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Short communication

Clozapine does activate nigrostriatal dopamine neurons in unanesthetized rats

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Abstract

Antipsychotic drugs are traditionally classified as typical or atypical on the basis of their property to cause or not to cause extrapyramidal side-effects. A widely accepted selectivity for the mesolimbic, vs. the nigrostriatal, dopaminergic system is postulated to underlie the existence of fewer or no extrapyramidal side-effects during treatment with atypical neuroleptics. In order to verify this hypothesis we examined the effect of acute clozapine on nigrostriatal dopaminergic neurons recorded from non-anaesthetised and from chloral hydrate-anaesthetised rats. Extracellular single-unit recording coupled with antidromic activation from the neostriatum was used. Intravenous administration of cumulative doses of clozapine (1.25–10 mg/kg) increased the firing rate of nigrostriatal dopaminergic neurons in non-anaesthetised rats, but failed to significantly modify the activity of the same units under chloral hydrate anesthesia. These results indicate that acute clozapine activates nigrostriatal dopamine cells in non-anaesthetised rats and cast doubts about a direct link between the lack of significant extrapyramidal side-effects and the selectivity of atypical neuroleptics, such as clozapine, for the mesolimbic dopamine system.

Keywords: Clozapine; Dopamine; Electrophysiology; Neuroleptic atypical; Substantia nigra pars compacta

1. Introduction

Clozapine has been described as an atypical antipsychotic drug, with proven superior efficacy for neuroleptic-resistant schizophrenic patients (for a review see Arnt and Skarsfeldt, 1998). It also has a favourable side-effect profile with minimal or no extrapyramidal symptoms such as parkinsonism, dystonia, akathisia and tardive dyskinesia. The commonly accepted hypothesis for the profile of clozapine as well as of other atypical antipsychotic drugs is its selective action on the mesolimbic dopaminergic system. Indeed, there are reports from several electrophysiological studies that atypical neuroleptics interact selectively with central dopaminergic systems, affecting the neuronal activity of mesolimbic, but not of nigrostriatal, cells (Chiodo and Bunney, 1983; White and Wang, 1983). These findings, coupled with some biochemical evidence, provided the grounds to support the idea that antipsychotic effects are mediated by mesolimbic, while extrapyramidal side-effects are mediated by nigrostriatal, dopamine systems.

However, results of behavioral rodent experiments and of more recent biochemical and microdialysis experiments provide only little support for this hypothesis and none for the notion that the clinical profile of atypical neuroleptics derives from a selective action on mesolimbic dopamine neurons (Kinon and Lieberman, 1996).

One possible explanation for these discrepancies might be that electrophysiological experiments were carried out under general anesthesia, which has been shown to influence basal activity and pharmacological responsiveness of nigrostriatal dopamine neurons (Kelland et al., 1990).

In order to verify whether the propensity of clozapine not to induce extrapyramidal side-effects reflects such selectivity, we examined the effect of acute clozapine on the electrical activity of nigrostriatal dopamine neurons in

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both non-anaesthetised and chloral hydrate-anaesthetised rats.

2. Materials and methods

2.1. Animals

Male Sprague Dawley albino rats (Charles River, Como, Italy) weighing 200 to 225 g were used in all experiments. All subjects were kept on a 12/12 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).

2.2. Anaesthetised rats

The rats in this group were anaesthetised with chloral hydrate (400 mg/kg i.p.) which was supplemented as needed through i.p. infusion via a butterfly needle throughout the experiment. Thereafter, chloral hydrate anaesthetised and non anaesthetised rats were prepared according to an identical protocol (see below).

