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Clozapine potently stimulates mesocortical dopamine neurons

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Abstract

The effects of clozapine on dopamine neurons projecting to the medial-prefrontal cortex, nuclei accumbens and caudatus were compared. Clozapine (1.25 mg/kg i.v.) maximally stimulated the firing rate and burst activity of dopamine neurons projecting to the medialprefrontal cortex. Much higher doses (5 and 10 mg/kg i.v.) were needed to stimulate mesoaccumbens and nigrostriatal dopamine neurons. The results suggest that the activation of mesocortical dopamine neurons is responsible for clozapine-induced dopamine release in the prefrontal cortex.

Keywords: Clozapine; Dopamine; Electrophysiology

Clozapine is the prototype of atypical antipsychotics, not producing extrapyramidal side-effects and being effective against the negative symptoms of schizophrenia and/or in patients resistant to traditional neuroleptic treatment (Kinon and Lieberman, 1996).

Consistent with its clinical profile, clozapine, unlike classic neuroleptics, is not cataleptogenic in laboratory animals and fails to antagonize stereotypies induced by dopamine receptor agonists, but, like classic neuroleptics, blocks conditioned avoidance response (Kinon and Lieberman, 1996).

It is postulated that the negative symptoms of schizophrenia depend on reduced dopaminergic activity in the prefrontal cortex whereas the beneficial effects of clozapine arise from an enhancement of dopamine release in this brain area. Consistently, microdialysis studies have shown that clozapine markedly increases dopamine release in the prefrontal cortex and this effect is attributed to the stimulation of 5-HT_{1A} receptors (Rollema et al., 1997), the inhibition of 5-HT_{2A} receptors or the preferential inhibition of cortical vs. striatal dopamine D_2 receptors (Lidow et al., 1998).

The purpose of this study was to investigate if clozapine-induced dopamine release might be mediated by the activation of dopamine neurons projecting to the prefrontal cortex.

To this aim, the effect of clozapine on the firing rate and pattern of antidromically identified mesoprefrontal cortical dopamine neurons was studied by extracellular single unit recording techniques and compared with the effect on mesoaccumbens and nigrostriatal dopamine neurons.

Experiments were carried out in non-anesthetized rats because general anesthetics modify both the firing rate and pattern of dopamine neurons and their response to neuroleptics, including clozapine (Mereu et al., 1984). Male Sprague–Dawley albino rats (200–225 g) were used in all experiments. All subjects were kept on a 12 h/12 h light/dark cycle with food and water available ad libitum. Experimental protocols were approved by the Ethical Committee at the University of Cagliari and performed in strict accordance with the E.C. regulations for the care and use of experimental animals (CEE No. 86/609). Electrophysiological experiments were performed as already described (Diana et al., 1998a,b). Firing rate and pattern analysis were performed as already described (Diana et al., 1989). Clozapine was dissolved in 5% tartaric acid and then diluted in saline solution. Injection volumes were 1 ml/kg of body weight. The statistical significance of the data was evaluated by analysis of variance (ANOVA) for repeated measures, while burst data were analysed with Students' *t*-test.

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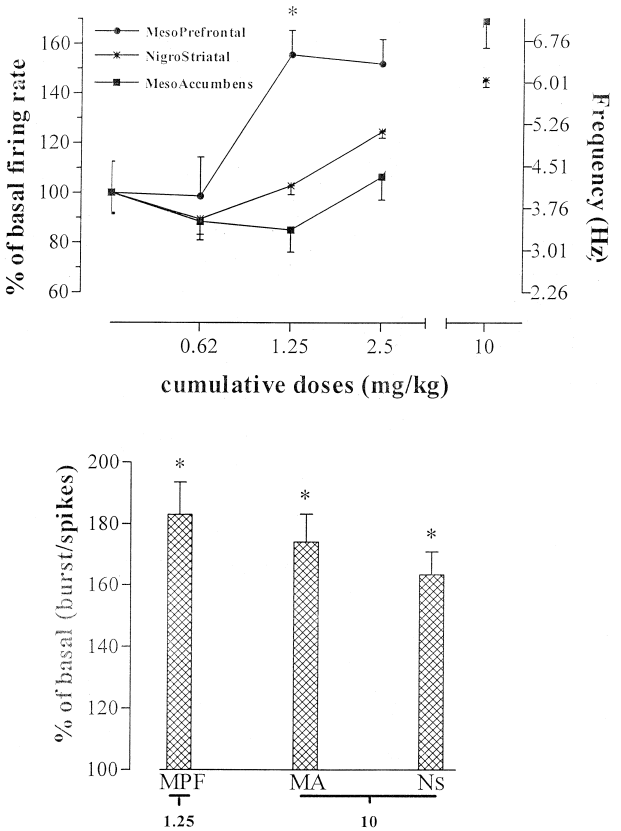

