector Laboratories; diluted 1:200) for 3 h at room temperature, in PBS containing 0.1% Triton X-100. The reaction product was visualized with diaminobenzidine in the presence of nickel. Sections were mounted, air dried, dehydrated and covered with acrytol. Omission of the primary antibody from the immunohistochemical procedure or preadsorption of this antibody with a synthetic peptide corresponding to the N-terminal antigenic sequence of the Fos protein (Genosys; OP-11-3210) eliminated Fos-like immunoreactivity.

Immunohistochemistry with a double fluorescent labeling procedure was used in studies aimed at determining the cell types of the prefrontal cortex in which Fos was induced by SR141716. Free-floating prefrontal cortex sections (30 μm) were first processed for Fos detection using a fluorescein-labeled tyramide signal amplification (TSA kit, NEN). Sections were incubated with the anti-Fos antibody (1:3000; 72 h at 4°C), washed in buffer (0.1 M Tris–HCl, 0.15 M NaCl and 0.05% Tween 20), and treated successively with the biotinylated rabbit anti-sheep secondary antibody (1:300; 2 h at 37°C), streptavidin-conjugated horseradish peroxidase (1:100; 30 min at room temperature), buffer (0.1 M Tris–HCl, 0.15 M NaCl and 0.05% Tween 20; 3×5 min) and fluorescein–tyramide (1:50; 5 min at room temperature). Subsequently, sections were processed using mouse monoclonal antibodies directed against microtubule-associated protein-2 (MAP-2; Sigma Chemical Co.; 1:500) or parvalbumin (Sigma Chemical Co.; 1:500), followed by incubation with a Texas Red-conjugated goat anti-mouse antibody (Jackson Immunoresearch, Westgrove, PA, U.S.A.; 1:1000). MAP-2 and parvalbumin labelings in the prefrontal cortex were used previously to identify pyramidal neurons and a subpopulation of GABAergic interneurons, respectively.[10,25] Sections were examined under a Leica confocal laser microscope (TCS 4D) configured with a Leica inverted microscope (DMIRBE) equipped with an argon/krypton laser. Fluorescein and Texas Red were excited through a ×40 oil immersion lens using 488/568 nm wavelengths and the emissions were collected using 520 nm (for fluorescein) or 590 nm (for Texas Red) long-pass filters.

Neurotensin radioimmunoassay

Brain tissues were extracted by sonic dismembranation in ice-cold 1 N HCl[33] in Low-Binding microcentrifuge tubes. After centrifugation, duplicate aliquots of supernatant were lyophilized and reconstituted in assay buffer, and neurotensin-like immunoreactivity contents were measured as described previously[30] using a radioimmunoassay kit (Peninsula). Sediments from dismembranation were dissolved in 3% NaOH and assayed for protein concentration using the Biorad micromethod. Neurotensin-like immunoreactivity contents are expressed as picograms of neurotensin-like immunoreactivity per milligram of protein.

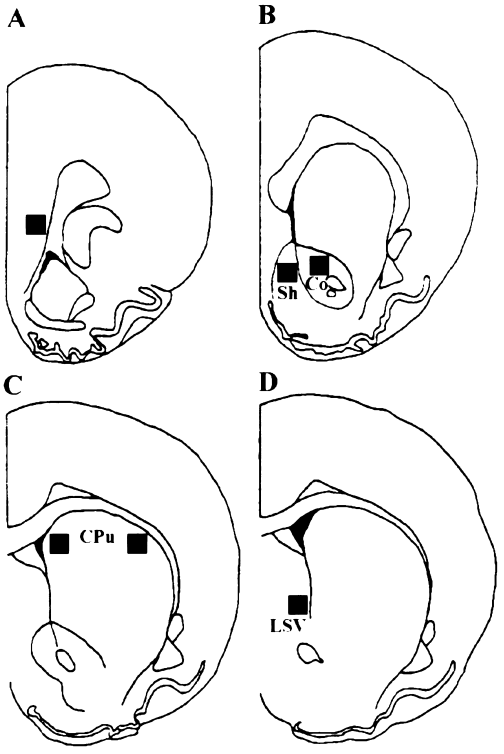

Fig. 1. Drawing of representative sections used for counting Fos-positive neurons in the prefrontal cortex (A), the shell (Sh) and core (Co) compartments of the nucleus accumbens (B), the dorsomedial and dorsolateral caudate–putamen (CPu; C) and the ventrolateral septum (LSV; D).

Dopamine microdialysis experiments

Brain microdialysis was performed in freely moving rats, as described previously.[62] In brief, two days prior to dialysis measurements, rats were anesthetized with equithesin (4% chloral hydrate, 6% pentobarbital, 4 ml/kg body weight) and a guide cannula was implanted stereotaxically 2 mm above the shell of the nucleus accumbens. Coordinates (in mm) were A +1.45, L 1 and V 5.5, according to the atlas of Paxinos and Watson.[45] On the day of the experiment, animals were placed in a Plexiglas cage, the obturator of the cannula was removed and the microdialysis probe (CMA 12, Carnegie Medicine AB, Stockholm, Sweden; length 2 mm) was placed in the shell of the nucleus accumbens. The probe was perfused at a constant flow rate of 2 μl/min with a gassed Ringer's solution (pH 7.4). After discarding the first 150 min perfusate, 30-μl samples were collected at 15-min intervals for 3 h in Eppendorf microtubes containing 5 μl 0.1 N $HClO_4$, 1 mM EDTA and 4 mM sodium metabisulfite. Four baseline samples were collected before administration of SR141716 (3 and 10 mg/kg, i.p.). The samples were immediately frozen and stored in a deep freeze (−80°C) no more than four days before assays. Dopamine levels were assayed in 10-μl samples by high-pressure liquid chromatography with electrochemical detection, as described in detail previously.[56]

Data analysis

Sections processed for chromogenic detection of Fos-like immunoreactivity were imaged through a Leica DMIRBE microscope, and the Fos-like immunoreactive signal was quantified with an image analysis system (Samba, Unilog, France) by counting the number of Fos-positive cells within