2.3. Non-anaesthetised rats

The rats were temporarily anaesthetised with halothane/room air inhalation anaesthesia and the femoral vein was cannulated for intravenous administration of pharmacological agents. The trachea was then exposed and incised to allow tracheal intubation with a Teflon catheter for artificial respiration. Inhalation anesthesia was disconnected and Gallamine triethiodide (20 mg/kg i.v.) was administered intravenously; once muscular paralysis was obtained, the rat was placed in a stereotaxic apparatus (Kopf) and the tracheal catheter was connected with a mechanical rodent ventilator (7025 Stoelting) set to deliver 90 strokes/min (3 ml/stroke). All incision and pressure points were infiltrated with a 2% solution of xylocaine and supplemented as needed. Body temperature was maintained constant at 38 ± 1°C by means of an electrically controlled heating pad. Thereafter (30–45 min after temporary anesthesia), rats were prepared as follows: the scalp was retracted and two burr holes were drilled, one for the placement of a recording electrode above the substantia nigra pars compacta (AP 6.04 mm posterior to bregma; L 1.8 mm from midline, and 6–7 mm from cortical surface) (Paxinos and Watson, 1982) and the other to introduce a Formvar coated stainless-steel bipolar electrode for antidromic identification in the neostriatum (AP 1.0 mm anterior to bregma; L 3.6 mm from midline; V 3.75 mm from dura matter; inclination 10°). Stimuli consisting of monophasic rectangular pulses (0.1–2.0 mA; 0.1–0.5 ms; 0.8 Hz) were generated. The stimulating current was monitored on the oscilloscope. Dopamine neurons were identified according to well established electrophysiological characteristics (Bunney et al., 1973) and by antidromic activation from the neostriatum including collision of an antidromically-elicited spike with spontaneously occurring action potentials (Guyenet and Aghajanian, 1978; Lipski, 1981). The extracellular neuronal signal from single neurons was amplified (Neurolog System) and displayed on a digital oscilloscope (Philips pm 3305) before storage on magnetic tape for off-line analysis of the data. Firing rate and pattern analysis were performed as already described (Diana et al., 1989). Clozapine (Sandoz, Milano, Italy) was dissolved in 5% tartaric acid and then diluted in saline solution. Injection volumes were 1 ml/kg of body weight. Basal firing rate was recorded for 5 min and drugs were administered i.v. at exponentially increasing doses at 120 s intervals. Changes in firing rate and burst rate were calculated by averaging the effects of the drugs for the 2 min

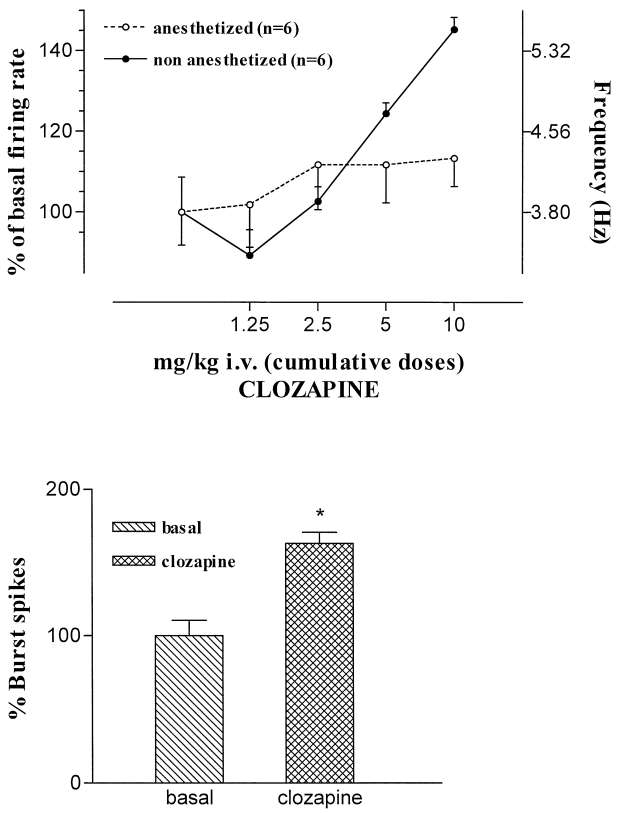

Fig. 1. (top) Dose–response curves depicting the effect of cumulative doses of i.v. clozapine on the firing rate of antidromically identified nigrostriatal dopamine neurons in anaesthetised and non-anaesthetised rats. Data are expressed as percentages (on left *Y* axis) and as means of firing rate (on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

after drug or vehicle administration and comparing them to the mean of the pre-drug baseline. The statistical significance of the data was evaluated by analysis of variance (ANOVA) for repeated measures, while burst data were analysed with Student's *t*-test.

3. Results

As shown in Fig. 1 (top) the i.v. administration of clozapine ($n = 6$) as a cumulative dose regimen (1.25–10 mg/kg) to non-anaesthetised rats increased the firing rate of nigrostriatal dopamine cells.

At the highest dose tested, a maximal increase of 45.34 ± 2.99% was observed (ANOVA for repeated measures, $F(4,25) = 3.5$; $P < 0.05$).

