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# Clozapine—A Serotonin Antagonist?

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FINK, H., R. MORGENSTERN AND W. OELSSNER. *Clozapine—A serotonin antagonist?* PHARMACOL BIOCHEM BEHAV **20**(4) 513–517, 1984.—The effect of clozapine on the central serotonergic transmission system was studied by investigation of open-field motility of rats after microinjection of drugs into nucleus accumbens and median raphe nucleus. Previous work has shown that LSD in low doses potentiates apomorphine-induced hypermotility and that this LSD effect is induced by a serotonin agonist action in median raphe nucleus. Clozapine, injected into median raphe nucleus (0.05 μg), suppressed the LSD effect in the same manner as serotonin antagonists did. Since α-adrenergic drugs, injected into median raphe nucleus, caused locomotor stimulant effects, an α-adrenalytic action of clozapine was excluded. Clozapine, injected into nucleus accumbens (0.2 μg), increased apomorphine-induced hypermotility, whereas the dopamine antagonist haloperidol suppressed it. Our results suggest a serotonin antagonist action of clozapine.

Antagonist Clozapine Median raphe Microinjection Open-field test Serotonin

CLOZAPINE, a dibenzodiazepine antipsychotic drug, has been used widely in the treatment of schizophrenia. The pharmacological profile of clozapine, however, differs basically from that of classical neuroleptics. Clozapine has hardly dopaminolytic, but strong cholinolytic and adrenolytic effects, and it acts on GABA-ergic mechanisms [3, 20, 21, 23, 24, 28]. A central antiserotonergic property is still questionable. Until now it has been unclear, which pharmacological property of clozapine preferentially contributes to its strong antipsychotic action.

Many effects of clozapine on dopaminergic transmission contrast with those of the typical neuroleptics and cannot be related to the therapeutic potency in man: In several animal tests, clozapine lacks a dopaminolytic activity [3]. In pharmacologically relevant doses clozapine decreases dopamine release [12,16]. Only high doses of clozapine increase dopamine turnover [19]. Furthermore, clozapine was shown to have no presynaptic dopamine receptor blocking ability [31]. Consequently, it can be suggested that clozapine does not act primarily on the dopaminergic system, but on another central transmission system.

The strong cholinolytic activity of clozapine does not contribute to the antipsychotic effect, and it has been shown that the cholinolytic activity does not interfere with a presumed dopaminolytic action [7, 10, 20].

Furthermore, it would appear that the actions of clozapine on α-adrenergic or GABA-ergic mechanisms are not solely responsible for the antipsychotic action in man since both α-adrenolytics and drugs affecting GABA-ergic transmission produce weaker therapeutic effects than clozapine.

Serotonergic mechanisms have to be taken into account in the expression of symptoms of schizophrenia. Therefore, the effect of clozapine on the central serotonergic transmission system is investigated in the present paper. Previously, we elaborated an animal model suitable for discovering central antiserotonergic drugs [7,13]. This model uses the effect of low doses of LSD to potentiate apomorphine hypermotility in rats. LSD, given alone, has no effect on locomotor activity [7]. The potentiating effect of LSD on apomorphine-induced hyperactivity was shown to be induced in the median raphe nucleus and was characterized as a serotonin agonist effect at the somatodendritic serotonin receptors [8]. Furthermore, this potentiating effect of LSD can be antagonized by serotonin antagonists [8]. In a further study clozapine was shown to suppress this LSD effect and a central serotonin antagonist activity of clozapine was suggested [25]. In the present microinjection experiments the attempt was made to verify this hypothesis.

## METHOD

### *Animals*

The experiments were performed on male Wistar rats (VEB Versuchstierproduktion Schönwalde) weighing 140±10 g. They were housed in groups of 10 animals per cage under a room temperature of 22±2°C and a 12 hours light-dark schedule. The rats received food and water ad lib.

### *Intracerebral Injection Technique*

Platforms with fixed guide cannulae were used for intracerebral injection (For details see [8]). Rats were anesthetized with sodium hexobarbitone, 140 mg/kg IP, and the platforms were screwed on the scull. On the next day an injection cannula with an outer diameter of 0.23 mm was slowly inserted through the guide cannulae into the target nuclei.

The coordinates of the injection cannula tips were according to König and Klippel [17] for median raphe nucleus:

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A=0.35; L=0; V=2.6, for nucleus accumbens: A=9.4; L=1.3; V=1.0. Drugs, vehicle or 0.9% NaCl were administered to the awake, hand held animals by a Hamilton microliter syringe (CR 700-20) in a volume of 1 $\mu$l over 30 sec with a further 30 sec for deposition of drugs. The intracerebral injection was followed by IP administration (1 ml/0.1 kg body weight). Seven min after IP drug administration the rat was transferred to the middle of the open-field box.

