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Short communication

The effect of E-5842, a σ receptor ligand and potential atypical antipsychotic, on Fos expression in rat forebrain

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Abstract

We examined the ability of E-5842 (4-(4-fluorophenil)-1,2,3,6- tetrahydro-1-[4-(1,2,4-triazol-1-il)butyl] pyridine citrate), a σ receptor ligand, to increase Fos protein expression in regions of rat forebrain. An acute administration of E-5842 increased levels of Fos in the medial prefrontal cortex and the nucleus accumbens, without affecting the levels of the protein in the striatum, an effect very similar to that of clozapine. Our results suggest that E-5842 may be an atypical antipsychotic with low propensity to produce extrapyramidal side-effects.

Keywords: Fos regulation; σ Receptor; Cortex, prefrontal; Antipsychotic, atypical; Schizophrenia; Immediate-early gene

1. Introduction

Antipsychotic medication is clearly useful in the treatment of schizophrenia (Deutch et al., 1991), but among the compounds used as antipsychotics, the neuroleptics also produce extrapyramidal side-effects and have almost no action on negative symptoms. By comparison, the atypical antipsychotic clozapine, produces its therapeutic effects without causing the extrapyramidal side-effects that are characteristic of typical antipsychotics (Matz et al., 1974; Kane et al., 1988; Baldessarini and Frankenburg, 1991).

The neuronal expression of Fos, the protein product of the immediate-early gene c-*fos* is increased by a plethora of physiological and pharmacological treatments (Morgan and Curran, 1991). It has been proposed that Fos immunodetection might be used to map functional pathways in the brain (Sagar et al., 1988) and neuroanatomical sites of drug action (Presely et al., 1990). In recent years, it has been described that acute administration of antipsychotic drugs increases the expression of Fos in different brain regions (Dragunow et al., 1990; Miller, 1990; Deutch et al., 1992; Nguyen et al., 1992; Robertson and Fibiger, 1992). Fos is potently increased in the dorsolateral striatum by typical, but not atypical antipsychotics (Robertson et al., 1994). In contrast, both types of antipsychotics increase Fos immunoreactivity in the nucleus accumbens, and only a reduced group of antipsychotics, including clozapine (Robertson and Fibiger, 1992), are able to increase levels of Fos in the medial prefrontal cortex. Based on these and other findings, it has been suggested that efficacy shown by clozapine against the negative symptoms of schizophrenia, the metabolic hypofrontality thought to be associated to these symptoms, and Fos expression in the prefrontal cortex could be related.

E-5842 (4-(4-fluorophenil)-1,2,3,6-tetrahydro-1-[4-(1,2,4-triazol-1-il)butyl] pyridine citrate) is a newly developed and potential atypical antipsychotic with potent affinity for the σ_1 receptor ($K_i = 4$ nM) and moderate to null affinity for other central nervous system receptors. E-5842 does not show any induction of extrapyramidal side-effects in rats even at very high doses (> 80 mg/kg, data not shown), but shows efficacy in most of the tests predictive of antipsychotic activity at doses between 5 and 15 mg/kg (Guitart et al., 1997). Based on the results observed in different tests, our data suggest that E-5842 could be classified as an atypical antipsychotic.

To further characterize its atypical properties, and taking into account the characteristic pattern of Fos induction in concrete areas of the brain by atypical antipsychotics, the effects of E-5842 on Fos induction, compared to those of haloperidol and clozapine on striatal, accumbal and frontocortical brain regions were studied. Western blot analysis was used to study and compare such effects.

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2. Materials and methods

All experiments were performed in male Sprague–Dawley rats (150–175 g). The animals were housed in temperature-controlled rooms (20–22°C) with a light/dark cycle (lights on from 0600 h to 1800 h). The animals were allowed ad libitum access to food and water and were handled several times the days before the experiment in order to minimize the stressing effect due to the experimental procedure.