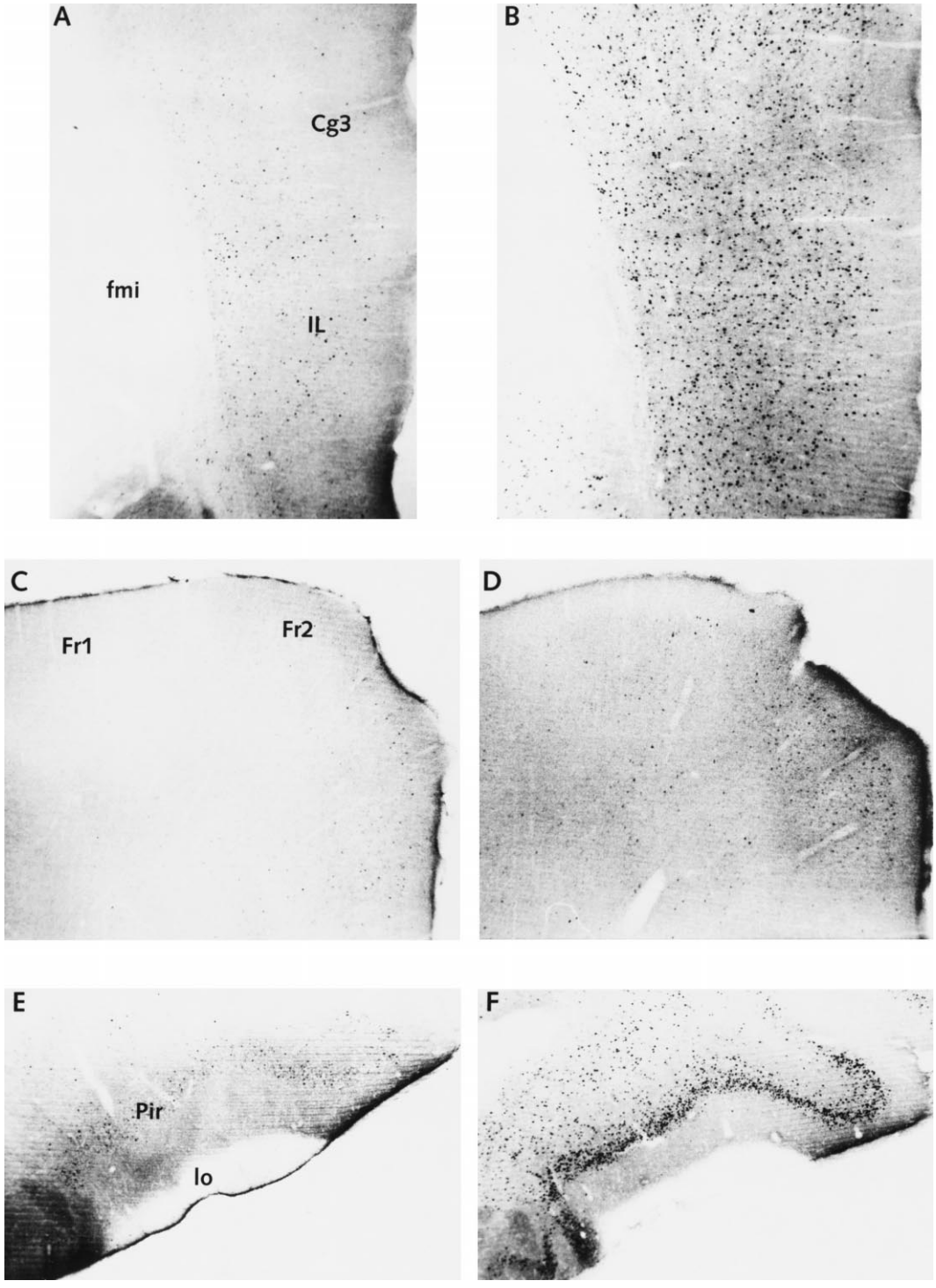

Fig. 2. Photomicrographs of brain sections showing Fos-like immunoreactive cells in the prefrontal (A, B), frontoparietal (C, D) and piriform cortices (E, F) of rats administered with vehicle (A, C, E) or SR141716 (10 mg/kg, i.p.; B, D, F). Cg3, cingulate cortex, area 3; fmi, forceps minor of the corpus callosum; Fr1, 2, frontal cortex, areas 1 and 2; IL, infralimbic cortex; lo, lateral olfactory tract; Pir, piriform cortex.

square areas, depicted in Fig. 1. Only visual objects that met the appropriate size and optical density criteria (cells showing grey levels between 0 and 160/170; total range: 0–255) were automatically counted as Fos-immunoreactive cells.

Data from neurotensin radioimmunoassay (presented as the mean ± S.E.M. of normalized values) and Fos immunohistochemistry (number of Fos-immunoreactive cells) were submitted to one-way ANOVA followed by Dunnett's *t*-test, with an accepted level of significance of $P < 0.05$. In microdialysis experiments, the dopamine levels in serial perfusates were converted to percentage of the mean value of the four baseline measurements.

RESULTS

*Distribution of SR*141716*-induced Fos-positive neurons in the brain*

SR141716 (10 mg/kg, i.p.) induced the accumulation of Fos-like immunoreactivity in several structures, including the prefrontal, cingulate (at the rostral level) and piriform cortices (Fig. 2), the ventral part of the lateral septum (especially at its more caudal level), the shell of the nucleus accumbens (dorsal part), the dorsomedial caudate–putamen (at rostral and caudal levels) (Fig. 3), the central amygdaloid nucleus and the lateral subdivision of the bed nucleus of the stria terminalis (Fig. 4). Enhanced Fos-like immunoreactivity was also found within the anterior olfactory nucleus, the claustrum, the thalamic paraventricular nucleus and the medial nucleus of the habenula (not shown). SR141716 failed to induce Fos-like immunoreactivity in other brain regions, such as the motor and sensorimotor areas of the frontoparietal cortex, the core compartment of the nucleus accumbens and the dorsolateral caudate–putamen (Figs 2, 3). There was no consistent increase in the Fos-like immunoreactive signal in brain regions such as the islands of Calleja, the globus pallidus, the ventral pallidum, the hippocampus, the paraventricular, dorsomedial and dorsolateral hypothalamic nuclei, the substantia nigra, the ventral tegmental area and the locus coeruleus (not shown). The vehicle-treated rats exhibited few Fos-positive neurons in these regions (Figs 2–4).

