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Effects of Clozapine on The Activity of Central Dopaminergic and Noradrenergic Neurons

M. SOUTO, J. M. MONTI AND H. ALTIER

Departamento de Farmacología y Terapeútica, Hospital de Clínicas, Montevideo, Uruguay

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SOUTO, M., J. M. MONTI AND H. ALTIER. *Effects of clozapine on the activity of central dopaminergic and noradrenergic neurons*. PHARMAC. BIOCHEM. BEHAV. **10**(1) 5–9, 1979.—The effects of clozapine on the spontaneous firing rate of noradrenergic (NE, locus coeruleus), dopaminergic (DA, zona compacta, ventral tegmental area) and non-dopaminergic (zona reticulata) neurons was studied in chloral hydrate anesthetized rats. Clozapine administered intraperitoneally significantly increased the spontaneous activity of NE and DA neurons. After a cumulative dose of 8 mg/kg clozapine, the increase of mean firing rate per min of zona compacta and ventral tegmental area neurons attained almost similar values. Amphetamine reversed the clozapine-induced stimulation of NE and DA neurons. Zona reticulata neurons were depressed by clozapine. Neither amphetamine nor physostigmine were effective in antagonizing the inhibition. Our results suggest that clozapine blocks central NE and DA receptors. Furthermore, they fail to reveal a smaller sensitivity of the striatum as compared to the limbic system to the effects of clozapine.

Clozapine Amphetamine Zona compacta Ventral tegmental area Locus coeruleus Single unit activity

THE INTRODUCTION of clozapine, a dibenzodiazepine derivative, in the treatment of psychoses raised considerable research and therapeutic interest because of its unique features. The drug differs from the classical neuroleptics in that it lacks a cataleptic effect and does not antagonize apomorphine- or amphetamine-induced stereotyped behavior in animals [18] and is free from extrapyramidal side effects in man [4, 12, 17].

Clozapine increases also the turnover of cerebral dopamine (DA) and norepinephrine (NE) in laboratory animals [5]. It has been proposed that the absence of catalepsy and extrapyramidal side effects after clozapine administration, could be related to a smaller blockade of the nigrostriatal system as compared to the meso-limbic one [3,6]. However, other studies failed to reveal that the limbic system possesses a greater sensitivity to clozapine than the striatum [10,20].

Aghajanian *et al.* [1] established that there is a direct correlation between the effects of DA and NE-receptor blockers on catecholamines (CA) turnover and their actions on CA neurons firing rate. In this connection, drugs which increase CA turnover lead to a compensatory increase of the firing rate of CA neurons via a neuronal feedback mechanism. The opposite effect is depicted with compounds which decrease CA turnover.

It was our purpose to comparatively determine the effects of clozapine on zona compacta (A9 nucleus) and ventral tegmental area (A10 nucleus)-DA neurons and on zona reticulata non-DA neurons firing rate. For control purposes, amphetamine was also included in our study.

The findings that clozapine inhibits the arousal reaction induced by electrical stimulation of NE-containing nuclei of the rat brain stem reticular formation [18] and increases central NE turnover [5,9] induced us to determine also the effects of clozapine on the locus coeruleus-NE neurons.

METHOD

Thirty-eight male Wistar rats weighing 180–250 g were used. They were anesthetized with chloral hydrate (Carlo Erba, Milan, 400 mg/kg, IP) and given additional injections of 1/3 the initial dose, approximately every 40 min. The animals were fixed to a stereotaxic apparatus and their body temperature was maintained at 37°C. A midsaggital scalp incision exposed the calvarium and a small hole was drilled in the skull overlying the locus coeruleus nucleus or the substantia nigra. Stainless steel microelectrodes for extracellular unit recording with a tip diameter of 1–2 μ and a resistance of 3–5 MΩ were made by the method of Green [15]. They were stereotaxically inserted [16] by means of a micromanipulator into the corresponding structures. Coordinates were: (1) locus coeruleus (extrapolated from König and Klippel [16]): P 1.6–1.8; L 1.0–1.2 and vertical approximately 6 mm beneath the skull surface; (2) zona compacta: A 1.8–2.4; L 1.8–2.4; H −1.5 to −3.0; (3) ventral tegmental area: A 1.7–2.2; L 0.5–1.0; H −2.0 to −3.0; (4) zona reticulata: A 1.8–2.4; L 1.8–2.4; H −2.0 to −3.2.

