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European Journal of Pharmacology 378 (1999) 31–37

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Electrophysiological effects of E-5842, a σ_1 receptor ligand and potential atypical antipsychotic, on A9 and A10 dopamine neurons

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Received 8 March 1999; received in revised form 28 May 1999; accepted 15 June 1999

Abstract

Extracellular single unit recording techniques were used to study the effects of the novel potential atypical antipsychotic E-5842, (4-(4-fluorophenyl)-1,2,3,6-tetrahydro-1-[4-(1,2,4-triazol-1-il)butyl]pyridine citrate), a preferential σ_1 receptor ligand, on the activity of dopamine cells in substantia nigra pars compacta (A9) and ventral tegmental area (A10) in anesthetized rats. Acute i.v. administration of E-5842 (up to 3.2 mg kg^{-1}) did not change the spontaneous activity of the dopamine neurons, which still responded to the inhibitory effect of a subsequent administration of high dose of apomorphine. Acute administration of E-5842 (20 mg kg^{-1}, i.p.) did not change the number of spontaneously active A9 or A10 dopamine cells. Chronic administration of E-5842 (20 mg kg^{-1} $day^{-1} \times 21$ days, s.c.) decreased the number of spontaneously active A10, but not A9, dopamine neurons. This effect was reversed by the administration of apomorphine, thus, indicating a possible depolarization inactivation phenomenon. Our results suggest an influence of E-5842 on dopaminergic neurotransmission, although the exact mechanism remains unknown. The effect of E-5842 on A10 is similar, in some ways, to the effects observed with several atypical antipsychotics and suggest the atypicality of the compound and that E-5842 may exert its antipsychotic effects without causing significant extrapyramidal side effects.

Keywords: Schizophrenia; E-5842; Depolarization inactivation; Atypical antipsychotic; Electrophysiology; Dopamine neuron

1. Introduction

Schizophrenia is a serious disorder of the central nervous system, and although its aetiology is still unknown, antipsychotic agents have proven to be efficacious in the treatment of psychotic disorders. For many years, it has been suggested that the pathophysiology of schizophrenia is related to a hyperactivity of the dopaminergic system of the brain (dopaminergic hypothesis of schizophrenia), and in fact, most of the active antipsychotic drugs have affinity for the dopamine D_2 receptor (Creese et al., 1976). Although classical antipsychotics may be active in the treatment of schizophrenia, they also induce extrapyramidal side-effects (Snyder et al., 1974), and many research attempts have been focused to the development of new agents with low extrapyramidal side-effects liability.

Electrophysiological studies have shown that chronic treatment with classical antipsychotics (e.g., haloperidol, chlorpromazine) decreased the number of spontaneously active dopamine cells in both the substantia nigra compacta (A9, which mainly project to the striatum), and the ventral tegmental area (A10, which project to limbic and cortical regions) (Bunney and Grace, 1978; Chiodo and Bunney, 1983). The reduction observed in the number of spontaneously active dopamine cells clearly depends on time of drug administration and has been attributed to the development of depolarization inactivation, a state characterized by a steady membrane depolarization above the action potential threshold, so that no action potential is generated (Bunney and Grace, 1978; Chiodo and Bunney, 1983). Long treatment with atypical antipsychotics (e.g., clozapine, seroquel, olanzapine, among others) induces depolarization inactivation of the A10 cells only (Chiodo and Bunney, 1983; White and Wang, 1983; Goldstein et al., 1993; Skarsfeldt, 1994; Stockton and Rasmussen, 1996). In addition, based on the hypothesis that psychotic disorders could be caused by a hyperfunction of the mesolimbic and mesocortical systems that originate in the ventral tegmental area (Hökfelt et al., 1974) it has been hypothesized that the decrease of activity in A10 dopamine cells after treatment could predict potential antipsychotics

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0014-2999/99/$ - see front matter
PII: S0014-2999(99)00440-9

therapeutically effective (Grace et al., 1997), while the decrease of activity in A9 dopamine cells after repeated treatment may correlate with the extrapyramidal side-effects associated with classical antipsychotics (Chiodo and Bunney, 1983).