Subregional distribution and cellular characterization of Fos-like immunoreactive cells in the prefrontal cortex

Following SR141716 (10 mg/kg) administration, the Fos-like immunoreactive signal was distributed predominantly in the median and deep layers of the infralimbic and prelimbic areas of the prefrontal cortex (Fig. 2). As illustrated in Fig. 5, the increase in the Fos-like immunoreactive signal occurred primarily in MAP-2-positive cells, presumably pyramidal neurons, while cellular co-localization with parvalbumin was rarely observed.

*Dose dependence of SR*141716*-induced Fos-like immunoreactivity*

SR141716 (1.0, 3.0 and 10 mg/kg) produced a dose-dependent increase in the number of Fos-like immunoreactive neurons in the prefrontal cortex, the nucleus accumbens shell, the ventrolateral septum and the dorsomedial caudate–putamen, a statistically significant level being achieved with the doses of 3.0 and 10 mg/kg (Fig. 6). By contrast, none of these doses produced a significant increase in the Fos-like immunoreactive signal in the nucleus accumbens core and the dorsolateral caudate–putamen (Fig. 6).

*Time dependence of SR*141716*-induced Fos-like immunoreactivity*

In the ventrolateral septum, taken as a representative structure, the induction of Fos-like immunoreactivity reached a significant level 1 h after SR141716 (10 mg/kg) injection, was maximal at 2 h and returned to near control levels by 4 h (Table 1). In contrast, the immunoreactive signal remained at very low levels in the dorsolateral caudate–putamen at all postinjection time intervals examined (Table 1).

*Pharmacological characterization of the effects of SR*141716 *on Fos-like immunoreactivity*

SCH23390, which had no significant effect on the Fos-immunoreactive signal when administered alone (0.1 mg/kg), reduced by 50–65% the number of Fos-like immunoreactive cells induced by cocaine (20 mg/kg) in the prefrontal cortex, the ventrolateral septum, the nucleus accumbens shell and the dorsomedial caudate–putamen (Fig. 7). In contrast, this D_1-like receptor antagonist failed to modify the increased Fos-like immunoreactivity provoked by SR141716 in these regions (Fig. 7). Although not assessed quantitatively, the SCH23390 pretreatment also failed to affect the SR141716 response in the piriform cortex and the central amygdala (not shown). Conversely, the D_2-like receptor agonist, quinpirole (0.25 mg/kg), significantly decreased the SR141716-inducing effects in the ventrolateral septum (−62%), the nucleus accumbens shell (−58%) and the dorsomedial caudate–putamen (−57%) (Fig. 8). This antagonistic effect of quinpirole was not observed in the prefrontal cortex (Fig. 8).

*Effects of SR*141716 *on neurotensin-like immunoreactivity*

Significant, dose-dependent increases in neurotensin-like immunoreactivity were induced in the nucleus accumbens, 18 h after the administration of 2.5 and 5 mg/kg SR141716A (154% and 174% of control values, respectively), whereas no increase was observed in the caudate–putamen at any dose of SR141716 (Table 2). For comparison, the administration of haloperidol (1 mg/kg) increased the amount of neurotensin-like immunoreactivity in both the nucleus accumbens and the caudate–putamen (230% and 148% of control values, respectively; Table 2).

*SR*141716 *did not affect dopamine release in the nucleus accumbens*

At none of the 12 sampling times examined over the 3-h collection period did the extracellular dopamine levels change in the shell of the nucleus accumbens following administration of SR141716

