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Inhibitory Action of Clozapine on Rat Ventral Tegmental Area Dopamine Neurons Following Increased Levels of Endogenous Kynurenic Acid

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The mode of action by which the atypical antipsychotic drug clozapine exerts its superior efficacy to ameliorate both positive and negative symptoms is still unknown. In the present *in vivo* electrophysiological study, we investigate the effects of haloperidol (a typical antipsychotic drug) and clozapine on ventral tegmental area (VTA) dopamine (DA) neurons in a situation of hyperdopaminergic activity in order to mimic tentatively a condition similar to that seen in schizophrenia. Increased DA transmission was induced by elevating endogenous levels of the *N*-methyl-D-aspartate receptor and α7* nicotinic receptor antagonist kynurenic acid (KYNA; by means of PNU 156561A, 40 mg /kg, i.v.). In control rats, i.v. administered haloperidol (0.05–0.8 mg/kg) or clozapine (1.25–10 mg/kg) was associated with increased firing rate and burst firing activity of VTA DA neurons. However, in rats displaying hyperdopaminergia (induced by elevated levels of KYNA), the effects of clozapine on VTA DA neurons were converted into pure inhibitory responses, including decrease in burst firing activity. In contrast, haloperidol still produced an excitatory action on VTA DA neurons in rats with elevated levels of endogenous brain KYNA. The results of the present study suggest that clozapine facilitates or inhibits VTA DA neurotransmission, depending on brain concentration of KYNA. Such an effect of clozapine may be related to its unique effect in also ameliorating negative symptoms of schizophrenia.

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INTRODUCTION

Schizophrenia has for decades been associated with dopaminergic (DA) hyperactivity (Abi-Dargham *et al*, 2000; see Carlsson *et al*, 2001). The beneficial effects of classic antipsychotic drugs (eg haloperidol), especially with regard to ameliorating the positive symptoms of schizophrenia, are thought to be related to a reduced DA neurotransmission within the limbic region of the brain (see Carlsson *et al*, 2001), in particular, to antagonism of DA-D_2 receptors. A profound blockade of DA-D_2 receptors in the basal ganglia is, however, also associated with side effects, such as extrapyramidal symptoms (EPS; Farde *et al*, 1992; see Gerlach, 2002). The antipsychotic drug clozapine, with remarkable efficacy in treatment-resistant schizophrenia, has very low incidence of EPS and has thus been classified as an atypical antipsychotic drug (Claghorn *et al*, 1987; Coward *et al*, 1989). Another beneficial effect of clozapine is its superior efficacy in also ameliorating negative symptoms. During the last decade, large efforts have been made to understand the mode of antipsychotic action of clozapine, but despite a large number of studies, our knowledge remains fragmentary. It is established that clozapine not only interacts with all DA receptors (the D_4 subtype showing the highest affinity (19 nM; Van Tol *et al*, 1991)), but also with other metabotropic receptors, for example, those for serotonin ($5HT_1$ and $5HT_2$; Canton *et al*, 1990), acetylcholine (Snyder *et al*, 1974), noradrenaline (see Coward, 1992), and histamine (see Brunello *et al*, 1995; see Coward, 1992). Previous studies also point to an interaction with some ionotropic receptors, for example, the *N*-methyl-D-aspartate (NMDA) receptor (Arvanov *et al*, 1997; Ossowska *et al*, 1999) and the $GABA_A$ receptor (Squires and Saederup, 1998). It has been suggested that clozapine has a preferential action on the mesolimbic DA system as compared to the nigrostriatal DA system (Andén and Stock, 1973; Bartholini, 1976; Chiodo and Bunney, 1983, 1985; Moghaddam and Bunney, 1990). Moreover, since clozapine is a potent serotonin 5-HT_2-receptor antagonist, it has been suggested that concurrent 5-HT_2 and DA-D_2 receptor antagonism may contribute to its atypical profile (see Deutch *et al*, 1991; Ichikawa *et al*, 2001; Meltzer and Nash, 1991).

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Previous electrophysiological studies show that all antipsychotic drugs, including clozapine and haloperidol, when acutely administered increase the firing rate and burst firing activity of ventral tegmental area (VTA) DA neurons (Gessa *et al*, 2000; Tung *et al*, 1991; White and Wang, 1983). However, most of these results derive from studies where naive control rats have been used. In the present *in vivo* electrophysiological study, we investigate the effects on VTA DA neurons of clozapine or haloperidol in a situation of hyperdopaminergia in order to mimic tentatively a condition similar to that occurring in schizophrenia. Increased DA transmission was pharmacologically induced by administration of the kynurenine 3-hydroxylase inhibitor PNU 156561A, thereby elevating endogenous brain levels of the NMDA-receptor antagonist kynurenic acid (KYNA). Previous studies demonstrate that such treatment is associated with increased firing rate and burst firing activity of rat midbrain DA neurons (Erhardt and Engberg, 2002; Erhardt *et al*, 2001a).