Extracellular unit potentials recorded between the microelectrode tip and a small clip inserted into the temporalis muscle were led to Grass 15 preamplifiers. The action potentials were displayed on a dual beam oscilloscope in parallel with a loudspeaker unit. After the isolation of single unit discharges, spontaneous activity was recorded for at least 10 min in order to determine if the firing rate was stable. For analysis the amplifier output of the unit responses was processed by a Schmidt trigger and gating unit (Tektronix 161 and 162) for write-out as square wave pulses on a polygraph. Only one unit was studied in each preparation. Clozapine (Sandoz) was dissolved in a few drops of glacial acetic acid, the final volume made up with distilled water and the pH adjusted to 6. d-Amphetamine sulfate (S K and F) and

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TABLE 1

EFFECTS OF CLOZAPINE ON THE FIRING RATE OF SUBSTANTIA NIGRA AND LOCUS COERULEUS NEURONS

Histological* location	Clozapine (cumulative dose) mg/kg	Mean spikes/min ± SE		p
		Control	10 min after last dose	
Zona compacta (10)	8	262 ± 26	359 ± 39	<0.02
Ventral tegmental area (8)	8	280 ± 31	356 ± 34	<0.05
Zona reticulata (7)	8	224 ± 36	124 ± 31	<0.01
Locus coeruleus (10)	4	92 ± 25	158 ± 22	<0.02

*Numbers in parentheses indicate number of neurons.

physostigmine sulfate (Merck) were dissolved in saline. All substances were injected intraperitoneally following the technique of cumulative drug administration. Solutions were prepared immediately before injection. At the end of the experiments electrode recording points were detected by passing a 5 μA DC current for 5 sec through the electrode tips and perfusing the brain with 10% Formalin and 2% potassium ferrocyanide to give a Prussian blue reaction. Frontal sections (15 μ thickness) were stained with cresyl violet and from these sections electrode locations were determined. Only data from brains where the electrode tips were within the limits of locus coeruleus or substantia nigra nuclei were included in the presentation of the results.

RESULTS

Neurons which according to the histology were located in the locus coeruleus-NE nucleus displayed positive-negative action potentials at a rate of 0.5–2.0 spikes/sec and reacted to light skin touch with a short lasting increase of firing rate [14]. Neurons histologically located in the dopaminergic (zona compacta, ventral tegmental area) and non-dopaminergic (zona reticulata) subdivisions of the substantia nigra showed as biphasic spikes firing at a rate of 2–7 spikes/sec. Dopaminergic neurons presented also bursting activity, with each burst characterized by spikes of progressively decreasing amplitude [7]. As described below, NE and DA neurons were also tentatively characterized by their responses to amphetamine injection [7].

Following the injection of clozapine, there was a significant increase of spontaneous firing rates of zona compacta (ZC) and ventral tegmental area (VTA) dopaminergic neurons. The onset of this effect was seen within 2–3 min after the administration of the drug (Figs. 1 and 2). The mean spontaneous firing of ZC neurons was increased by 137% of baseline rate 10 min after the injection of 8 mg/kg clozapine (cumulative dose, Table 1). Regarding VTA neurons the increase amounted to 127% of baseline rate after the same cumulative dose (Table 1). The facilitatory effect of clozapine on dopaminergic neurons was antagonized by amphetamine (Figs. 1 and 2). In separate experiments it could be also observed that clozapine was effective in reversing the amphetamine-induced depression of ZC neurons (n=3; Fig. 3). Zona reticulata (ZR)-nondopaminergic neurons were inhibited by clozapine. The drug produced a significant decrease of baseline firing (Fig. 4, Table 1). Neither amphetamine (n=2) nor physostigmine (n=3) were effective in antagonizing the clozapine-induced depression (Figs. 4 and 5).

The spontaneous activity of locus coeruleus cells was increased to 171% of baseline rate after the administration of 4 mg/kg clozapine (cumulative dose). Small doses of amphetamine (2–4 mg/kg) readily reversed the clozapine-induced facilitation of unit activity (Fig. 6; Table 1).