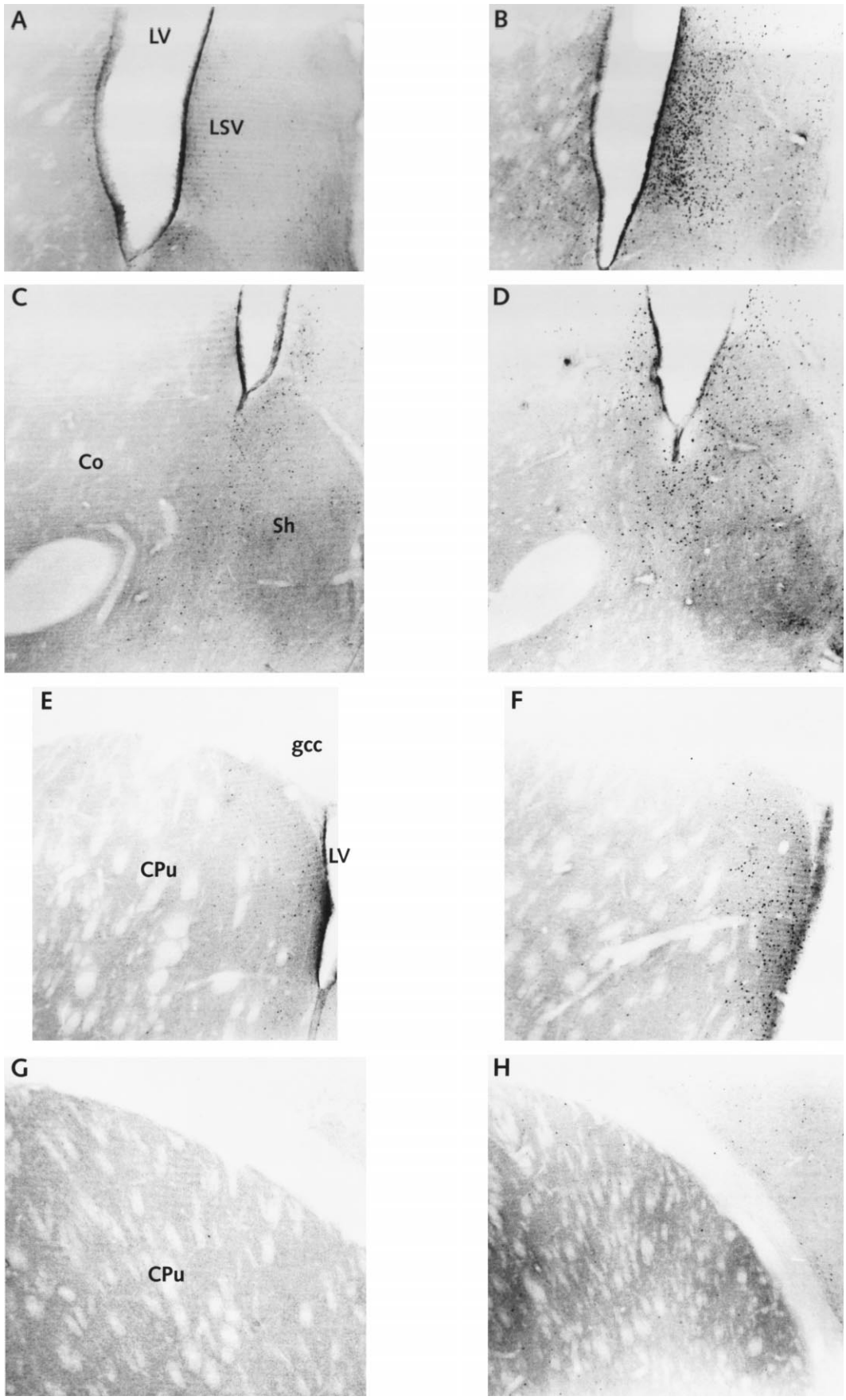

Fig. 3.

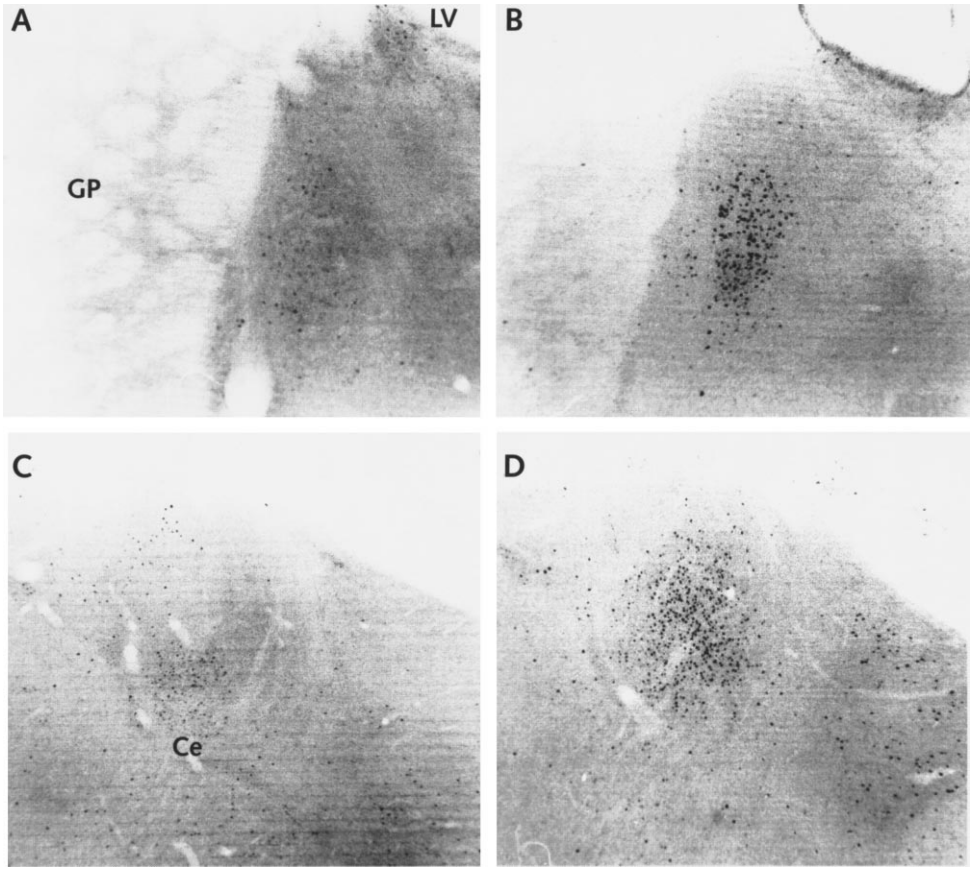

Fig. 4. Photomicrographs of brain sections showing Fos-like immunoreactive cells in the bed nucleus of the stria terminalis (A, B) and the central amygdaloid nucleus (C, D) of rats administered with vehicle (A, C) or SR141716 (10 mg/kg; B, D). Ce, central amygdaloid nucleus; GP, globus pallidus; LV, lateral ventricle.

(3 and 10 mg/kg). For example, the changes were of $+1 \pm 11\%$ ($n=4$) and $21 \pm 12\%$ ($n=4$), respectively, at 60 min compared with the vehicle-treated group ($+10 \pm 9\%$; $n=4$). Before treatment, the mean ($\pm$ S.E.M.) dopamine baseline levels were 1.1 ± 0.2 and 1.28 ± 0.25 pg/μl perfusate in rats treated with 3 and 10 mg/kg of SR141716, respectively, and 1.51 ± 0.11 pg/μl perfusate in vehicle-treated rats.

DISCUSSION

The main finding of the present study is that SR141716 increased Fos expression in the rat brain with a regional pattern of distribution and pharmacological characteristics that are reminiscent of those of atypical neuroleptic drugs. Like these drugs, SR141716 was also able to selectively increase the level of neurotensin-like immunoreactivity in the nucleus accumbens.