MATERIALS AND METHODS

Animals

Experiments were performed on male Sprague–Dawley rats (B&K Universal AB, Sollentuna, Sweden; weighing between 200 and 250 g). The animals were housed in groups of five, and free access to food and water was provided. Environmental conditions were checked daily and maintained under constant temperature (25°C) and 40–60% humidity in a room with a regulated 12-h light/dark cycle (lights on at 0600). Experiments were approved by and performed in accordance with the guidelines of the Ethical Committee of Northern Stockholm, Sweden and all efforts were made to minimize the number of animals used and their suffering.

Surgery

Before surgery, rats were pretreated with PNU 156561A (dissolved in 10% β-cyclodextrin) or vehicle i.v. The animals were placed in a restrainer and a temporary cannula was inserted into a lateral tail vein. Following drug administration, the cannula was removed and the rats were placed individually in a Plexiglas cage. About 5 h later, rats were anesthetized (chloral hydrate; 400 mg/kg, intraperitoneally) and then mounted onto the ear bars of a conventional stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) so that the skull was set in a horizontal plane and the nose was secured using a clamp at the front of the frame. For i.v. administration, a cannula was again inserted into a lateral tail vein and additional injections of chloral hydrate were given as they were required to maintain a stable level of anesthesia. Also, clozapine and haloperidol were given through the lateral tail vein. Throughout the experiments, the body temperature of the animals was maintained at 37°C by means of a thermostatic heating pad. The skull surface was exposed and a 3-mm burr hole was drilled with its center located approximately 3 mm anterior to the lambda and 0.7 mm lateral to the midline.

Extracellular Single Unit Recording

Following careful removal of the dura, a glass microelectrode with a tip diameter of approximately 1–2 μm (filled with 2 M sodium acetate saturated with Pontamine Sky Blue) was lowered by means of a hydraulic microdrive (David Kopf Instruments, Tujunga, CA, USA) into the region of VTA, according to the stereotaxic coordinates from the atlas of Paxinos and Watson (1998). The *in vitro* impedance of the electrode was generally 5–8 MΩ, measured at 135 Hz in 0.9% saline. Single unit potentials were passed through a high input-impedance amplifier and filters. The impulses were discriminated from background noise and fed into a computer, and simultaneously displayed on a digital storage oscilloscope, monitored on an audio monitor and on a strip chart recorder (Gould). All DA neurons were found 7.5–8.5 mm from the brain surface and all DA neurons fulfilled the neurophysiological characteristics (triphasic action potential of more than 2.0 ms, basal firing rates of 1 and 10 Hz, and frequent occurrence of burst firing) previously described for DA neurons in the VTA (Wang, 1981). Only one DA neuron was studied in each rat. The position of the electrode was marked at the end of each experiment by iontophoretic ejection of Pontamine Sky Blue. The brains were subjected to conventional histological procedures for verification of recording sites. All recording sites were found within the boundaries of the VTA. Anatomical subdivisions (nucleus parabrachialis pigmentosus (PBP) or nucleus paranigralis (PN)) were assessed according to the atlas of Paxinos and Watson (1998).

Data Analysis

The distribution of spikes was analyzed online utilizing a Macintosh computer. The software used for the analysis of firing was written in-house using a high-level object-oriented programming language called 'G' (Lab VIEW; National Instruments, Austin, TX, USA). The software was designed to sample and analyze the intervals of an arbitrary number of TTL pulses (corresponding to spikes passing through the discriminating filter) using a time resolution of 1 ms. An interspike interval was designated as the time (in ms) elapsed between the rising edges of two sequential TTL pulses. In order to avoid artifacts in the sampling procedure, the spike analyzer ignored time intervals below 20 ms. The onset of a burst was determined as an interspike interval shorter than 80 ms and the termination of a burst by the next interval longer than 160 ms (Grace and Bunney, 1984a, b). The software program also sorted the intervals of recorded spikes and divided them into 3 ms bins and displayed the results as an interspike time interval histogram (ISH) with regard to the number of intervals corresponding to each bin. The intervals were analyzed with regard to the number of bursts that occurred during a 100-spike sampling period along with the calculation of the percentage of spikes fired in bursts. Firing rate, percentage of spikes fired in bursts and variation coefficient (calculated as the ratio between the standard deviation and the mean interval of an ISH and used as a measure of the regularity of firing; Werner and Mountcastle, 1963), were expressed as the median of at least three consecutive ISHs.