Rats were used only once. After experiments animals were decapitated. The removed brains were fixed with 10% formalin and later sectioned into 30 $\mu$m serial slices. These were stained with toluidine blue and examined microscopically. Data were discarded if the location of cannula tips was not within the nucleus.

### *Measurement of Locomotor Activity*

The experiments were performed in a sound proof, diffusely illuminated room between 8.00 a.m. and 11.00 a.m. The white open-field cage consisted of a 1×1 m area. An array of 10 infrared light beams divided this area into 36 equal squares. The detector signals, produced by interrupting these photobeams, were fed into digital logic providing an automatic counting of squares crossed by the animal during a 5 min observation period.

### *Drugs*

The following drugs were used: apomorphine hydrochloride (SPOFA), clozapine (Sandoz), clonidine (AWD), haloperidol (Gedeon Richter), lysergic acid diethylamide tartrate (LSD) (SPOFA), phentolamine (CIBA). Haloperidol was dissolved in two drops of acetic acid and the pH adjusted to 5–6 with 1 N NaOH. Clozapine was dissolved in tartaric acid in a final concentration of 0.01%. Phentolamine was obtained as an injectable solution. All other drugs were dissolved in 0.9% NaCl. All doses were calculated as the salt. Data values are presented as mean±standard error of the mean (S.E.M.). The statistical significance was assessed using the nonparametric Mann-Whitney test.

## RESULTS

### *Effect of Intra-Accumbens Injected Clozapine on Hypermotility Induced by Apomorphine and LSD*

For the nucleus accumbens experiments neither spontaneous locomotor activity nor drug induced hyperactivity were influenced by the surgery, by the guide cannula platform or by saline or vehicle injection. Saline treated animals crossed about 40 squares. Following systemic administration of 1 mg/kg apomorphine or combined administration of apomorphine with 0.1 mg/kg LSD the animals showed a stimulation of locomotor activity, varying between 90 and 100 and between 150 and 170 crossed squares, respectively (Fig. 1).

Clozapine, injected bilaterally into the nucleus accumbens in a dose of 0.2 $\mu$g, failed to influence spontaneous locomotor activity, but caused a further increase of apomorphine-induced hyperactivity by about 30%. The LSD-induced potentiation effect was not affected. A higher dose of clozapine, 0.8 $\mu$g, decreased slightly but not significantly the LSD potentiated apomorphine-induced hyperactivity.

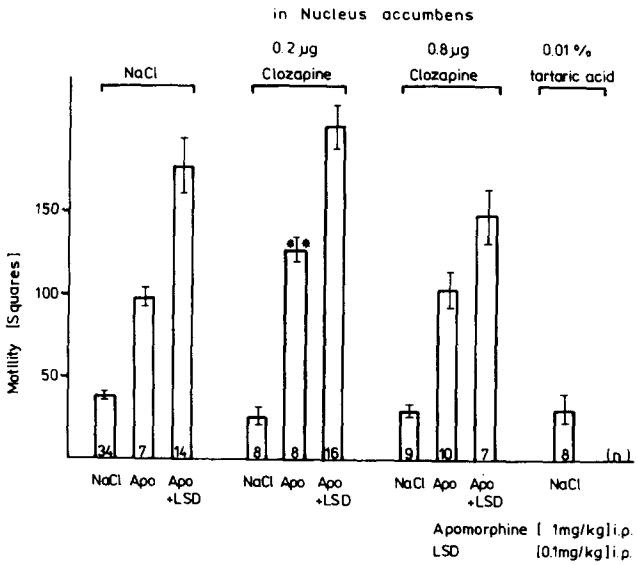


FIG. 1. Effect of clozapine injected into nucleus accumbens on apomorphine-induced hypermotility and on LSD-induced potentiation effect. Crossed squares of an open-field were counted for 5 min. Columns represent mean±S.E.M. n=number of animals, $^{**}p<0.005$ versus corresponding control.

### *Effect of Intra-Accumbens Injected Haloperidol on Hypermotility Induced by Apomorphine and LSD*

Bilateral injection of 0.1 $\mu$g haloperidol into the nucleus accumbens did not influence spontaneous locomotor activity but depressed, in a dose dependent manner, the hyperactivity induced by IP injected apomorphine alone or in combination with LSD (Fig. 2). However, the LSD-induced potentiation effect remained.