The clozapine-induced stimulation of the firing rate in non-anaesthetised rats was associated with an augmented burst activity (Student's *t*-test, $n = 6$, $T = 4.89$; $P < 0.0005$; Fig. 1, bottom). Augmented electrical activity lasted up to 1 h after the last dose of clozapine and was reversed towards pre-drug values by i.v. administration of apomorphine (10–40 μg/kg).

On the contrary, clozapine failed to produce a significant increase in the electrical activity of nigrostriatal dopamine neurons in chloral hydrate-anaesthetised rats ($n = 6$).

This lack of effect was significant when compared with results for non-anaesthetised rats (ANOVA for repeated measures, $F(4,1) = 2.75$; $P < 0.05$) (Fig. 1, top).

Although clozapine did not change the basal firing rate under chloral hydrate anaesthesia, the doses of apomorphine required to inhibit the electrical activity of nigrostriatal dopamine cells by 40% were higher in clozapine- ($n = 6$) than in saline-treated ($n = 6$) rats, being 40 and 10 μg/kg respectively (ANOVA for repeated measures, $F(1,40) = 10.82$, $P < 0.001$) (Fig. 2).

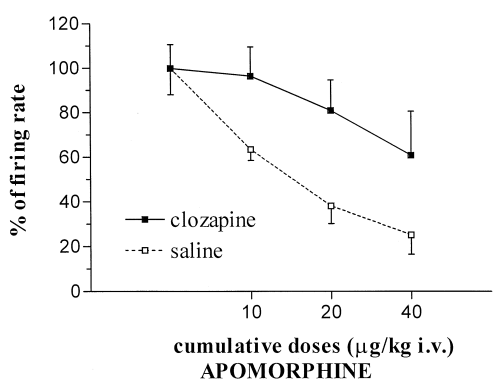

Fig. 2. Dose–response curves depicting the inhibitory effect (ANOVA, see Section 3) of cumulative doses of apomorphine on the firing rate of antidromically identified nigrostriatal dopamine neurons in clozapine-treated (10 mg/kg i.v.) anaesthetised rats. Data (mean ± S.E.M.) are expressed as percentages of the basal firing rate.

4. Discussion

The present results show that clozapine, the prototype of atypical neuroleptics, stimulates the firing rate of antidromically identified nigrostriatal dopamine neurons in non-anaesthetised rats.

However, we have also shown, in agreement with previous observations, that clozapine fails to activate nigrostriatal dopamine neurons in chloral hydrate-anaesthetised animals (Hand et al., 1987).

The finding that clozapine does stimulate nigrostriatal dopamine neurons in non-anaesthetised but not in anaesthetised rats suggests that chloral hydrate blunts the pharmacological responsiveness of dopamine neurons (Kelland et al., 1990), possibly by inhibiting the long-loop feedback pathway activated in response to blockade of dopamine actions at postsynaptic sites in the neostriatum.

Furthermore, in line with previous results (Hand et al., 1987), the finding that clozapine prevented the inhibitory effect of apomorphine on dopamine cell activity suggests that chloral hydrate anaesthesia does not suppress the ability of clozapine to block dopamine autoreceptors.

Since neuroleptic-induced excitation of dopamine cells is believed to be the response to both blockade of dopamine action at both pre- (Pucak and Grace, 1994, 1996) and post-synaptic receptors, the failure of clozapine to activate nigrostriatal dopamine neurons has been interpreted as an indication of the lack of inhibition of dopaminergic receptors by atypical neuroleptics. Conversely, the selective functional blockade of mesolimbic system ascribed to its selective activation has been considered to be predictive of the clinical atypical antipsychotic activity (for a review see Arnt and Skarsfeldt, 1998).

Our results indicate that clozapine increases the firing rate of nigrostriatal dopamine neurons and thus its action is not selective for the mesolimbic dopamine system. Therefore, the mechanism underlying the lack of its cataleptogenic effect in experimental animals, and of extrapyramidal side-effects in humans, is not linked to the anatomically selective blockade of dopaminergic transmission by clozapine.

Since clozapine has a high affinity and antagonistic activity at different muscarinic and serotonin receptors, including 5-HT_{2A} receptors, the interaction with any one and/or a specific combination of these receptors might account for the lack of cataleptic response in experimental animals and of extrapyramidal side-effects in humans. Accordingly, both muscarinic and 5-HT_{2A} receptor antagonists have been shown to counteract the haloperidol-induced catalepsy in rats and the extrapyramidal side-effects in humans.

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