Drugs

The following drugs were used: chloral hydrate (Merck, Darmstadt, Germany), PNU 156561A [(*R*,*S*)-2-amino-4-oxo-4(3′-f′-dichlorophenyl) butanoic acid] (kindly donated by Dr C Speciale, Pharmacia & Upjohn, Milano, Italy), *β*-cyclodextrin, clozapine (Sigma, St Louis, MO, USA), and haloperidol (Janssen Pharmaceutical, NV, 2340 Beerse, Belgium).

Statistical Analysis

Statistically significant differences regarding firing rate, burst firing activity, and variation coefficient were established using the Kruskal–Wallis analysis of variance followed by the Wilcoxon signed rank test or the Mann–Whitney *U*-test. Significance was assumed for all values where $P<0.05$.

RESULTS

I.V. administration of the atypical antipsychotic drug clozapine ($n=11$; 1.25–10 mg/kg) or the classic antipsychotic drug haloperidol ($n=7$; 0.05–0.8 mg/kg) produced a dose-dependent increase in firing rate and in the percentage of spikes fired in bursts of VTA DA neurons (Table 1, Figure 1). No significant effects on the regularity of firing, expressed as the variation coefficient, were observed by either drug (Figures 3 and 4). Clozapine or haloperidol produced a significant increase in the mean number of bursts (recorded during a 100-spike sampling period) as well as in the mean number of spikes per bursts when compared with predrug levels (see Table 1). Moreover, two of nine DA neurons were excluded from analysis due to induction of depolarization block after 0.2 and 0.4 mg/kg haloperidol, respectively.

Administration of PNU 156561A (40 mg/kg, i.v., 5–7 h) has previously been shown to elevate endogenous levels of KYNA three- to five-fold (Erhardt and Engberg, 2000, 2002; Erhardt *et al*, 2000, 2001a, b, 2002a) and to increase significantly firing rate and percent spikes fired in bursts of rat midbrain DA neurons (Erhardt and Engberg, 2002; Erhardt *et al*, 2001a). However, in the present study efforts were made to avoid recording of DA neurons with very high frequency and burst firing activity, that is, basal firing rate over 10 Hz and basal percentage of spikes fired in bursts over 90%. In line with our previous study (Erhardt and Engberg, 2002), 25% of all VTA DA neurons in rats with elevated levels of KYNA occasionally showed depolarization-block characteristics. Such neurons were excluded from pharmacological analyses. In spite of these limitations, a tendency toward higher basal frequency and higher basal percentage of spikes fired in bursts were found in rats with elevated KYNA levels (Figures 2, 3a, b), although no statistical significances were obtained due to the relatively low numbers of neurons analyzed.

Pretreatment with PNU 156561 A (40 mg/kg, i.v., $n=8$, 5–7 h) was found not only to antagonize the increase in firing rate and percentage of spikes fired in burst of VTA DA neurons induced by clozapine (1.25–10 mg/kg, i.v.), but also to reverse the action of clozapine, leading to a tendency in reduction in firing rate and to a significant decrease in the

Table 1 Effects of clozapine and haloperidol on firing rate and spike distribution of dopamine neurons in the VTA, in controls, and in rats pretreated with PNU 156561A