### *Effect of Clozapine Injected into the Median Raphe Nucleus on the LSD-Induced Potentiation Effect*

Injection of saline or 0.01% tartaric acid into the median raphe nucleus influenced neither spontaneous nor apomorphine-stimulated locomotor activity. LSD injected into the median raphe nucleus in a dose of 0.05 $\mu$g was shown to be itself ineffective [8] but potentiated the apomorphine-induced hyperactivity. The animals crossed about 160 squares (Fig. 3). Clozapine in a dose of 0.05 $\mu$g injected into the median raphe nucleus failed to influence spontaneous as well as apomorphine-stimulated locomotor activity. After simultaneous administration of 0.05 $\mu$g clozapine and 0.05 $\mu$g LSD into this raphe nucleus the potentiation effect of LSD was completely abolished (Fig. 3).

### *Effect of Intra-Raphe Injected Phentolamine and Clonidine on Spontaneous and Apomorphine-Stimulated Motility*

Phentolamine in a dose of 0.1 $\mu$g injected into the median raphe nucleus did not alter spontaneous locomotor activity but caused a strong increase of apomorphine-induced hyperactivity, i.e., 170 squares were crossed (Fig. 4). Clonidine in a dose of 0.3 $\mu$g injected into the median raphe nucleus induced a more than two-fold increase in spontane-

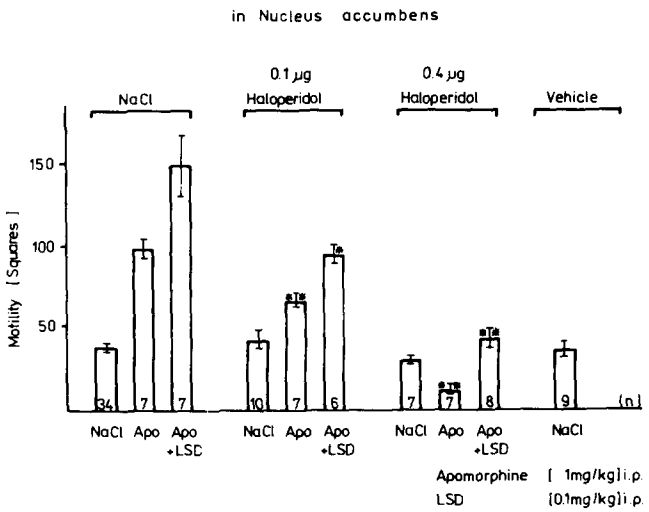


FIG. 2. Effect of haloperidol injected into nucleus accumbens on apomorphine-induced hypermotility and on LSD-induced potentiation effect. Crossed squares of an open-field were counted for 5 min. Columns represent mean±S.E.M. n=number of animals, $^{**}p<0.005$, $^{*}p<0.025$ versus corresponding controls.

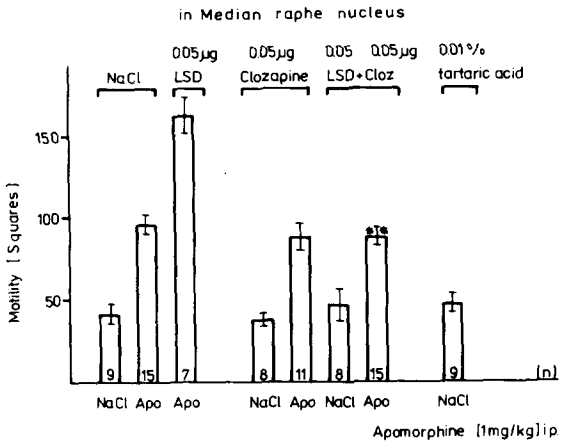


FIG. 3. Effect of clozapine injected into median raphe nucleus on LSD-induced potentiation effect. Crossed squares of an open-field were counted for 5 min. Columns represent mean±S.E.M. n=number of animals, $^{**}p<0.005$ versus corresponding control.

ous locomotor activity and a significant increase in apomorphine-induced hyperactivity, too (Fig. 4).

## DISCUSSION

In previous studies we demonstrated that the model of LSD-potentiated apomorphine hypermotility is suitable for investigating the effects of psychotropic drugs on dopaminergic and serotonergic transmission systems [13,25]. Several lines of evidence indicate that the LSD-induced potentiation effect represents a central serotonergic behavioral effect [8]. An agonist action of LSD at somatodendritic serotonin receptors in the median raphe nucleus has been