Rats were injected subcutaneously with saline, haloperidol (1 mg/kg), clozapine (30 mg/kg) or E-5842 (20 mg/kg) and killed by decapitation 2 h later, a time point that has been widely used to study Fos expression. The haloperidol and clozapine doses were chosen on the basis of clinical equivalency, the clozapine dose being 30 times greater than the haloperidol dose (Fitzgerald et al., 1995), which is consistent with an approximately 30-fold difference in clinical doses of the two antipsychotics. Brains were quickly removed and cooled immediately in ice-cold physiological buffer.

At the level of various brain regions, coronal sections (1 mm thick) were obtained, and punches of medial prefrontal cortex, nucleus accumbens and dorsolateral striatum were excised. The areas that were excised are shown as dark circles in Fig. 1. Bilateral punches were pooled from individual rats. Tissue samples were immediately homogenized in 150–200 μl of electrophoretic mobility shift assay buffer (Korner et al., 1989) containing: 20 mM Hepes, pH 7.9, 0.4 M NaCl, 20% glycerol, 5 mM $MgCl_2$, 0.5 mM EDTA, 0.1 mM EGTA, 1% Nonidet P-40, 10 μg/ml leupeptin, 0.1 mM *p*-aminobenzamidine, 1 μg/ml pepstatin, 0.5 mM phenylmethylsulfonyl fluoride, and 5 mM dithiothreitol. Brain homogenates were then incubated on ice for 25 min, centrifuged at 15,000 rpm for 25 min at 4°C in an Eppendorf centrifuge 5417R and the supernatants were removed for immunoblotting. After protein determination, aliquots of brain extracts (containing 40–50 μg of protein) were subjected to sodium dodecyl sulphate (SDS)/polyacrylamide gel electrophoresis. After electrophoretic transfer to nitrocellulose, Fos was immunolabelled with the c-Fos antiserum (1:1000) (sc-52, Santa Cruz Biotechnology, Santa Cruz, CA), incubated with goat anti-rabbit immunoglobulins (IgG) (1:2000) conjugated to horseradish peroxidase (Vector Laboratories, Burlingame, CA), and developed with the Renaissance chemiluminescence reagent of DuPont NEN. Nitrocellulose filters were then exposed to Reflection autoradiographic films (DuPont NEN, USA). Relative Fos protein levels were analyzed by measuring bands optical densities and using computer-assisted densitometry (Bio-Rad Fluor-S MultiImager).

Optical densities values were analyzed by Student's unpaired *t*-tests. As the intensity of the bands shows variations among different experiments, optical densities were then expressed as mean percentage of control (saline) values (±S.E.M.) for graphic clarity. All the experiments in rats adhered strictly to the European Community Guide for the Care and Use of Laboratory Animals.

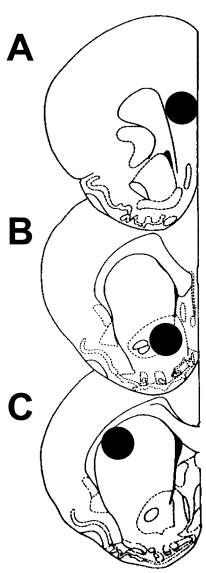

Fig. 1. Drawings showing the brain regions (dark circles) punched out to perform Fos expression experiments in the medial prefrontal cortex (A), nucleus accumbens (B), and dorsolateral striatum (C).

3. Results

Fig. 2A corresponds to an autoradiogram representative of a typical experiment involving E-5842 administration, and shows the significant increase in Fos levels in the nucleus accumbens and the medial prefrontal cortex (138% and 186% of saline injected group, respectively) after a single E-5842 injection. On the other hand, Fig. 2A also shows that levels of Fos were not significantly affected by E-5842 in the dorsolateral striatum (110% of saline group). A single subcutaneous injection of haloperidol or clozapine affected Fos levels in a characteristic way, as it has been previously described. A clear increase in levels of the protein was observed in the dorsolateral striatum and the nucleus accumbens after 2 h of haloperidol administration. Increased levels of Fos induced by clozapine administration were detected in the nucleus accumbens (Fig. 2B) and, as it was expected, in the medial prefrontal cortex. Haloperidol was unable to induce any significant change in Fos levels in this brain area.