	Controls					PNU 156561 A				
		Clozapine					Clozapine			
	Controls ($n=11$)	1.25 mg/kg ($n=8$)	2.5 mg/kg ($n=8$)	5 mg/kg ($n=7$)	10 mg/kg ($n=6$)	Control ($n=8$)	1.25 mg/kg ($n=6$)	2.5 mg/kg ($n=7$)	5 mg/kg ($n=7$)	10 mg/kg ($n=6$)
Firing rate (Hz)	3.7 ± 0.5	6.0 ± 0.8*	6.4 ± 0.8*	5.7 ± 1.0*	6.2 ± 1.7*	5.5 ± 0.9	4.9 ± 0.7	4.6 ± 0.5	4.2 ± 0.6	3.7 ± 0.5
Mean % of spikes fired in burst	19.1 ± 7.0*	42.4 ± 10.0*	48.0 ± 11.6*	52.1 ± 12.1*	58.0 ± 12.2*	32.9 ± 11.2	13.3 ± 10.1^{+}	6.3 ± 4.3^{++}	9.4 ± 5.9^{++}	8.2 ± 8.2^{+}
Mean number of bursts	6.1 ± 1.8	8.9 ± 2.3*	8.1 ± 2.0	11.1 ± 2.1	11.2 ± 2.9	6.5 ± 1.9	3.5 ± 2.6	2.0 ± 1.4*$^{+}$	3.7 ± 2.4^{+}	2.5 ± 2.5^{+}
Mean spikes per burst	3.1 ± 0.3	3.8 ± 0.6	6.7 ± 1.9	5.6 ± 2.0	9.3 ± 3.6	4.8 ± 0.8	3.6 ± 0.2	2.8 ± 0.2	3.1 ± 0.3	3.3 ± 0.0
		Haloperidol					Haloperidol			
	Controls ($n=7$)	0.05 mg/kg ($n=7$)	0.1 mg/kg ($n=7$)	0.2 mg/kg ($n=7$)	0.4 mg/kg ($n=7$)	0.8 mg/kg ($n=6$)	Control ($n=4$)	0.05 mg/kg ($n=4$)	0.1 mg/kg[a] ($n=4$)	0.2 mg/kg[a] ($n=4$)	0.4 mg/kg[a] ($n=4$)	0.8 mg/kg[a] ($n=4$)
Firing rate (Hz)	3.9 ± 0.6	4.3 ± 0.8	4.9 ± 0.6	5.1 ± 0.7*	6.5 ± 1.0*	6.8 ± 0.9*	5.9 ± 1.5	5.9 ± 1.5	—	—	—	—
Mean % of spikes fired in burst	28.9 ± 7.1	36.4 ± 8.0*	43.1 ± 7.3*	40.2 ± 11.0	49.3 ± 12.0*	52.6 ± 11.7*	28.1 ± 12.3	33.3 ± 11.3	—	—	—	—
Mean number of bursts	10.7 ± 3.0	11.9 ± 2.9	14 ± 3.0*	12.0 ± 2.7	11.0 ± 3.0	11.7 ± 2.9	7.0 ± 2.5	8.3 ± 2.2	—	—	—	—
Mean spikes per burst	3.2 ± 0.3	3.8 ± 0.6	3.8 ± 0.6	3.7 ± 0.5	5.9 ± 1.8	6.5 ± 1.9	6.2 ± 2.9	8.2 ± 5.1	—	—	—	—

Values represent means ± SEM. Statistics: *$P<0.05$ *vs* corresponding predrug value (the Wilcoxon signed rank test) and $^{+}P<0.05$, $^{++}P<0.01$ *vs* corresponding control value (the Mann–Whitney *U*-test).
[a]Values not obtained due to depolarization block.

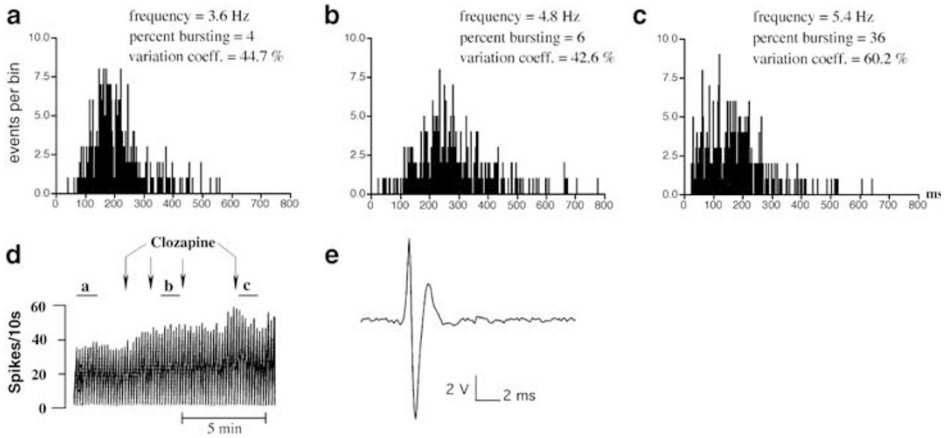

Figure 1 Extracellular recording from a spontaneously bursting DA neuron in the VTA depicting the effect of i.v. administration of clozapine. (a) ISH before drug administration. (b) ISH after administration of clozapine (2.5 mg/kg, i.v.). (c) ISH after administration of clozapine (10 mg/kg, i.v.). (d) Cumulative rate histogram showing the action of clozapine (1.25 + 1.25 + 2.5 + 5 mg/kg, injected at arrows) on the firing rate. Horizontal bars indicate the time periods where the three ISHs were recorded. (e) Spontaneous action potential from the same DA neuron.

