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Chronic Clozapine Selectively Decreases Prefrontal Cortex Dopamine as Shown by Simultaneous Cortical, Accumbens, and Striatal Microdialysis in Freely Moving Rats

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HERNANDEZ, L. AND B. G. HOEBEL. *Chronic clozapine selectively decreases prefrontal cortex dopamine as shown by simultaneous cortical, accumbens, and striatal microdialysis in freely moving rats.* PHARMACOL BIOCHEM BEHAV 52(3) 581-589, 1995.—We used microdialysis to study the acute and chronic effects of clozapine on the metabolism of dopamine (DA) in terminal areas of the mesocortical, mesolimbic, and nigrostriatal systems simultaneously. In the acute experiment, groups of four rats received the following doses: 0 (vehicle), 10, 20, and 40 mg/kg of clozapine subcutaneously, which resulted in a dose-related increase in extracellular DA, 3,4-dihydroxyphenalacetic acid (DOPAC), and homovanillic acid (HVA) in the prefrontal cortex (PFC). In the nucleus accumbens (NAC) and striatum (STR), no significant changes were observed at any dose. In the chronic experiment, six rats received 20 mg/kg of clozapine and a control group received vehicle daily for 30 days. After 30 days of treatment, DA, DOPAC, and HVA were significantly lower in the PFC, and unchanged in the NAC or STR. The 30th clozapine injection failed to increase DA, DOPAC, or HVA in any of the three regions. We conclude that clozapine acted selectively on the mesocortical system, and that this may underlie clozapine's therapeutic, antipsychotic effect.

Microdialysis Neuroleptics Clozapine Dopamine Schizophrenia Rats

THE DOPAMINERGIC theory of schizophrenia was proposed on the basis of two types of neuropharmacologic evidence. First, the antipsychotic drugs that relieved symptoms of schizophrenia increased the synthesis, release, and metabolism of dopamine (DA) (1,15,59,85,86) and increased the electrophysiologic activity of dopaminergic neurons (13). Second, the clinical potency of these drugs was well correlated with their affinity for dopamine receptors (23,43,69,73). However, not all of the neuroleptics act the same way. Typical neuroleptics such as haloperidol tend to produce extrapyramidal side-effects of the type seen in Parkinson's disease (4,74). Eventually, tardive dyskinesia may develop as a result of irreversible damage to the basal ganglia (21,22). In contrast, atypical neuroleptics such as clozapine do not produce such pronounced extrapyramidal side-effects (29,70) and may bind differentially to various dopamine receptors (42). It was also clear that acute administration of neuroleptics has only a weak antipsychotic effect (20,58); prolonged treatment of 2 or more weeks is usually necessary for the antipsychotic effect. This clinical observation underlined the importance of long-term administration in experimental studies aimed at clarifying the mechanism of neuroleptic action.

Refinement of the dopamine theory of schizophrenia has become necessary as these and other new experimental observations have been revealed. Currently, evidence suggests that only selected populations of dopamine neurons are involved in the pathogenesis of schizophrenia. Well-known subdivisions of the dopaminergic system respond differently to the typical and the atypical neuroleptics. For example, some cells of the mesocortical and mesolimbic systems in the ventral tegmental area enter a refractory state because of sustained depolarization, as a result of prolonged treatment with atypi-

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cal or typical neuroleptics (18,19,82). The cells of origin of the nigrostriatal system in the pars compacta of the substantia nigra show depolarization blockade after long-term administration of typical, but not atypical neuroleptics. Neurons of the mesocortical system seem to lack autoreceptors regulating the synthesis and release of dopamine (6,7). In this respect, they are unlike the nigrostriatal and mesolimbic cells.

Biochemical studies in brain homogenates also reveal differences in the response of dopamine systems to neuroleptics. For instance, systemic acute injections of typical neuroleptics are effective in increasing 3,4-dihydroxyphenalacetic acid (DOPAC) and homovanillic acid (HVA) in homogenates of the striatum (STR), nucleus accumbens (NAC), and prefrontal cortex (PFC). (5,10,67,68). By contrast, atypical neuroleptics only increase DOPAC and HVA in homogenates of the NAC and PFC (2,44). Based on this and other evidence, it was suggested that the mesolimbic system might be more relevant to schizophrenia than the nigrostriatal system (24,50,55,77).

Brain homogenate studies after chronic administration of neuroleptics have yielded mixed results according to the region and the species studied (44,48,52,61). In general, chronic neuroleptics increase DOPAC or HVA in PFC homogenates but have little effect on the STR or the NAC. These findings strongly suggest that the mesocortical system might have an important role in the genesis of schizophrenia.

The development of in vivo techniques has allowed measurement of extracellular DA, DOPAC, and HVA in localized brain regions (26,34,35,78). This complements and extends the information obtained with classical biochemical and electrophysiologic techniques.

Acute administration of typical neuroleptics such as haloperidol increases DA, DOPAC, and HVA in the PFC, NAC, and STR in rats as shown by brain microdialysis (31,39,84). Atypical neuroleptics such as sulpiride and clozapine increase DA, DOPAC, and HVA in the STR (11,38,39,60). In vivo voltammetry confirmed that acute administration of typical and atypical neuroleptics increases dopamine in the STR and NAC (8,49).

Long-term effects of neuroleptics have not been thoroughly examined with in vivo measurement techniques. In vivo voltammetry studies suggested that long-term administration of haloperidol decreased dopamine in the NAC and STR. Clozapine decreased DA in the NAC but not in the STR (9,46). Microdialysis studies found that chronic haloperidol decreased DA, DOPAC, and HVA in the PFC, and decreased DOPAC and HVA in the STR (31,33,37). In the NAC, however, one study found that chronic haloperidol did not affect DA, DOPAC, or HVA, (31); but in another study, DA and DOPAC decreased (37). The microdialysis study of chronic clozapine has shown negative results [i.e., no effect on DA, DOPAC, or HVA in the STR and NAC (37)].