*Regional distribution of SR*141716*-induced Fos-like immunoreactivity*

Like most typical and atypical neuroleptic drugs,[11,23,51,53,58,68] SR141716 increased Fos-like immunoreactivity in several limbic regions, such as the ventrolateral septum, the shell subdivision of the nucleus accumbens, the piriform cortex, the bed nucleus of the stria terminalis, the central amygdala and the habenula. SR141716 also selectively elevated Fos-like immunoreactivity in the dorsomedial part of the caudate–putamen. Converging anatomical, electrophysiological and behavioral data indicate that this brain region is associated with cognitive processes.[5,13,18,69] As distinct from classical neuroleptic drugs, but like atypical compounds such as clozapine,[10,11,51,53,58,68] SR141716 elevated Fos-like immunoreactivity in the prefrontal cortex and was unable to affect the dorsolateral caudate–putamen and the core

Fig. 3. Photomicrographs of brain sections showing Fos-like immunoreactive cells in the ventrolateral septum (A, B), the nucleus accumbens (C, D), the dorsomedial (E, F) and dorsolateral (G, H) caudate–putamen of rats administered with vehicle (A, C, E, G) or SR141716 (10 mg/kg, i.p.; B, D, F, H). Co and Sh, core and shell compartments of the nucleus accumbens, respectively; Cpu, caudate–putamen; gcc, genu of the corpus callosum; LSV, lateral septal nucleus, ventral part; LV, lateral ventricle.

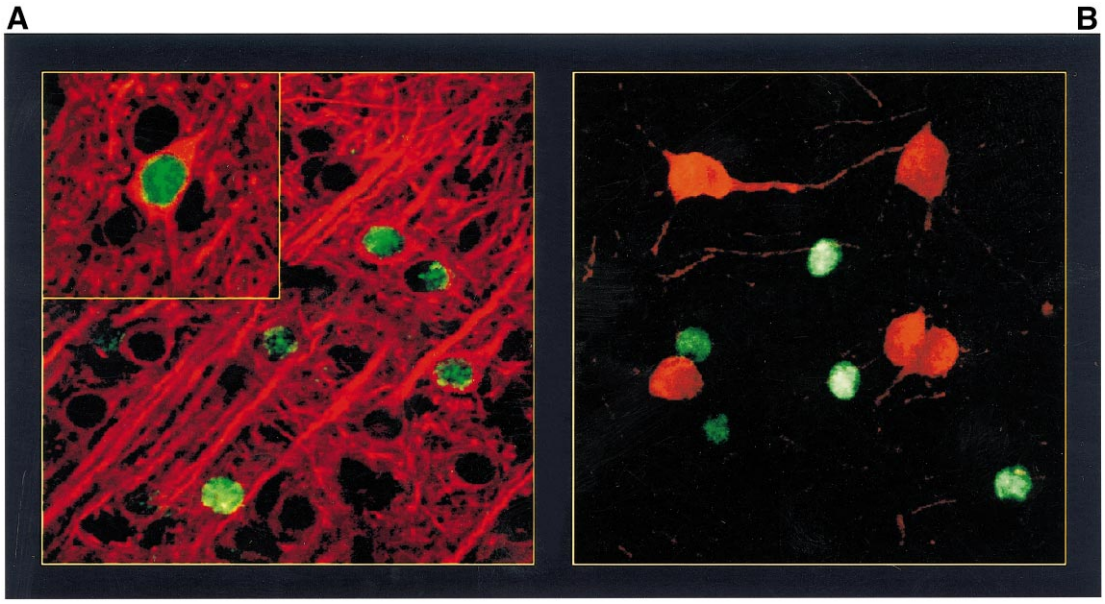

Fig. 5. Typical example of cellular localization of Fos-like immunoreactivity in the prefrontal cortex of SR141716-treated rats. Fos-like immunoreactivity (green nuclei) is seen in MAP-2-counterstained pyramidal neurons (A and insert), but not in parvalbumin-positive cells (B). Confocal microscopic images were acquired sequentially through the green channel (Fos) and the red channel (MAP-2 or parvalbumin), and subsequently merged for the visualization of double labeling. Magnifications: ×70 (A, B); ×75 (insert).

compartment of the nucleus accumbens, brain areas associated with motor behaviors.[4,70] The lack of effect of SR141716 in these structures was unlikely to result from possible different time-courses of induction, since the immunoreactive signal remained unchanged at all postinjection times examined (i.e. 1, 2 or 4 h). However, in contrast to clozapine,[23,58] SR141716 failed to induce Fos expression in the islands of Calleja and the hypothalamic nuclei. SR141716 was also devoid of activity in the hippocampus, the substantia nigra, the ventral tegmental area or the locus coeruleus, brain areas reportedly insensitive to neuroleptic treatments with respect to Fos expression.[51]

Finally, both the subregional and the cellular locations of Fos protein induced by SR141716 in the prefrontal cortex were in harmony with those reported for clozapine.[10] The Fos-immunoreactive signal was distributed predominantly in the infralimbic and prelimbic areas, and mainly co-localized with pyramidal cells. In contrast, very few parvalbumin-like immunoreactive neurons, presumptive GABAergic interneurons,[25] expressed Fos protein in these subregions after the SR141716 challenge.

Taken together, our results on the distribution of Fos-positive cells extend to the prefrontal cortex, the dorsomedial caudate–putamen and the ventrolateral septum, the brain regions reported by Rodriguez de Fonseca *et al.*[55] to be affected by SR141716. They also provide further precision in the subregional distribution of Fos-like immunoreactivity, in particular within cortical structures and in the caudate–putamen. Finally, they show the apparent similarities between SR141716 and atypical neuroleptic drugs at the anatomical level.

*Pharmacological characteristics of SR*141716-*induced Fos-like immunoreactivity*

A host of pharmacological and physiological stimuli can increase c-fos expression in the brain.[43] It is well established that amphetamine or cocaine, as well as cannabinomimetics, elevate c-fos expression through the indirect stimulation of D_1-like receptors, as revealed by the ability of the D_1-like receptor antagonist, SCH23390, to suppress this effect.[21,41,42] In marked contrast, the c-fos-inducing effect of neuroleptic drugs is impervious to D_1-like receptor blockade, but highly sensitive to D_2-like receptor stimulation,[10,23,39,40,42,54] thus suggesting that neuroleptic drugs increase c-fos expression by blocking inhibitory D_2-like receptors. Remarkably, we found that the stimulatory effects of SR141716 on Fos expression shared with neuroleptic drugs an identical sensitivity to stimulation of D_2-like vs blockade of D_1-like receptors. Indeed, the D_1-like receptor antagonist, SCH23390, at a dose which markedly reduced the cocaine responses, did not affect SR141716-induced Fos-like immunoreactivity in the prefrontal cortex, the lateral septum, the nucleus accumbens and the dorsomedial caudate–putamen. A similar lack of sensitivity of the SR141716-induced response to SCH23390 was also observed, although not assessed quantitatively, in the piriform cortex and the central amygdala. Conversely, the D_2-like receptor agonist, quinpirole,