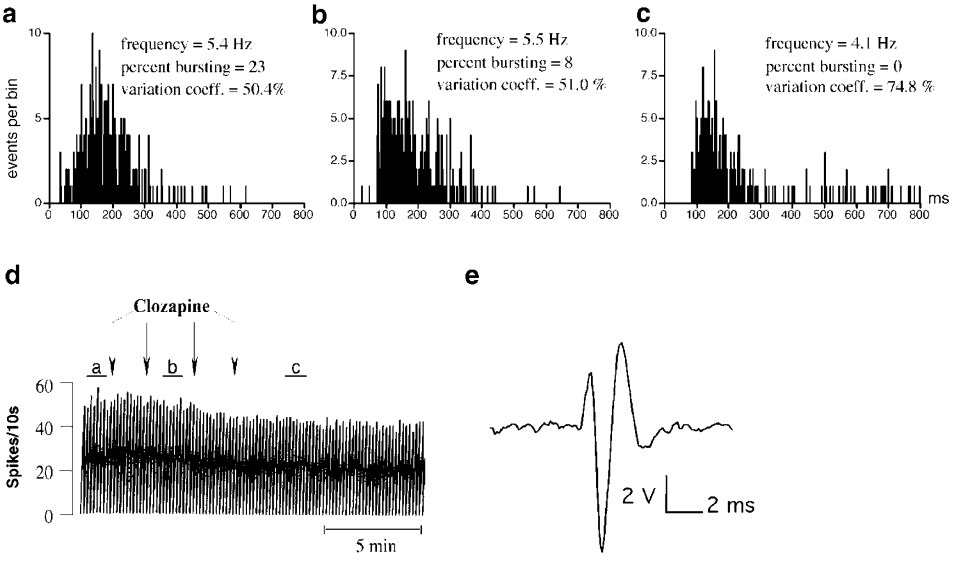

Figure 2 Extracellular recording from a spontaneously bursting DA neuron in the VTA depicting the effect of i.v. administration of clozapine following pretreatment with PNU 156561 A (40 mg/kg, i.v., 6 h). (a) ISH before drug administration. (b) ISH after administration of clozapine (2.5 mg/kg, i.v.). (c) ISH after administration of clozapine (10 mg/kg i.v). (d) Cumulative rate histogram showing the action of clozapine (1.25 + 1.25 + 2.5 + 5 mg/kg, injected at arrows) on the firing rate. Horizontal bars indicate the time periods where the three ISHs were recorded. (e) Spontaneous action potential from the same DA neuron.

percentage of spikes fired in burst (Table 1, Figure 2). This reduction in burst firing activity was observed in seven of eight DA neurons, irrespective of predrug basal characteristics. Furthermore, four of seven spontaneously bursting neurons were converted into nonbursting neurons by clozapine. Thus, this pretreatment decreased the average number of burst during a 300-spike sampling period as well as the number of spikes within a burst (see Table 1). No significant effects on the regularity of firing expressed as the variation coefficient was observed (Figures 3).

In contrast to clozapine, the effects of haloperidol (see Figures 4) on VTA DA neurons after pretreatment with PNU 156561A seemed to be clearly potentiated, since haloperidol forced all DA neurons recorded into depolarization block already after 0.1 mg/kg haloperidol (see Table 1).

DISCUSSION

The present study describes an interaction between endogenous KYNA and the response of VTA DA neurons to various antipsychotic drugs. KYNA is a noncompetitive antagonist of the NMDA receptor ion-channel complex, acting on the strychnine-insensitive glycine recognition site (Birch *et al*, 1988), with an IC_{50} in the low micromolar range ($IC_{50} = 7.9\,\mu M$; Ganong and Cotman, 1986; Kessler *et al*, 1989; Parsons *et al*, 1997). Furthermore, a recent study showed that KYNA blocks the α7* nicotinic receptor with the same IC_{50} value as for the glycine-site of the NMDA receptors (Hilmas *et al*, 2001).