The pattern of Fos immunoreactivity in some forebrain regions is very similar for clozapine and E-5842, and clearly different from that of haloperidol (Fig. 2B). It is important to point out the big increase in Fos immunoreactivity in the medial prefrontal cortex induced by E-5842. Taken together, these results clearly show the similarities of E-5842 with clozapine and other atypical an-

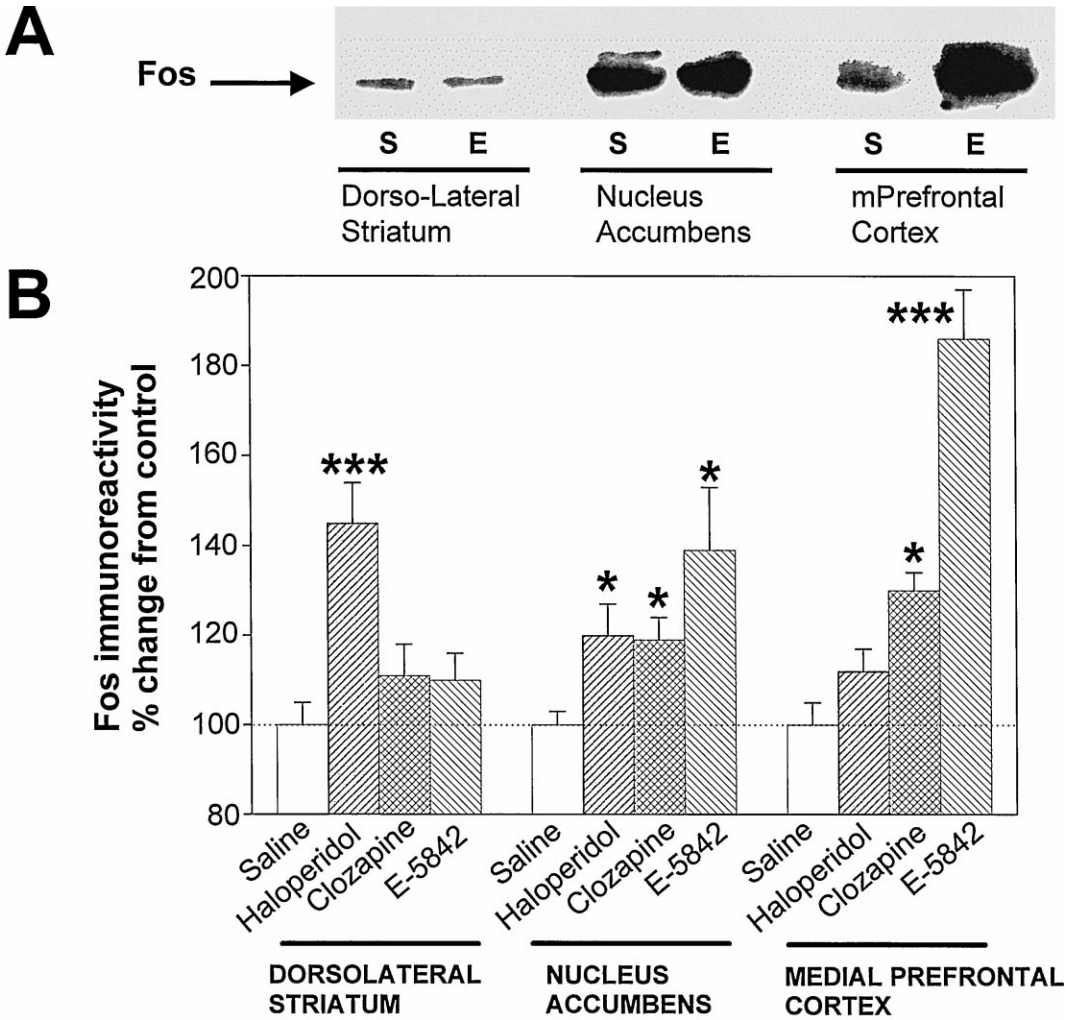

Fig. 2. Regulation of Fos protein immunoreactivity by acute treatment with haloperidol (1 mg/kg), clozapine (30 mg/kg), and E-5842 (20 mg/kg). (A) Typical autoradiogram showing the effect of an acute dose of E-5842 on Fos levels in different brain areas. S (saline), E (E-5842). (B) Differential Fos-like immunoreactivity produced by typical (haloperidol) and atypical (clozapine, E-5842) antipsychotics. Each bar represents the mean ± S.E.M. of data from 8–12 animals per treatment group. *Significantly different from saline control ($P < 0.05$), ***significantly different from saline control ($P < 0.001$).

tipsychotics and further suggest the atypicity of our compound.