DISCUSSION

Our results show that clozapine has a significant activating effect on the firing rate of DA and NE neurons. This effect is probably related to an action on postsynaptic sites, since presynaptic receptor blocking properties were not observed with this compound [19].

After a cumulative dose of 8 mg/kg, the mean firing rate per min attained similar values in ZC and VTA. In this connection our findings correlate well with the turnover studies by Carlsson [10] and Wilk and Glick [20], who also failed to reveal a smaller sensitivity of the striatum as compared to the limbic system to the receptor blocking effects of clozapine. The facilitatory action of clozapine on DA neurons was antagonized by amphetamine. Furthermore, as previously described by Bunney and Aghajanian [8], clozapine reversed the amphetamine-induced depression of ZC neurons. The same authors reported that they could not obtain increased firing rates of ZC cells after intravenous clozapine. The reasons for these differences between laboratories are not evident at present. The inhibition by amphetamine of ZC and VTA neurons in the clozapine-pretreated preparations could be related to the increased

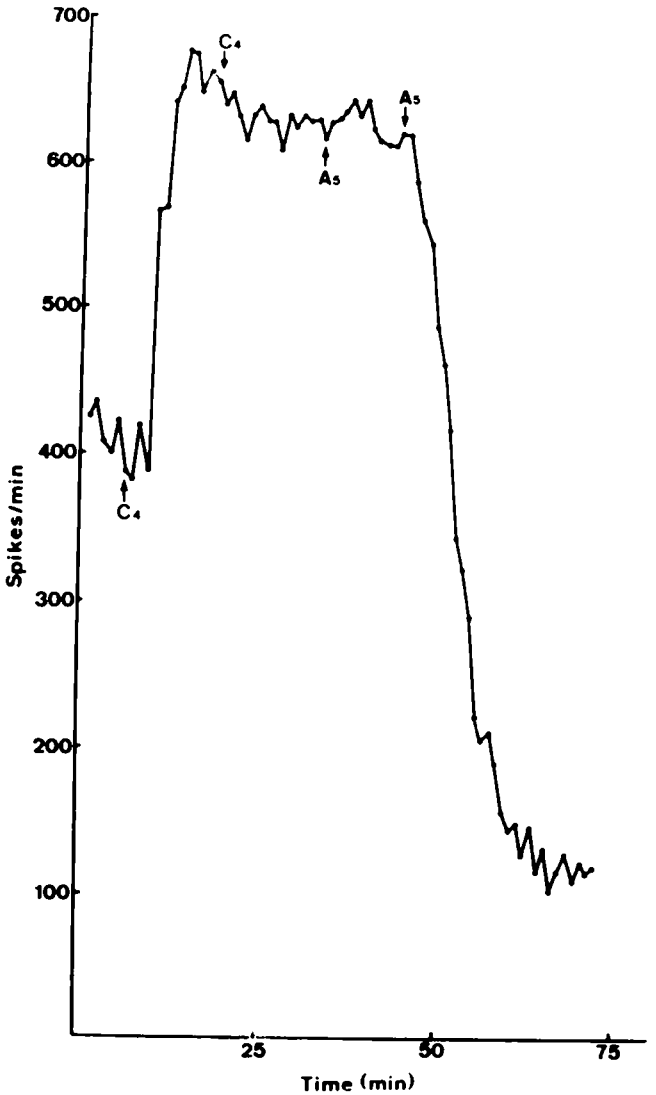

FIG. 1. Effect of clozapine on spontaneous firing rate of a zona compacta dopaminergic neuron. Abscissa: time in min; ordinate: spikes per min. C: clozapine; A: amphetamine. The numbers adjacent to the letters indicate mg/kg of drug administered. Clozapine-induced increase of cell activity was reversed by amphetamine.