Fig. 1. (top) Dose–response curves for the cumulative doses of i.v. clozapine on the firing rate of antidromically identified mesoprefrontal, mesoaccumbens and nigrostriatal dopamine neurons in non-anesthetized rats. Data are expressed as percentages (left, *Y* axis) and as means of firing rate (right, *Y* axis) ± S.E.M. $* P < 0.002$ ANOVA, see results. (bottom) Effect of clozapine on the burst firing of mesoprefrontal (MPF), mesoaccumbens (MA) and nigrostriatal (NS) dopamine neurons in non-anesthetized rats. The effect was observed at the highest dose administered (10 mg/kg i.v.) for both mesoaccumbens and nigrostriatal dopamine neurons, and at the dose of 1.25 mg/kg i.v. for mesoprefrontal dopamine neurons. Data are expressed as percentages (means ± S.E.M.). $* P <$ 0.0005 with respect to pre-drug level (Student's *t*-test).

The basal firing rate and bursting pattern of dopamine neurons projecting to the prefrontal cortex, nuclei accumbens and caudatus were not different. The intravenous administration of clozapine ($n = 6$) at the dose of 1.25 mg/kg significantly increased the firing rate of mesoprefrontal dopamine neurons by 55.5 ± 9.8% (ANOVA for repeated measures, $F(2,45) = 6.98$; $P < 0.002$). A lower dose of 0.625 mg/kg was ineffective while a higher dose of 2.5 mg/kg produced no further increase. The increase in firing rate was associated with a proportional increase in burst firing (84.95 ± 10.43%, Students' *t*-test $P < 0.0005$). On the other hand, much higher doses of clozapine were needed to activate the firing rate of mesoaccumbens and nigrostriatal dopamine neurons. Maximal stimulation of 69.9 and 45.35%, respectively, was observed with the dose of 10 mg/kg (ANOVA for repeated measures, $F(5,30) =$ 22.05, $P < 0.01$ and $F(4,25) = 3.5$, $P < 0.05$ respectively) (Fig. 1). Higher doses produced no further stimulation (results not shown). Changes in firing rate were associated with parallel modifications in burst activity (74.2 and 63.4%, for mesoaccumbens and nigrostriatal, respectively). Interestingly, the 1.25 mg/kg dose of clozapine that maximally stimulated the firing rate of mesoprefrontal dopamine neurons produced a modest non-significant reduction in the firing rate of mesoaccumbens dopamine neurons.

The results indicate that clozapine potently stimulates the firing rate and the burst firing of dopamine neurons projecting to the prefrontal cortex.

Since stimulation of dopamine neurons in bursts has been shown to cause an increase in dopamine release far greater than that after regularly spaced stimuli, a clozapine-induced increase, both in the firing rate and burst firing, may for a large part sustain the increased release of dopamine in the prefrontal cortex.

Our results are in contrast to previous observations that clozapine fails to activate nigrostriatal dopamine neurons in the chloral hydrate-anesthetized preparation (Hand et al., 1987).

A possible explanation for this discrepancy is that chloral hydrate inhibits the excitatory feedback systems in response to blockade of postsynaptic dopamine receptors in the striatum.

Irrespective of the mechanisms involved, the selective stimulation of mesocortical dopamine neurons indicates that the mechanisms regulating the activity of the different populations of dopamine neurons are dissociable.

An important problem is whether neuronal activation represents the compensatory response to the blockade of dopamine receptors, therefore reflecting inhibition of dopamine neurotransmission, or whether clozapine, by activating dopamine neurons, e.g., via 5-HT_{1A} receptors, causes a release of endogenous dopamine onto unblocked receptors.

This problem is extremely relevant for the interpretation of the role of cortical dopamine in the negative symptoms of schizophrenia.

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