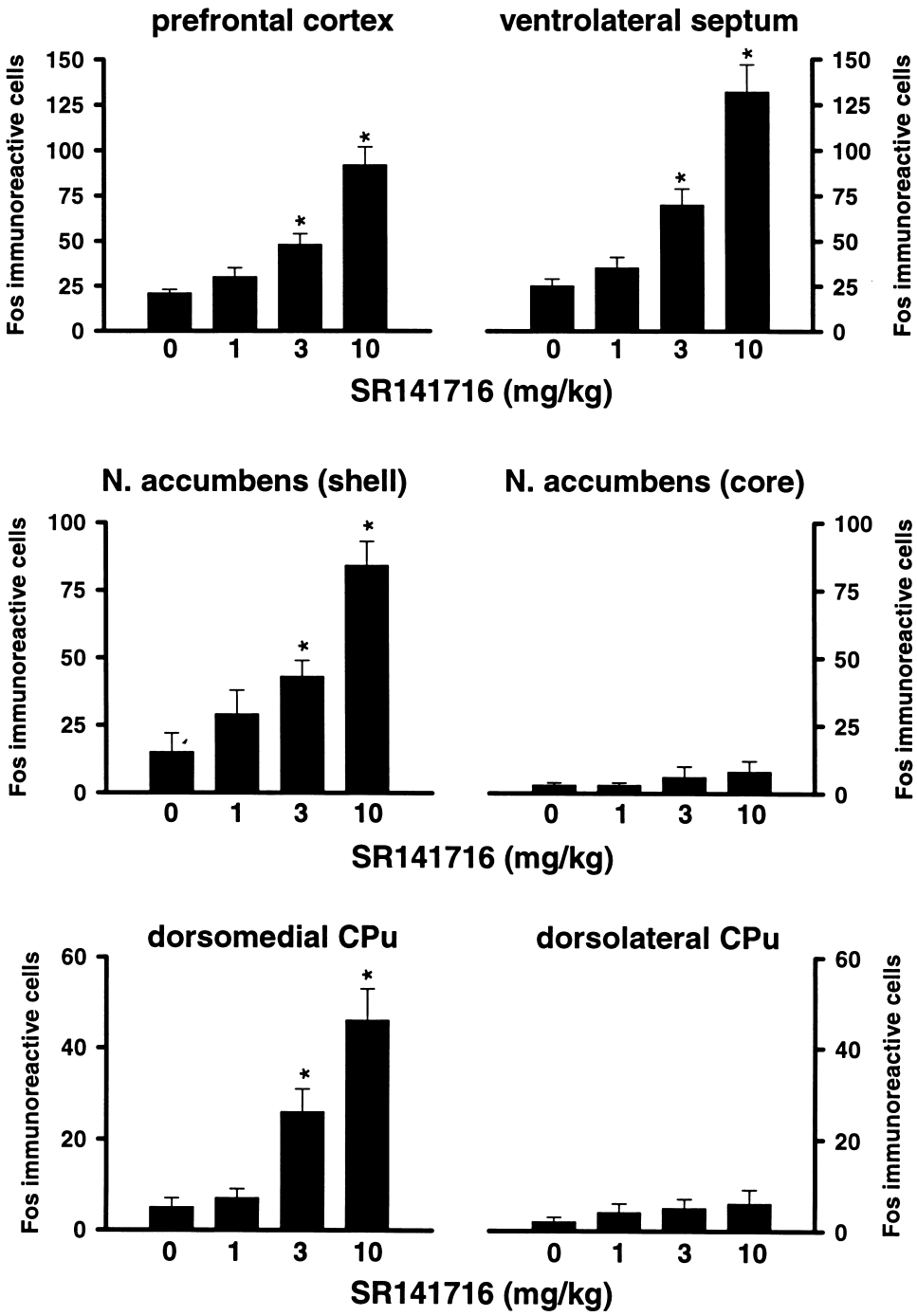

Fig. 6. Number (mean ± S.E.M.) of Fos-positive neurons after administration of vehicle ($n = 4$) or SR141716 at the doses of 1 ($n = 5$), 3 ($n = 4$) or 10 ($n = 4$) mg/kg. CPu, caudate–putamen. *$P < 0.05$ compared with vehicle-treated rats.

strongly decreased the ability of SR141716 to stimulate Fos expression in the nucleus accumbens, the lateral septum and the dorsomedial caudate–putamen. Collectively, these pharmacological experiments suggest that the action of SR141716 is likely to occur via reduced D_2-like receptor-mediated function. However, quinpirole was unable to reduce the Fos-inducing effect of SR141716 in the prefrontal cortex. This could be consistent with the atypical D_2-like receptor pharmacology described in this brain region.[66] Moreover, Guo *et al.*[23] reported a significant, but marginal (about 20%), inhibitory effect of quinpirole on clozapine-induced Fos expression in the prefrontal cortex compared with the marked reduction (60–100%) of the clozapine response in the nucleus accumbens, the medial

Table 1. Number of Fos-positive cells in the ventrolateral septum and the dorsolateral caudate–putamen of rats perfused immediately or 1, 2 and 4 h after SR141716 (10 mg/kg) administration

Brain region	Postinjection time interval (h)			
	0	1	2	4
Lateral septum	17 ± 5	65 ± 7*	93 ± 13*	30 ± 13
Dorsolateral caudate–putamen	6 ± 2	12 ± 5	14 ± 6	7 ± 3

Data are mean ± S.E.M. ($n = 3$ animals at each time interval). *$P < 0.05$ compared with rats perfused immediately after SR141716 injection.