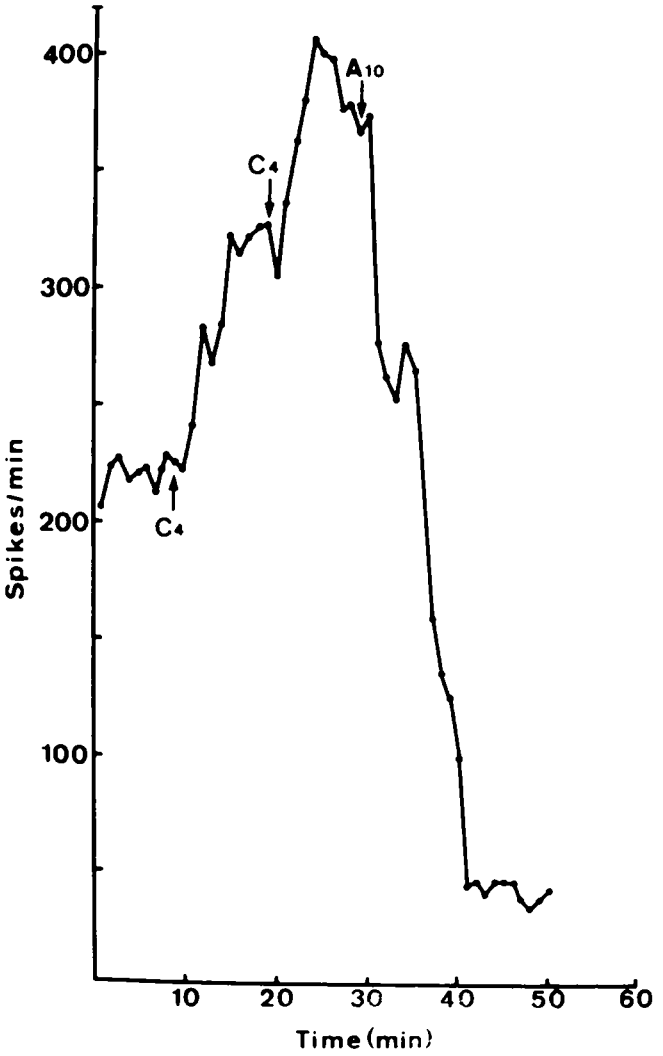

FIG. 2. Effect of clozapine on spontaneous firing rate of a ventral tegmental area dopaminergic neuron. Abbreviations as in Fig. 1. Clozapine-induced increase of firing rate was reversed by amphetamine.

availability of DA to postsynaptic receptors [13] which would start a neuronal feedback inhibition of DA neurons. Zona reticulata non-dopaminergic neurons were depressed by clozapine and this effect could not be reversed by amphetamine. Thus, ZR neurons showed an opposite response to clozapine as compared to DA neurons. The absence of response of ZR cells to systemic amphetamine and to microiontophoretically delivered DA [2] tend to suggest that in this instance the effects of clozapine are not related to CA mechanisms.

On the other hand, ZR neurons are excited by acetylcholine, while ZC neurons show no response to this neurotransmitter [2]. Furthermore, clozapine shows a weak central anticholinergic action [18] and it could be inferred that its inhibitory effect on ZR neurons is related to the blockade of a cholinergic facilitatory input. However, our findings showing that physostigmine does not antagonize the inhibition produced by clozapine argues against this suggestion.

Locus coeruleus-NE neurons showed similar responses to clozapine and amphetamine as compared to the DA neurons. However, they were much more sensitive to these compounds. In this connection, it took 4 times as much clozapine to produce a relatively comparable degree of facilitation of the ZC and VTA neurons as it did to facilitate the NE cells.

Drowsiness is a common side effect among patients receiving clozapine [11,17]. Furthermore, the drug has a strong inhibitory effect on the arousal reaction induced by electrical stimulation of the brain stem reticular formation [18]. Turnover studies [5] and our own findings tend to support the view that clozapine blocks NE-receptors and this could be related to the sedative effects of the drug.