E-5842 (4-(4-fluorophenyl)-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 very high affinity for the σ_1 receptor ($K_i = 4$ nM), and moderate affinity for other central nervous system receptors, including several dopamine receptors (e.g., K_i for the dopamine D_2 receptor > 1000 nM using raclopride as radioligand and $K_i = 510$ nM using YM-09151-2 as radioligand; K_i for the dopamine D_3 receptor $= 418$ nM; K_i for the dopamine D_4 receptor > 1000 nM), (K_i for the serotonin 5-HT_2 receptor $= 817$ nM) (Guitart et al., 1998). E-5842 showed to be active in different animal models predictive of antipsychotic activity either using behavioural tests (e.g., conditioned avoidance responding, apomorphine induced climbing) or biochemical approaches (Guitart and Farré, 1998), and its profile suggests a low extrapyramidal side-effects liability (Guitart et al., 1998). The possible involvement of σ receptors in schizophrenia has been suggested, based on the psychotomimetic actions of σ receptor agonists (Tam, 1983), the density of σ receptors detected in various mesencephalic and limbic areas (Weissman et al., 1988, 1991), and the action of several σ receptor ligands on dopaminergic neurons albeit their low affinity for different dopamine receptors (Steinfels and Tam, 1989; Zhang et al., 1992; Poncelet et al., 1993).

In order to evaluate the effect of acute administration of E-5842, the activity of dopaminergic neurons in the A9 and A10 areas was investigated by using extracellular single-unit recording techniques. On the other hand, and taking into account that many of the therapeutic and side effects of antipsychotics seen in humans are observed after weeks of treatment (Beckman et al., 1979; Crow et al., 1980), the effect of a repeated treatment (21 days) with E-5842 was also studied using electrophysiological techniques. These experiments were performed in order to clarify whether the electrophysiological profile of E-5842 is also similar to that of the atypical antipsychotics. The similarities between pharmacological profiles of E-5842 and atypical antipsychotics have already been shown using other experimental approaches.

2. Materials and methods

2.1. Animals and surgery

Male Wistar rats, weighing 250–280 g at the beginning of the study, were anesthetized with chloral hydrate (400 mg kg^{-1}, i.p.). A catheter was placed in the peritoneum for maintenance of anaesthesia and supplemental doses of chloral hydrate were administered when needed. One femoral vein was also cannulated for drug administration. The rats were placed on a stereotaxic instrument, and a hole was drilled in the skull over the ventral tegmental area (A10 area) (5.2 mm posterior to bregma, 0.7 mm lateral to midline), and/or the substantia nigra (A9 area) (5.2 mm posterior to bregma, 2.2 mm lateral to midline) (Paxinos and Watson, 1986). Body temperature was maintained between 36.5 ± 0.3°C during all the experiment with the aid of a heating pad. At the end of each experiment, the brain was removed, coronal frozen sections obtained at 50 μm intervals, and stained with toluidine blue to locate the end and the tracks of the electrodes. All the procedures involving animals and their care were conducted in strict conformity with the European Community Guide for the Care and Use of Laboratory Animals (EEC Council Directive 86/609, OJ L 358, 1, Dec. 12, 1987).

2.2. Dopamine cell recording and identification

Single glass micropipettes were pulled and filled with a solution of 2 M NaCl. The impedance of the electrode was usually 2–4 MΩ, measured at 135 Hz in vitro. The recordings of extracellular electric activity were made using a Dagan 2400 amplifier (bandpass settings: 300 Hz and 3 kHz) connected to the micropipette and monitored on a digital Axotape device. An electric micromanipulator (WPI, model DC3001) was used to perform successive vertical penetrations (ventral 6.5 to 8.5 mm below the cortical surface, for both A10 and A9 areas) through the cranial hole until a stable recording was obtained from an identified dopaminergic neuron. Cells encountered in the A10 or A9 areas were considered as dopaminergic on the basis of the well established criteria (Bunney et al., 1973; Wang, 1981). In brief, cells were considered dopaminergic if they possessed these characteristics: (1) a regular or decreasing amplitude bursting firing-pattern; (2) a slow rate of 1–9 Hz, long action potentials of 2.5–4.5 ms with; and (3) a triphasic wave-form with an initial positive phase usually containing a notch, a second great negative component and a little positive last phase.