Previous studies have shown that the concentration of KYNA is elevated in the cerebrospinal fluid of schizophrenic patients (Erhardt *et al*, 2001c) as well as in the post-

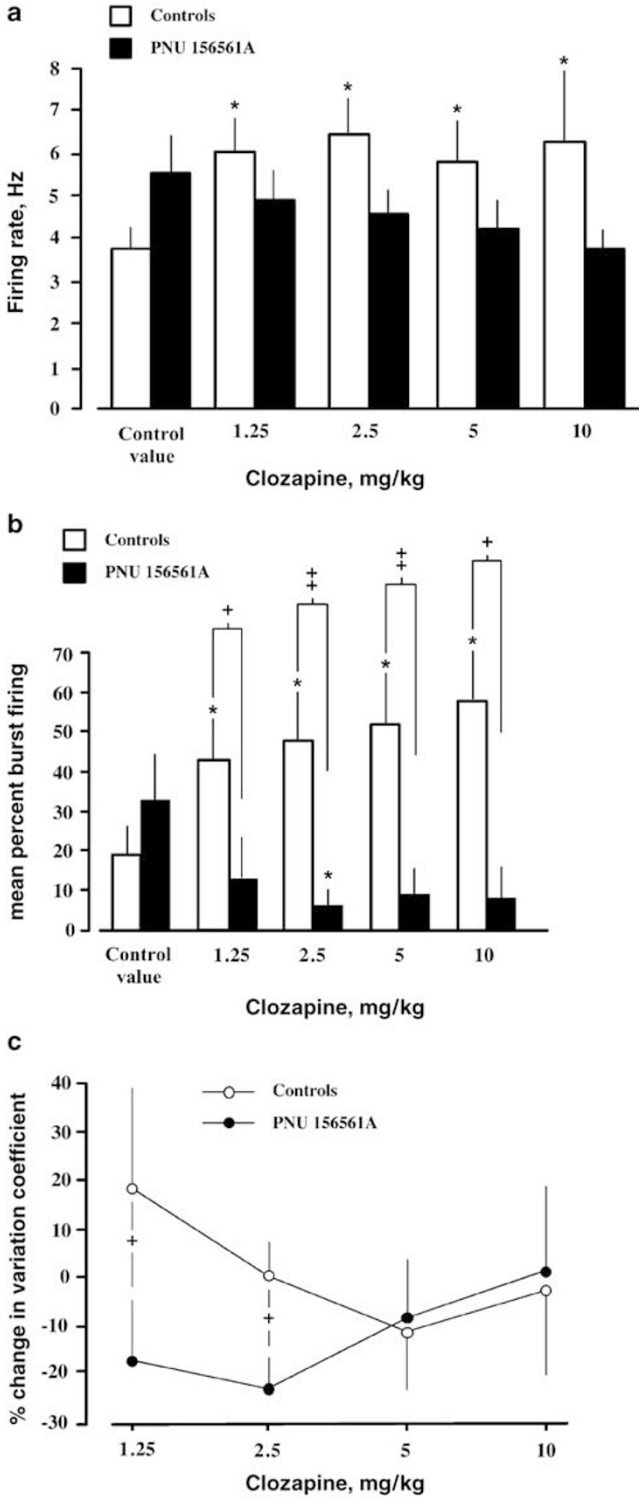

Figure 3 Effects of incremental doses of i.v. administered clozapine (1.25–10 mg/kg) in control rats and rats pretreated with PNU 156561 A (40 mg/kg, i.v., 5–7 h) on (a) the firing rate, (b) the percent burst firing activity and (c) the regularity of firing (assessed by the variation coefficient). Each value represents mean ± SEM from six to 11 VTA DA neurons. Statistic: $^{*}P<0.05$ (Wilcoxon signed rank test) *vs* corresponding predrug value and $^{+}P<0.05$, $^{++}P<0.01$ *vs* corresponding control value (the Mann–Whitney *U*-test).