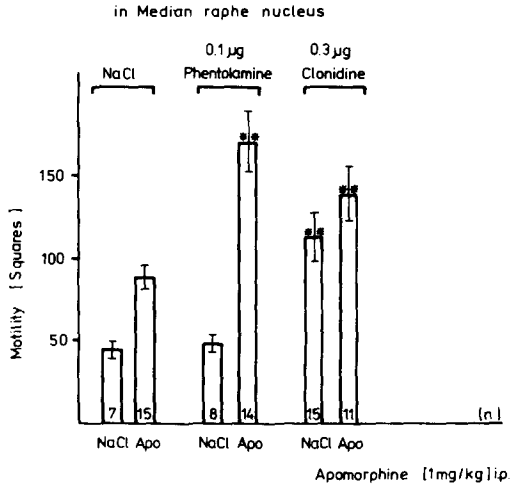


FIG. 4. Effect of phentolamine and clonidine injected into nucleus accumbens on apomorphine-induced hypermotility and LSD-induced potentiation effect. Crossed squares of an open-field were counted for 5 min. Columns represent mean±S.E.M. n=number of animals, $^{**}p<0.005$ versus corresponding controls.

well established [1,11]. The activity of serotonergic neurons is inhibited by LSD and a decreased serotonergic input into mesolimbic target structures is suggested to be responsible for the potentiation of dopaminergic-induced hypermotility. More recently we showed that clozapine suppressed the LSD-induced potentiation effect [24]. Hence, an antiserotonergic effect of clozapine can be suggested. The results of the present study strongly support this hypothesis of a marked central antiserotonergic activity of clozapine. The major evidence is given by the following:

(1) Similar to clozapine, serotonin antagonists, e.g., cyproheptadine, danitracen, pizotifen, mianserin were shown to suppress the LSD-induced potentiation effect [7,13]. By contrast, the classical neuroleptic haloperidol shifted the dose-response curve of apomorphine potentiated by LSD to the right, confirming the strong dopaminolytic property of haloperidol without any serotonin antagonist effect [25].

(2) Microinjection of clozapine into the median raphe nucleus suppressed the LSD-induced potentiation. The similarity of clozapine-induced locomotor effects after systemic or intra-raphe administration directs attention to the median raphe nucleus as a site for producing this clozapine effect. Since the median raphe is known as the site integral to serotonergic-induced locomotor effects this finding points also to an antiserotonergic property of clozapine. In support of this view another similarity should be mentioned: serotonin antagonists have been shown to suppress the LSD effect both after systemic and after intra-raphe administration [7,13].

(3) A primary action of clozapine at adrenergic or GABA-ergic systems within the median raphe nucleus has to be taken into account, because clozapine has $\alpha$-adrenolytic activity and may influence the GABA-ergic transmission [21, 23, 24, 28]. Furthermore, noradrenergic terminals and GABA-ergic neurons were shown to occur within the raphe nuclei [2,27]. However, there is a clear difference between the effects of clozapine and those of $\alpha$-adrenergic and

GABA-ergic drugs: The GABA antagonists picrotoxin and bicuculline induced convulsions, whereas muscimol induced a continuous sterotyped running without any apparent explorative behavior after injection into the median raphe nucleus (in preparation).

Clonidine and phentolamine caused locomotor stimulant effects, too. It should be noted, however, that the effects of adrenergic drugs, injected into the median raphe nucleus, differ from the sedative effects after systemic administration. These contrasting effects are not surprising, since the median raphe is not known as the site for originating the effects of adrenergic drugs.

(4) Injection of clozapine into the nucleus accumbens caused an increase of dopaminergic-induced hypermotility. This effect cannot be explained by the cholinolytic property of clozapine, because cholinolytics fail to induce hypermotility when injected into the nucleus accumbens [15,26]. Since intra-accumbens injected serotonin antagonists are known to increase the dopaminergic-induced hyperactivity this effect of clozapine further supports the hypothesis of clozapine acting as a serotonin antagonist, devoid of strong dopaminolytic properties [4]. In the literature there are only findings of an action of systemically administered clozapine on hypermotility induced by intra-accumbens injected dopamine [6, 14, 29]. Despite some differences in experimental designs most findings point to the failure of clozapine to inhibit a dopamine-induced hypermotility. In contrast, intra-accumbens injected haloperidol showed the typical dopaminolytic effect without any antiserotonergic activity.

In conclusion, our method was shown to be suitable for demonstrating directly a central antiserotonergic property of clozapine. Our results are consistent with results of receptor binding studies and of investigations in biological systems in which spinally or peripherally induced serotonergic effects can be demonstrated [5, 9, 10]. The results of investigations using the model of hind limb flexor-reflex of spinal rats indicate an antiserotonergic effect of clozapine, too ([22], Rawlow, personal communication). Further on, experiments with the quipazine-induced twitch of mylohyoideus muscle also suggest such property of clozapine [18]. The importance of the serotonin antagonist property of clozapine, contributing to the antipsychotic effect in man, however, has to be verified finally in controlled clinical studies.

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