2.3. Time course of firing rate cell study

A discriminator of action potentials model WD-2 (Axon Instruments, Foster City, CA) connected to a counter was used to measure the discharge frequency of the neuron (potentials every 10 s). The basal activity was calculated by averaging the rate for 2 min prior to the first drug administration. The effects on the groups were calculated from the 60-s period of maximal change in rate after each administration, and was expressed as the percentage response compared to the basal frequency. Only one cell per rat was used. The position of electrode tip was marked at the end of the experiment by passing a high intensity current (1.5 mA) through the micropipette for several

minutes and changing the polarity every 4 s. The percentages obtained for different doses of each treatment were subjected to variance analysis for repeated measurements (Hardy, 1989) and subsequent multiple comparisons using the SAS GLM procedure (SAS Statistical Package, SAS Institute, Cary, NC).

2.4. Drug treatment protocols

Acute i.v. administration. Cumulative doses of clozapine (0.2–3.2 mg kg^{-1}), E-5842 (0.2–3.2 mg kg^{-1}), or vehicle were intravenously administered through the femoral route. The administrations were performed every 2 min so that each administration doubled the previously accrued total. Then, and before sacrificing the animal, successive administrations of apomorphine (20 μg kg^{-1}, i.v.) were made until complete inhibition of the neuron was obtained.

Acute i.p. administration. Previous studies of our laboratory (e.g., brain microdialysis studies regarding dopamine measurements) indicate that a maximal effect of an intraperitoneal administration of E-5842 is achieved between 20 and 40 min after injection. E-5842 (20 mg kg^{-1}), or vehicle (physiological saline solution) were intraperitoneally administered 30 min before starting the electric recordings on dopamine cells.

Chronic s.c. administration of E-5842. For chronic treatments, ALZET 2ML4 osmotic minipumps (Alza, Palo