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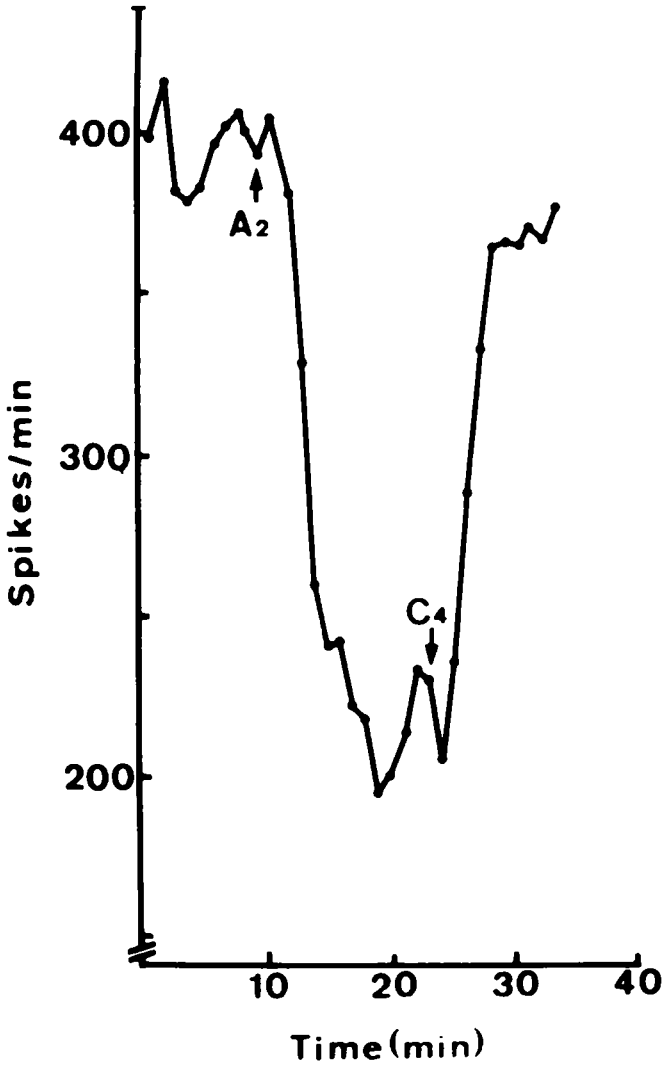

FIG. 3. Reversal of amphetamine depression of spontaneous activity of a zona compacta neuron by clozapine. Abbreviations as in Fig. 1. Clozapine injection resulted in a rapid recovery from depression.

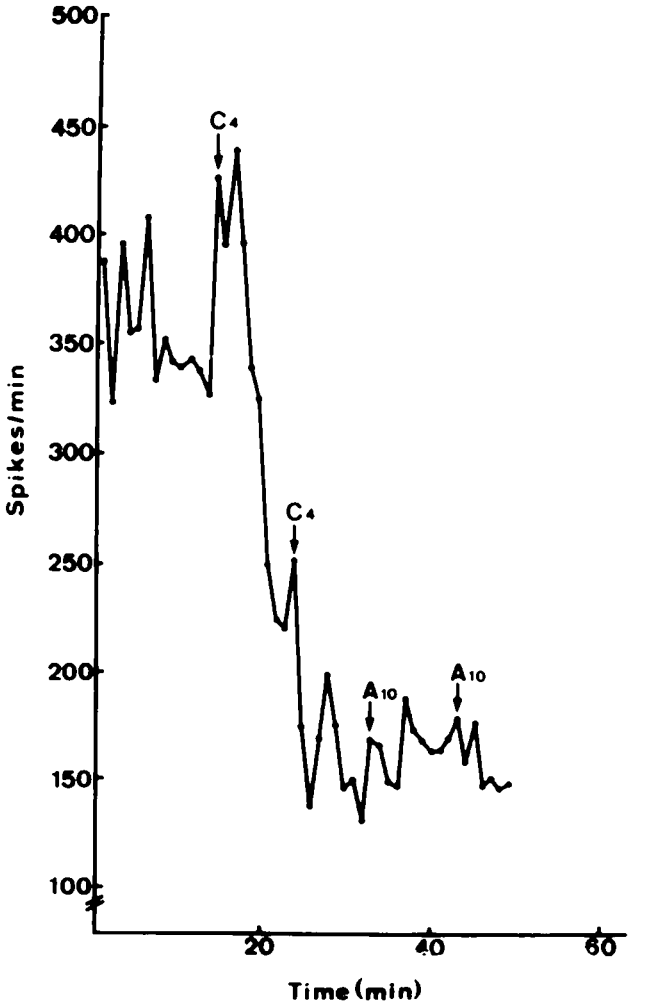

FIG. 4. Effects of clozapine and amphetamine on the firing rate of a zona reticulata neuron. Abbreviations as in Fig. 1. Clozapine injected in a total dose of 8 mg/kg depressed cell activity. Amphetamine did not return activity to baseline levels.