4. Discussion

The distribution of Fos-positive cells after haloperidol or clozapine administration has been widely described (Dragunow et al., 1990; Deutch et al., 1992; Nguyen et al., 1992; Robertson and Fibiger, 1992). Most of the studies addressing such issue have used immunoreactivity in brain tissue sections and counting of the positive nuclei marked on each section. Using a different approach, we have studied levels of Fos protein by immunoblotting techniques. Our results show that very similar results can be achieved using either one of the two methodologies. Administration of E-5842, a new potential atypical antipsychotic, enhanced levels of Fos protein in different brain areas. E-5842 administration elevated the immunodetectable levels of Fos in the nucleus accumbens and the medial prefrontal cortex. The nucleus accumbens is the only brain region where c-Fos expression is increased after administration of any of the known antipsychotics, suggesting that it may be a critical site of antipsychotic activity. On the other hand, only the administration of a few atypical antipsychotics induces an increase in the number of Fos-like immunoreactive cells in the medial prefrontal cortex. It has been proposed that hypofrontality is associated with the negative symptoms of schizophrenia (Berman et al., 1986; Weinberger, 1988) and hence, antipsychotics able to increase c-*fos* expression in this brain area would be active in the treatment of negative symptoms. As it occurs with clozapine, E-5842 was very active in inducing Fos in the medial prefrontal cortex.

Interestingly, E-5842 did not induce any significant change in Fos levels in the dorsolateral striatum. This brain area is involved in the control of movement and it has been suggested that antipsychotic-induced increases in Fos expression in this area would be associated to antipsy-

chotics devoid of extrapyramidal side-effects (Robertson and Fibiger, 1992). In fact, some of the commercially available antipsychotics with low propensity to induce extrapyramidal side-effects at therapeutic doses (olanzapine, risperidone), may increase Fos-like immunoreactivity in the dorsolateral striatum when used at doses higher than the therapeutic (Fink-Jensen and Kristensen, 1994; Robertson and Fibiger, 1996), indicating thus, a narrow therapeutic margin in patients. Although E-5842 has not been tested in patients yet, it does not induce Fos expression in the striatum at a dose that induces a huge increase in medial prefrontal cortex Fos levels. The Fos pattern induction by E-5842 would suggest that our compound would behave as a good antipsychotic with possible efficacy on the negative symptoms of schizophrenia and devoid of extrapyramidal side-effects.

It seems to be widely accepted that Fos induction in the dorsolateral striatum would be mediated by dopamine D_2 receptors blockade. Our results clearly support this point of view, since E-5842 has almost null dopamine D_2 receptor binding ($K_i > 1000$ nM). As many other antipsychotics, E-5842 increases Fos immunoreactivity in the nucleus accumbens, although the exact mechanism of action responsible for the increase in this area is not clear. The dopamine D_3 receptor has been implicated (Guo et al., 1995; Hurley et al., 1996), but E-5842 has also a very low affinity for the dopamine D_3 receptor ($K_i = 418$ nM). Up to date, the mechanism underlying the antipsychotic action of E-5842 is not known and little is known about its in vivo affinity for the different dopamine receptors, although no possibility can be discarded. Given its high affinity for the σ_1 receptor and almost null affinity for other receptors, E-5842 could represent a new type of antypsychotic drug. Moreover, it has been described (Dahmen et al., 1996) that EMD 57445, another σ receptor ligand (with no affinity for other receptors), is able to induce the expression of c-*fos* with a pattern similar to that obtained with atypical antipsychotics. Further studies are required to define the direct or indirect interaction of E-5842 with different neurotransmitter systems in the brain and to define the exact mechanism that triggers the characteristic pattern of Fos induction in the medial prefrontal cortex and the nucleus accumbens.

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