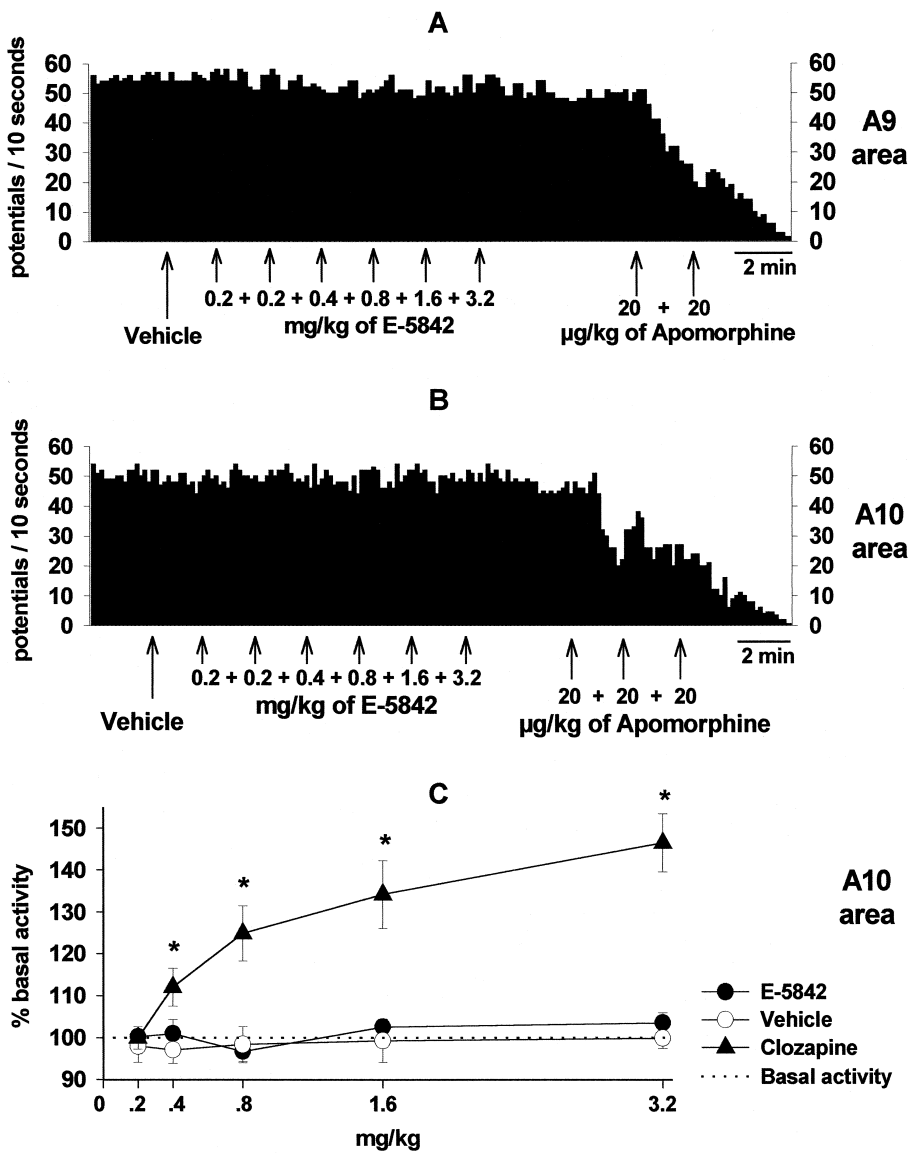

Fig. 1. Effect of acute i.v. administration of E-5842 on the electric activity of mesencephalic dopaminergic neurons. (A) and (B) show the time course of discharge frequency of two identified dopaminergic neurons in A9 and A10, respectively. Successive cumulative doses of E-5842 do not alter the spontaneous activity of the neurons, while a response to a posterior apomorphine administration is recorded. (C) shows the effect of clozapine compared to E-5842 in A10 ($^{*}P < 0.05$, each point of the graph represents the mean of four values ± S.E.M.).

Alto, CA) containing a solution of E-5842 or vehicle (physiological saline solution) were subcutaneously implanted to rats under light anaesthesia with halothane. Animals treated with E-5842 received 20 mg kg^{-1} day^{-1} of the compound. Electrophysiological recordings were performed 21 days after the implantation, with the pump still present in the animal.

2.5. Cell population study

In both groups of repeatedly treated (21 days) and acutely treated animals, the electrode was passed through eight tracks in either the A10 or A9 areas and the number of spontaneously active dopamine neurons was counted. Then, apomorphine (50 μg kg^{-1}, i.v.) was administered and the number of remaining spontaneously active A10 or A9 dopamine neurons counted again in four different tracks.

The initial track was located 5.5 mm posterior to bregma and 0.5 mm (when A10) or 2 mm (when A9) lateral to the midline. Each subsequent track was separated by 0.2 mm. The electrode tracks sequence was kept constant from animal to animal. Each neuron was recorded for about 1 min to establish the basal firing rate. Because of the influence of chloral hydrate on the basal dopamine neuron activity, the recordings were not performed in the first 10–15 min after each additional administration of choral hydrate. The position of the last micropipette tip was marked by passing a high intensity current through the electrode for several minutes, and changing the polarity every 4 s.

Results (cells by track or basal firing rate) were analyzed by comparing the drug-treated groups to the related control group and post apomorphine application using two-way analysis of variance. In addition, paired or non-paired *t*-tests were applied when necessary.

2.6. Drugs

Apomorphine was purchased from Sigma (St. Louis, MO). Clozapine was obtained from Research Biochemicals Int. (Natick, MA). E-5842 was synthesized at Laboratorios Esteve (Barcelona).

3. Results

3.1. Firing rate study

The average basal firing rate (spontaneous activity) of dopamine neurons for A9 and A10 were calculated to be of 45.3 ± 5.2 and 48.3 ± 4.1 potentials every 10 s, respectively. A typical response of these dopaminergic neurons to incremental administrations of E-5842 is shown in Fig. 1A and B. Increasing cumulative doses of E-5842 (0.2 to 3.2 mg kg^{-1}, i.v.) or a similar volume of the vehicle did not induce any significant change in the spontaneous firing rate of the dopaminergic neurons of A10 (Fig. 1B and C) or A9 (Fig. 1A and data not shown). According to previous reports (White and Wang, 1983; Goldstein et al., 1993), acute administration of clozapine dose dependently augmented the spontaneous firing rate of active A10 dopamine neurons (Fig. 1C). A significant increase in neuronal activity was already found at 0.4 mg kg^{-1} when compared with the control group. The averaged results for cumulative doses of E-5842 are included for comparison (Fig. 1C).