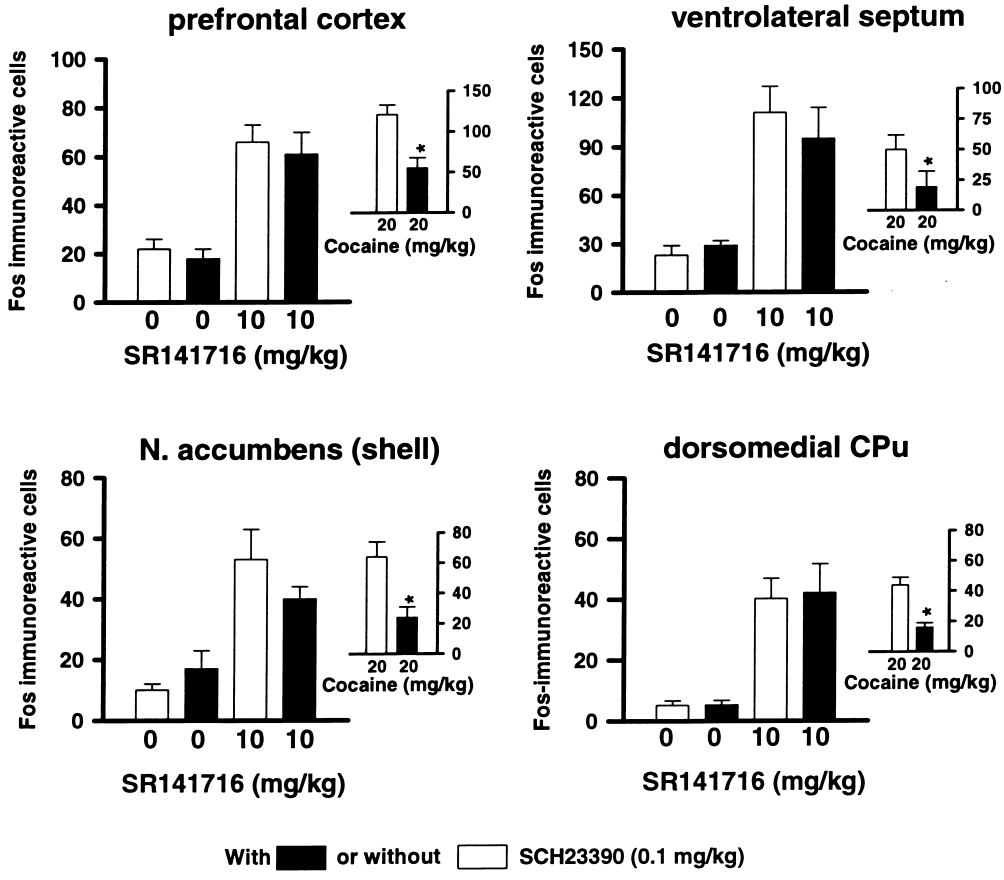

Fig. 7. Lack of effect of SCH23390 on Fos-like immunoreactivity induced by SR141716. Rats were treated with vehicle ($n = 5$), 0.1 mg/kg SCH23390 ($n = 4$), 10 mg/kg SR141716 ($n = 6$), 20 mg/kg cocaine ($n = 6$), 0.1 mg/kg SCH23390 plus 10 mg/kg SR141716 ($n = 5$) or 0.1 mg/kg SCH23390 plus 20 mg/kg cocaine ($n = 5$). Data are number (mean ± S.E.M.) of Fos-positive neurons counted within the indicated brain regions. Cpu, caudate–putamen. *$P < 0.05$ compared with cocaine alone.

caudate–putamen and the lateral septum. Finally, the ability of SR141716 to elevate the level of neurotensin-like immunoreactivity in the nucleus accumbens further supported the notion that the SR141716-induced Fos expression probably resulted from disinhibition of neuronal systems expressing D_2-like receptors. Indeed, both neuroleptic drugs and reserpine increased neurotensin mRNA or neurotensin-like immunoreactivity in this brain region,[29,33,38,39,60] where neurotensin and D_2-like receptors could be co-localized on enkephalin-containing neurons.[2,31,32] In agreement with data reported for atypical neuroleptic drugs,[29,39] the stimulatory effects of SR141716 on neurotensin-like immunoreactivity were observed in the nucleus accumbens but not in the caudate–putamen, which is consistent with the selective limbic distribution of c-fos expression after SR141716 challenge. Current double-labeling experiments in the nucleus accumbens are in progress to assess whether enkephalin/neurotensin-containing neurons, which express D_2 receptors, are only those affected by SR141716 in comparison to substance P neurons expressing D_1 receptors.