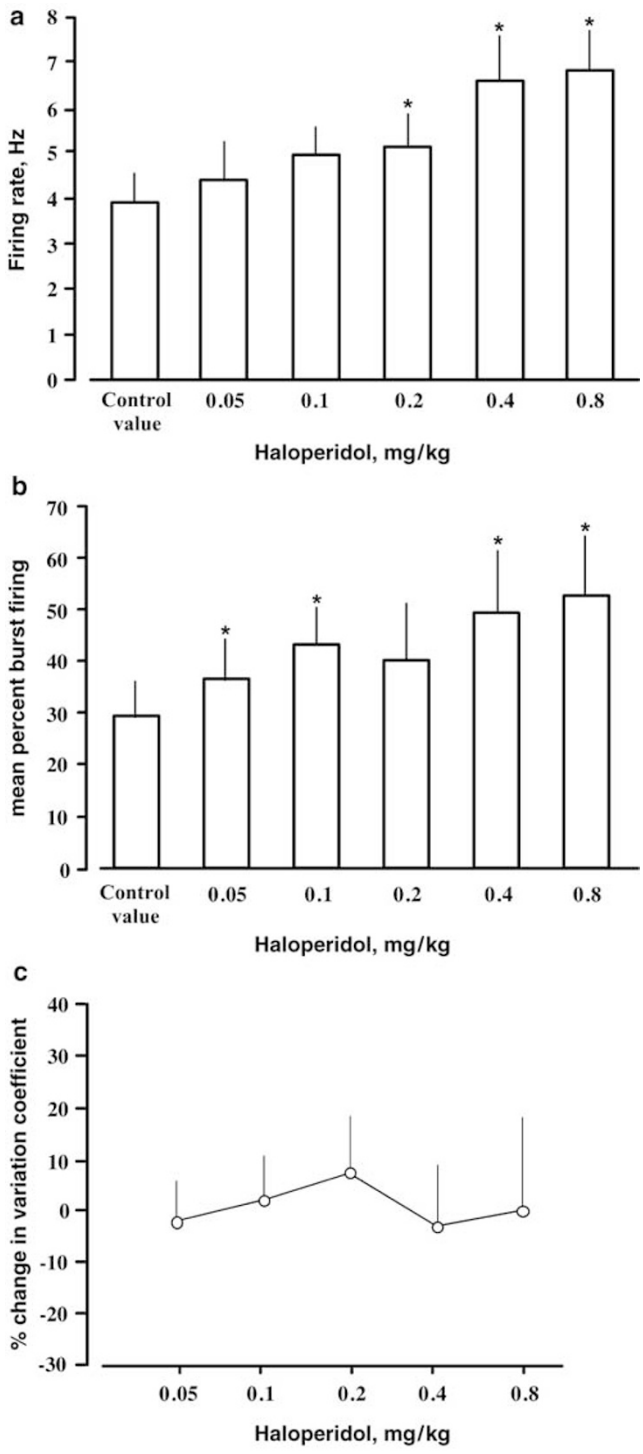

Figure 4 Effects of incremental doses of i.v. administered haloperidol (0.05–0.8 mg/kg) in control rats on (a) the firing rate, (b) the percent burst firing activity, and (c) the regularity of firing (assessed by the variation coefficient). Each value represents mean ± SEM from six to seven VTA DA neurons. Statistic: $^{*}P<0.05$ (the Wilcoxon signed rank test) *vs* corresponding predrug value.

mortem brain of schizophrenics (Schwarcz *et al*, 2001). Interestingly, a three- to five-fold increase in endogenous levels of rat brain KYNA is associated with dramatic effects on neuronal firing of midbrain DA neurons, including

increased firing rate and burst firing activity (Erhardt and Engberg, 2002; Erhardt *et al*, 2001a).

The results of the present study show that i.v. administration of clozapine or haloperidol increases the firing rate and burst firing activity of VTA DA neurons. These findings are in excellent agreement with previous *in vivo* electrophysiological studies (Gessa *et al*, 2000; Tung *et al*, 1991; White and Wang, 1983) and it is generally accepted that antipsychotic drugs increase DA cell firing rate by blockade of somatodendritic DA autoreceptors (Pucak and Grace, 1994, 1996). However, in a situation of hyperdopaminergia induced by elevation of brain KYNA (Erhardt and Engberg, 2000, 2002; Erhardt *et al*, 2000, 2001a, b, 2002a; Speciale *et al*, 1996), the excitatory effects of clozapine observed in control rats were converted into pure inhibitory responses by the drug. In contrast, the excitatory action of haloperidol on VTA DA neurons was even more pronounced in rats with elevated levels of endogenous brain KYNA, since administration of the drug in low doses was associated with a depolarization block of all DA neurons recorded. This finding is in sharp contrast with a previous electrophysiological study showing that i.c.v. administration of KYNA blocks the excitatory action of systemically administered haloperidol on VTA DA neurons (Tung *et al*, 1991). The discrepancy between these results may be related to different routes of elevating KYNA levels in the brain (Erhardt and Engberg, 2000).