The cumulative dose–response effect of apomorphine administration after vehicle injection was also studied. The

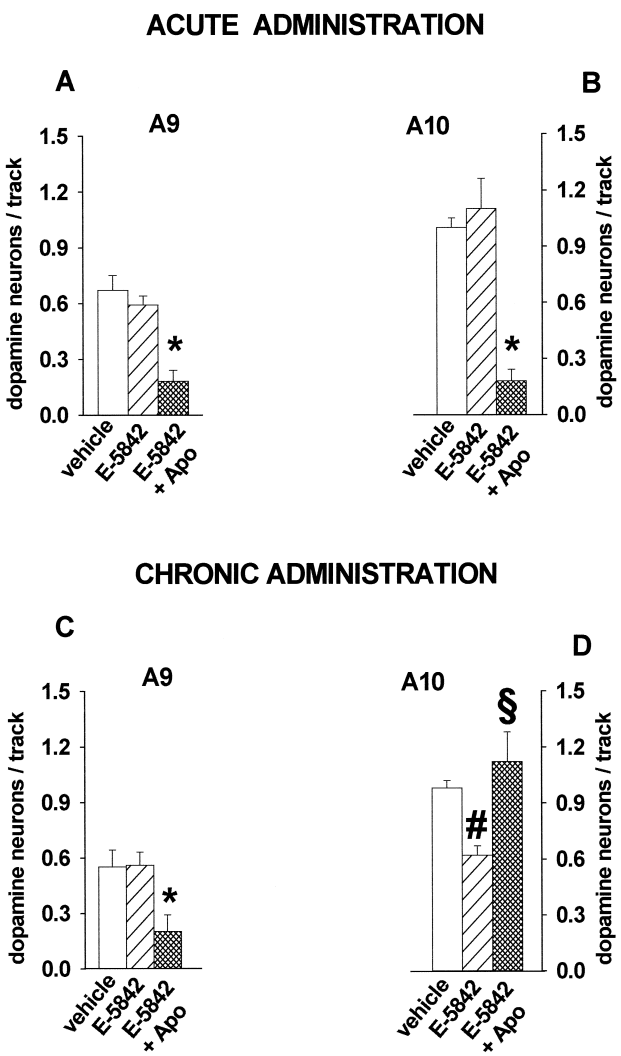

Fig. 2. The effects of acute (A and B) and chronic (C and D) treatment with E-5842 (20 mg kg^{-1}) on the number of spontaneously active mesencephalic dopaminergic neurons. Values express the mean ± S.E.M. * indicates apomorphine (Apo, 50 μg kg^{-1}, i.v., in all cases given acutely) values that were significantly different from the respective drug-treated groups ($P < 0.05$). # represents values that are significantly different from the vehicle treated group ($P < 0.05$). § indicates apomorphine values that are significantly different from the E-5842 treated group ($P < 0.05$) ($n = 4–6$ rats per group).

IC_{50} for apomorphine inhibition of A10 dopamine neurons was of 17 μg kg^{-1}, i.v.

In some experiments, rats were treated with two consecutive 0.5 mg kg^{-1} (i.v.) D-amphetamine injections until the neurons exhibited half of spontaneous basal activity. The subsequent administration of E-5842 (up 3.2 mg kg^{-1}, i.v., cumulative doses) did not modify the inhibitory effect of D-amphetamine (data not shown).

3.2. Cell population studies

In vehicle administered animals, the mean number of encountered spontaneously active dopamine neurons per track in anaesthetized rats was of 0.67 ± 0.18 and 1.00 ± 0.07 for A9 and A10, respectively (mean ± S.E.M.). An acute administration of E-5842 (20 mg kg^{-1}, i.p.) did not affect the number of dopamine neurons encountered (Fig. 2A,B). In both areas, the administration of apomorphine provoked a significant decrease in the number of active neurons after E-5842 administration (Fig. 2A,B).

In rats chronically treated with E-5842 (20 mg kg^{-1} daily for 21 days, s.c.), at the end of the treatment, the number of spontaneously active dopamine neurons of A10 was reduced by about 38% when compared to that of control (vehicle treated) animals (Fig. 2D, hatched bar compared to white bar). The number of active neurons of A9 was unchanged after E-5842 treatment (Fig. 2C). The reduction observed in A10 was clearly reversed by a subsequent administration of a low dose (50 μg kg^{-1}, i.v.) of apomorphine, thereby suggesting a state of depolarization inactivation in A10 (Fig. 2D). Such effect was not observed in A9 (Fig. 2C).

The average firing rate (Hz) for the dopaminergic neurons in A9 (Fig. 3) and A10 (Fig. 3) was not significantly changed after either acute (Fig. 3A and B) or chronic treatment with either E-5842 (20 mg kg^{-1}, s.c., daily for 21 days) or vehicle (Fig. 3C and D). In all the experimental conditions tested, the administration of apomorphine (50 μg kg^{-1}, i.v.) was accompanied by a decrease in the firing rate of the remaining active neurons (Fig. 3A–D).