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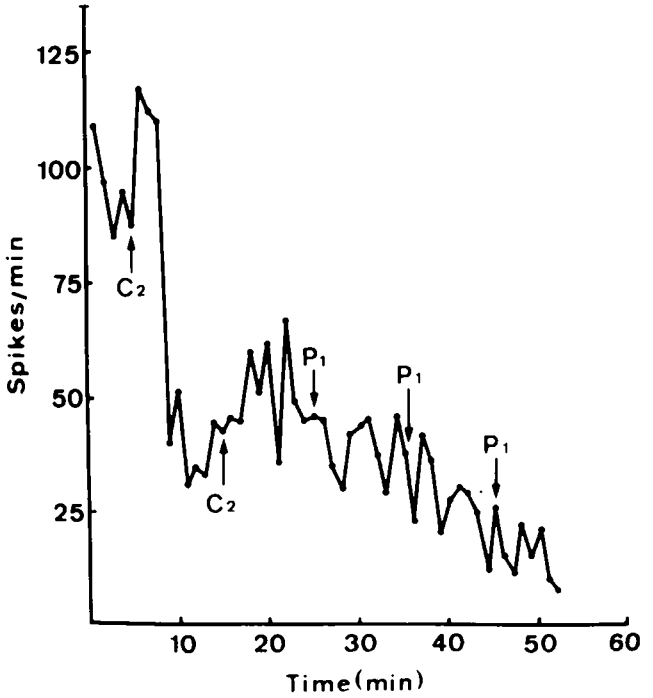

FIG. 5. Effects of clozapine and physostigmine on the firing rate of a zona reticulata neuron. Abbreviations as in Fig. 1. P: physostigmine. Clozapine in a dose of 2 mg/kg slowed the neuron. Further administration of the drug produced no additional depression. Physostigmine was ineffective in reversing the clozapine-induced inhibition.

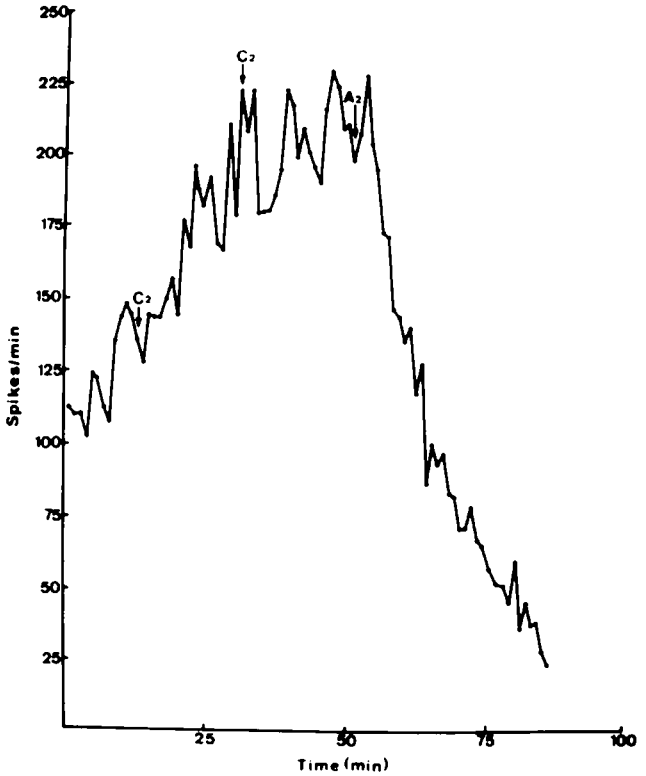

FIG. 6. Effect of clozapine on spontaneous firing rate of a locus coeruleus noradrenergic neuron. Abbreviations as in Fig. 1. The clozapine-induced increase of firing rate was antagonized by amphetamine.

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