The potentiated effect of haloperidol and the conversion of clozapine's effects into an inhibitory response seen in rats with elevated brain levels of KYNA were unexpected and point to a profound difference in the mode of action between these drugs. Judging from previous electrophysiological studies, a decreased glutamatergic tone in the brain is associated with an increase of neuronal activity of midbrain DA neurons. Thus, systemic administration of NMDA-receptor antagonists, for example, MK 801, phencyclidine and ketamine as well as elevated KYNA levels in brain are associated with an increased neuronal activity of these neurons (Erhardt and Engberg, 2002; Erhardt *et al*, 2001a; French, 1994; French *et al*, 1993; Murase *et al*, 1993). These paradoxical effects of glutamate receptor antagonists are thought to be induced by an inadequate balance of afferent regulation by GABAergic and glutamatergic projections, for example, from the prefrontal cortex and/or subcortical areas (Carr and Sesack, 2000; Kalivas *et al*, 1993; Phillipson, 1979; Sesack *et al*, 1989). In particular, it is suggested that KYNA primarily reduces the activity of GABAergic projections to the VTA, thereby activating VTA DA neurons by a decreased GABAergic tone (Erhardt and Engberg, 2002; Erhardt *et al*, 2002b). With regard to haloperidol, an inadequate balance between GABAergic and glutamatergic afferents, as induced by elevated KYNA levels, may promote the drug to induce depolarization block by its potent antagonism at somatodendritic DA-D_2 receptors.

The excitatory and inhibitory effects of i.v. administered clozapine on VTA DA neurons in control rats and in rats with elevated levels of KYNA, respectively, show striking similarities with the effects of i.v. administered nicotine on these neurons (Erhardt *et al*, 2002a). However, the effects of nicotine on VTA DA neurons were suggested to be the result of an interaction with glutamatergic mechanisms and unrelated to an activation of $\alpha7^*$ nicotinic receptors, since addition of the glycine-site agonist D-cycloserine to PNU 156561A pretreated rats restored the excitatory action of nicotine (Erhardt *et al*, 2002a). Judging from the present results, the inhibitory action of clozapine in rats with elevated brain levels of KYNA appears to be dissociated from an effect on DA receptors, and rather related to interference with glutamatergic or cholinergic mechanisms. Present data do not allow any definitive conclusion regarding the mechanism behind the inhibitory action of clozapine on VTA DA neurons. Hypothetically, the effect could be mediated by (I) activation of presynaptic $\alpha7^*$ nicotinic receptors located on glutamatergic afferents (McGehee *et al*, 1995; Wonnacott *et al*, 2000; leading to a potentially increased glutamate release by clozapine) (II) activation of postsynaptic $\alpha7^*$ nicotinic receptor, located on cell soma or dendrites of VTA DA neurons or (III) displacement of KYNA at postsynaptic NMDA receptors located on cell soma or dendrites of GABAergic neurons projecting to VTA DA neurons, thereby restoring the balance between GABAergic and glutamatergic projections to the VTA. An interaction between clozapine and the $\alpha7^*$ nicotinic receptor is supported by the demonstration of a clozapine-induced release of acetylcholine in the prefrontal cortex (Ichikawa *et al*, 2002). In favor of an interaction by clozapine with glutamatergic mechanisms, treatment augmentation studies with agents acting at the glycine-site of the NMDA receptor have shown that glycine and D-cycloserine improve negative symptoms when added to conventional antipsychotic drugs, but not when added to clozapine (Evins *et al*, 2002; Goff *et al*, 1999, Heresco-Levy *et al*, 1998, 1999; Javitt *et al*, 1994; Tsai *et al*, 1998). This indicates that clozapine may be an agonist or partial agonist at the glycine-site of the NMDA receptor or an inhibitor of the glycine transporter and such actions may contribute to its unique clinical efficacy. Interestingly, judging from the results of the present study, clozapine resembles the effect of D-cycloserine on VTA DA neurons, that is, an excitatory action in control rats and an inhibitory effect in rats with elevated levels of KYNA (Erhardt and Engberg, 2002). Preliminary data from our laboratory also point to an interaction of clozapine with the glycine transporter (unpublished data).

In conclusion, the present study demonstrates profound differences between haloperidol and clozapine with regard to their effects on the neuronal activity of VTA DA neurons as revealed by elevation of endogenous brain KYNA levels. Thus, in a situation of hyperdopaminergia, the excitatory action of haloperidol is potentiated, whereas clozapine facilitates or inhibits VTA DA neurotransmission, depending on the pre-existent DA tone. Tentatively, these actions of clozapine and haloperidol may also contribute to differences in clinical efficacy between these drugs.

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