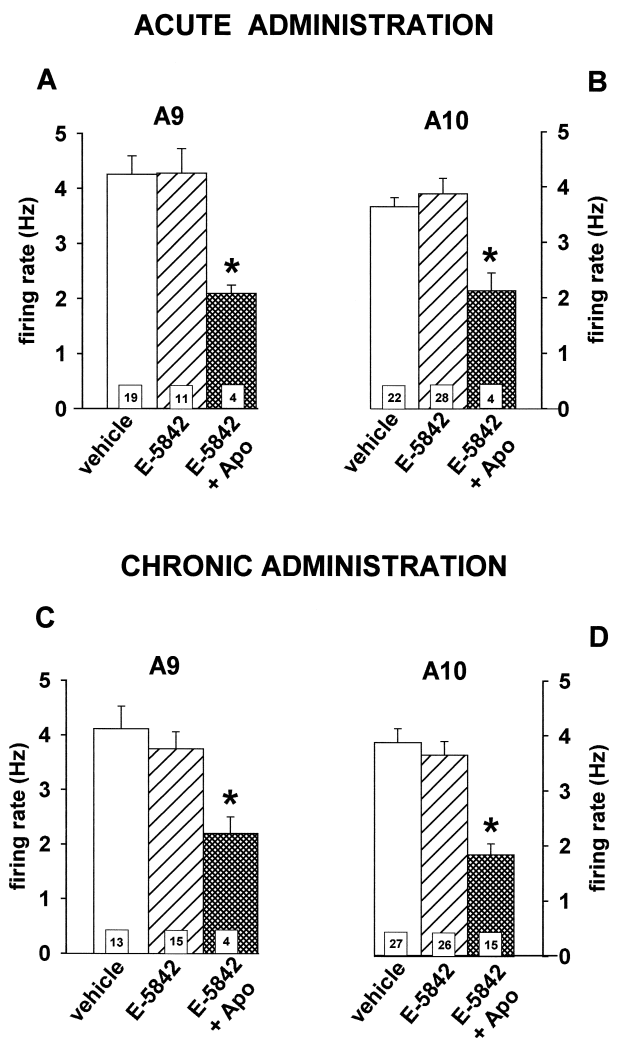

Fig. 3. Effects of the acute (A and B) and chronic (C and D) administration of E-5842 (20 mg kg^{-1}) on the firing rate (Hz, number of cell-firings s^{-1}) on the spontaneous active A9 (A and C) and A10 (B and D). Bars indicate mean ± S.E.M. (the *n* is indicated in the bars). Asterisks indicate statistically significant differences from the E-5842 treated groups ($P < 0.05$). (Apo, Apomorphine, 50 μg kg^{-1}, i.v., in all cases given acutely).

4. Discussion

Acute systemic administration of E-5842 did not change either the firing rate or the amount of spontaneously active dopaminergic neurons of the A9 or A10 areas. Even at the highest dose used (3.2 mg kg^{-1}, i.v.), no effect was seen, while the same dose of clozapine significantly increased the firing rate of these neurons. Classical antipsychotics have been reported to increase the spontaneous firing rate of A9 dopamine neurons and reverse dopamine agonist-induced suppression of these cells to above basal levels (Chiodo and Bunney, 1983), while atypical antipsychotics fail to increase the firing rate of A9 cells above basal levels. In contrast to many of the previously studied typical and atypical antipsychotics (Chiodo and Bunney, 1983), acute administration of E-5842 failed to increase the number of spontaneously active dopamine neurons in A10, an effect similar to that observed with BMY 14802 (Watchel and White, 1988). This could be interpreted as to indicate that E-5842 does not directly interact with the dopamine autoreceptors (extensively localized over the cell membrane of these neurons), in accordance with the relatively low binding affinity of the compound for the dopamine D_2 receptor. However, it has to be pointed out that under our experimental conditions, the IC_{50} for apomorphine inhibition of midbrain dopamine neurons is about 17 μg kg^{-1} when apomorphine is administered after vehicle injection, while as shown in Fig. 3, 50 μg kg^{-1} of apomorphine inhibited both A9 and A10 units by approximately 50%. Altogether, these data clearly suggest that an interaction of

E-5842 with the dopamine receptors should not be completely discarded, although the exact mechanism of such interaction remains unclear.