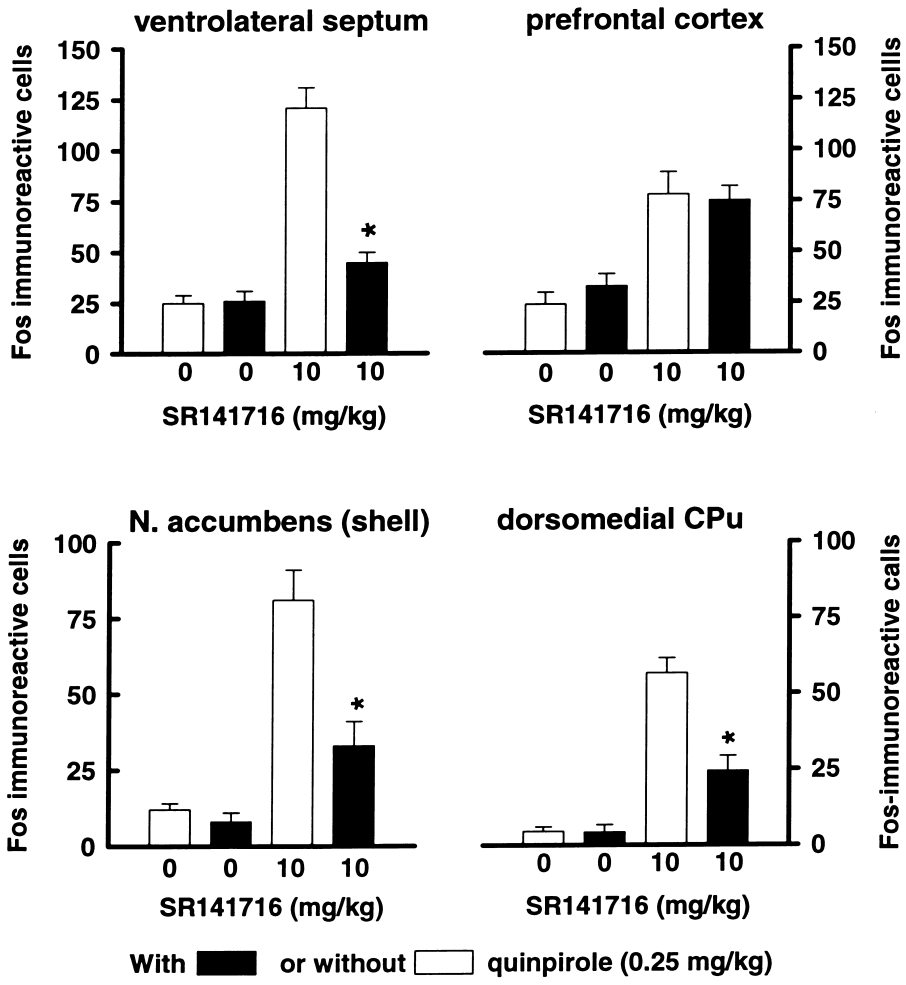

Fig. 8. Inhibitory effect of quinpirole on Fos-like immunoreactivity induced by SR141716. Rats were treated with vehicle ($n = 5$), 0.25 mg/kg quinpirole ($n = 4$), 10 mg/kg SR141716 ($n = 6$) or 0.25 mg/kg quinpirole plus 10 mg/kg SR141716 ($n = 4$). Data are number (mean ± S.E.M.) of Fos-positive neurons counted within the indicated regions. Cpu, caudate–putamen. *$P < 0.05$ compared with SR141716 alone.

Table 2. Effect of SR141716 and haloperidol on neurotensin-like immunoreactivity in the nucleus accumbens and the caudate–putamen

Treatment	mg/kg	Neurotensin-like immunoreactivity (% of control)	
		Nucleus accumbens	Caudate–putamen
Vehicle		100 ± 11	100 ± 12
SR141716	1.25	112 ± 19	113 ± 23
	2.5	154 ± 31*	101 ± 29
	5	174 ± 25*	73 ± 14
Haloperidol	1	230 ± 51*	148 ± 18*

Data are mean ± S.E.M. ($n = 7$–10 animals per treatment), expressed as percentage of control (vehicle). *$P < 0.05$ as compared to vehicle-treated rats. Values of neurotensin-like immunoreactivity in vehicle-treated rats were 274 ± 54 and 95 ± 9 pg/mg protein in the nucleus accumbens and caudate–putamen, respectively.

*Possible mechanisms of action of SR*141716

SR141716 displays a very high selectivity for CB_1 vs CB_2 receptors and is devoid of affinity for more than 30 non-cannnabinoid receptors, including not only dopamine receptor subtypes,[50] but also adenosine A2A or sigma receptors, which have been implicated in the induction of Fos expression in mesolimbic areas.[9,47] Thus, the Fos-inducing effects of SR141716 probably resulted from the blockade of an endogenous cannabinoid tone on CB_1 receptors.

SR141716 increased Fos expression in corticolimbic structures enriched with CB_1 receptors, but was found not to affect brain structures such as the dorsolateral caudate–putamen, whilst still displaying the highest density of CB_1 receptors in the brain.[26,35,67] The marked topographical selectivity of the Fos-inducing effects of SR141716 suggests that an endogenous cannabinoid tone is probably more efficient in corticolimbic than motor-related areas. A cannabinoid tone has also been suggested

to exist in other brain regions, including the hippocampus and the substantia nigra.[20,22] These brain regions are poorly sensitive to neuroleptic drugs,[51] and remarkably were not affected by SR141716 with respect to Fos expression.

Since converging electrophysiological[15,16,19] and biochemical[41,64] data indicate that cannabinoid agonists exert a stimulatory action on dopamine cells, it is tempting to speculate that the Fos-inducing effect of SR141716 resulted from a reduction of dopamine release. This hypothesis, however, is not further supported by our microdialysis data on awake animals, showing that SR141716 did not affect dopamine release in the shell of the nucleus accumbens when administered at 3 and 10 mg/kg, doses which stimulate Fos expression in this brain structure. Alternatively, as CB_1 receptors were found on neuronal fibers in the septum and the nucleus accumbens,[67] the Fos-inducing effect of SR141716 in these brain regions could result from a presynaptic action on non-dopaminergic endings. We are currently examining whether glutamatergic systems may contribute to the Fos-inducing effects of SR141716 since (i) cortical stimulation was shown to increase Fos expression in efferent structures via glutamate release[17] and (ii) glutamate receptors were involved in the induction of Fos by haloperidol.[3]

Another possibility would be that SR141716 acted at the postsynaptic level on neurons expressing D_2-like receptors. In that case, given that the effects of SR141716 are restricted to a fraction of cells in many brain regions, the postsynaptic action of SR141716 may only involve a subpopulation of D_2-like bearing neurons subjected to a high endogenous cannabinoid tone. Since CB_1 receptors are linked to inhibition of adenylyl cyclase,[28] the blockade of the endogenous tone on CB_1 receptors would result in an increase in cyclic AMP levels and enhanced c-fos expression via the activation of downstream elements, such as cyclic AMP response element binding protein.[43] Under this hypothesis, the ability of quinpirole to reduce the Fos-inducing effect of SR141716 might be related to its capacity to decrease cyclic-AMP levels through stimulation of D_2-like receptors.[61]

CONCLUSIONS

The present study shows that the anatomical and pharmacological profile of the Fos-inducing effect of SR141716, together with its ability to selectively increase neurotensin-like immunoreactivity in the nucleus accumbens, is reminiscent of that of atypical antipsychotic compounds. In line with the cannabinoid hypothesis of schizophrenia,[14] our results suggest that blockade of CB_1 receptors could have beneficial effects on this pathology.

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