The repeated administration (21 days) of E-5842, produced a selective decrease in the number of spontaneously active dopamine neurons in the A10 area, while no such effect was observed in the A9 area. These findings are similar to previous results obtained with many atypical antipsychotics and are in contrast to the effects of typical antipsychotics, which inactivate both A9 and A10 cells. As in the case of other antipsychotics, this phenomenon is probably due to the induction of depolarization inactivation, and the effect of chronic E-5842 treatment on A10 dopamine neurons is reversed by apomorphine (Skarsfeldt and Perregaard, 1990; Goldstein et al., 1993; Stockton and Rasmussen, 1996). Depolarization inactivation of dopamine neurons may be elicited by some depolarizing agents or by electrical stimulation, and the decreased activity of A9 and A10 neurons observed after chronic administration of antipsychotic drugs has been postulated to be due to a chronic state of strong depolarization. Depolarization block is typically reverted by hyperpolarizing agents like apomorphine. Apomorphine probably reverses the effect of antipsychotics by causing previously nonfiring dopamine cells to became active (Bunney and Grace, 1978). In this respect, it is interesting to point out that apomorphine (50 $\mu g\ kg^{-1}$) is able to reverse the depolarization block despite the moderate binding affinity of E-5842 for the dopamine D_2 receptor. It is also important to note that while acute administration of E-5842 failed to alter the number of spontaneous active neurons in A10, it was able to induce a decrease of such activity after repeated administration. The possible interaction of E-5842 with a neurotransmitter system other than the dopaminergic should not be discarded. Glutamatergic pathways could be involved in the pathophysiology of schizophrenia (Coyle, 1996), and glutamatergic and dopaminergic systems are reciprocally modulating each other (Karreman and Moghaddam, 1996). In vitro and in vivo experiments have shown that glutamate agonists, mainly through *N*-methyl-D-aspartate (NMDA) receptors may alter the firing rate of dopaminergic neurons (Wang and French, 1993; Meltzer et al., 1997) and ligands may modulate NMDA responses (Debonnel and de Montigny, 1996). In view of these experimental facts, the possibility of an interaction between E-5842 and the glutamatergic transmission should be left open.

After chronic administration of E-5842, the firing rate of the encountered A9 and A10 neurons were not significantly different from those of vehicle-treated animals. This result is in contrast with the observed increased firing rates of the remaining active A10 neurons after repeated clozapine treatment (Chiodo and Bunney, 1983), but in agreement with the effect observed with the atypical antipsychotic olanzapine (Stockton and Rasmussen, 1996). The reason for the selectivity of the effects of E-5842 in A10 respect to A9 dopamine neurons remains unknown, and probably can not be accounted only for its direct effect on σ receptors, as it has been stated above. The administration of different σ receptor ligands, with different degrees of selectivity for the σ receptor, has produced inconsistent and controversial results in electrophysiological and neurochemical studies (Piontek and Wang, 1986; Steinfels and Tam, 1989; Engberg and Wikström, 1991; Zhang et al., 1992, 1993; Poncelet et al., 1993; Shepard et al., 1994; Debonnel and de Montigny, 1996), despite the localization of σ binding sites described in brain (Iyengar et al., 1990; Jansen et al., 1991) In fact, different compounds with different binding profiles (see Arnt and Skarsfeldt, 1998 for a review) have been reported to decrease the number of spontaneously active dopamine cells in both A9 and A10 areas, or exclusively in the A10 area. Whether activity on one or more receptors is needed to account for the depolarization block of the referred neurons is still unknown. The fact that, after chronic E-5842 administration, the number of active neurons in A10 is decreased while their firing rate remains unchanged, would suggest different regulatory influences on the dopaminergic neurons. Apomorphine-induced reversal of the effects of chronically administered E-5842 on A10 dopamine neurons suggests that similarly to other atypical antipsychotics, E-5842 affects the activity of A10 neurons through depolarization inactivation, an experimental phenomenon that has been hypothesized to parallel with therapeutic efficacy. Moreover, its lack of effect on A9 dopamine neurons would suggest the low liability of E-5842, a σ_1 receptor ligand, to induce extrapyramidal symptoms. In conclusion, our results are similar to those obtained for several atypical antipsychotics and suggest the therapeutic efficacy of the compound and the absence of motor side effects after a prolonged treatment.

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