In summary, both acute and chronic administration of atypical neuroleptics give disparate results when in vivo measurement techniques are used. In an effort to clarify the mechanism of action of the atypical neuroleptic clozapine, we used triple microdialysis in freely moving rats to test both the acute and long-term effect of clozapine. A dose-response study for the acute effect and a chronic study for the long-term effect revealed that the mesocortical system is the one most affected as reported in an earlier abstract (32).

METHODS

Subjects and Surgery

We individually housed 20 male Sprague-Dawley rats, weighing between 300 and 350 g, with food and water ad lib in a room with temperature controlled at 25°C and a 15 L : 9 D cycle. On the day of surgery, the rats received an intraperitoneal (IP) injection of atropine followed 30 min later by IP pentobarbital (20 mg/kg) and ketalar (40 mg/kg); then, three guide cannulae were implanted in the brain of each rat. The cannulae were aimed to the PFC, NAC, and posterior part of the STR. The coordinates were A 11.2 mm, L 0.5 mm, and V 1.5 mm for the PFC; A 10.0 mm, L 1.2 mm, and V 4.0 mm for the NAC; and A 8.7 mm, L 3.0 mm, and V 4 mm for the STR relative to the interaural axis, midsagittal suture, and leveled surface of the skull. Microdialysis probes were inserted at least 7 days after recovery from surgery and extended an additional 5 mm beyond the guide shaft.

Microdialysis

Microdialysis probes of the narrow, concentric type were made of fused silica capillary (150 μm OD × 75 μm ID), inside 26-ga stainless-steel tubing with a 4-mm reconstituted cellulose tubular tip with a molecular weight cutoff of 6000. Probe details and recovery characteristics have been reported elsewhere (35). On the day of microdialysis perfusion, three probes were connected by their inlet tubes to three syringe pumps through a triple swivel joint. This swivel joint allowed free movement to the rat during the perfusion. The outlet of each probe was connected to a vial clipped to the tether line 5 cm above the rat's head. The three probes were inserted into the brain of the awake rat and the animal placed in the perfusion chamber. The perfusion solution, which flowed at 1 μl/min was a modified Ringer's solution made of 146 mM NaCl, 3.4 mM KCl, and 2.0 mM $CaCl_2$ at pH 6.0. Samples were collected from each probe every 20 min and analyzed in three high-performance liquid chromatography (HPLC) systems. All of these systems were equipped with rapid refill pumps (model 222D; SSI, State College, PA), injection valves (model 7125; Rheodine, Cotati, CA) and 10-cm-long, 3.2-mm-bore, 3-μm packing ODS columns (Browlee, Foster City, CA). Two systems were equipped with coulometric detectors (model 5100A; ESA, Chelmsford, MA) and one with an amperometric detector (model 400; EG&G Princeton Applied Research Corp., Princeton, NJ). The mobile phase for the coulometric systems was a 40-mM phosphate buffer at pH 3.6, with 238 μM EDTA, 1.3 mM heptane sulfonic acid as an ion-pairing reagent, and 6% v/v methanol. The mobile phase for the amperometric system was a 150 mM acetate buffer at pH 3.1, with 100 μM EDTA, 1.38 mM octanesulfonic acid as an ion-pairing reagent, and 3% v/v acetonitrile. Neurochemicals were measured in the reduction mode on the second detection electrode of the coulometric detectors with the potential set as follows: guard cell: +500 mV, electrode 1: +100 mV, and electrode 2: −350 mV. In the amperometric detector the neurochemicals were oxidized at 750 mV against an Ag-AgCl reference electrode. The peaks of the different neurochemicals were identified by their retention time and compared to the height of the peaks of standard solutions which were injected at the beginning and end of the experiment every day.

We conducted two experiments. In the first, a dose-response study was performed to calibrate the acute effect of clozapine. We divided 16 rats into four groups to receive one of the following doses of clozapine: 0 (vehicle injection), 10, 20, and 40 mg/kg, subcutaneously. Clozapine (Sandoz, Basel, Switzerland) was dissolved in 0.1 N hydrochloric acid, and 0.1 N sodium hydroxide was added until the solution became clouded. Then, another drop of 0.1 N HCl was added to redissolve the precipitate. To obtain baseline, samples were collected starting at least 3 h after probe insertion until in

five consecutive, 20-min samples, the neurochemicals showed < 10% variation. Then, the clozapine or vehicle at the same pH was injected and eight more samples were collected. For the second experiment, two more rats were added to the group that received 20 mg/kg and two more to the group that received vehicle. This provided two groups of six rats each. After acute measurements, the microdialysis probes were removed and saved in refrigerated nanopure bacteria-free water for reinsertion 1 month later after chronic clozapine treatment. The rats were returned to their home cage and received daily injections of clozapine or vehicle. After 29 injections (including the first one during the first microdialysis session for the acute study), the probes were reinserted and a microdialysis session was performed before, during, and after injection of clozapine or vehicle on the last day.

For histology, after the animals received an overdose of pentobarbital, their brains were perfused with saline and formalin, frozen, sliced, and photographed as wet, unstained sections. The tracks of the probes were located on the Paxinos and Watson atlas (62).

For data analysis, to minimize interprobe and interanimal variations, data were expressed in the acute as well as the chronic experiment as a percent of the first sample in the acute experiment.

The dose-response relation was calculated by regression analysis, and the significance of fit by analysis of variance (ANOVA). The effect on neurochemicals was assessed by one-way ANOVA followed by Newman-Keuls t-test when appropriate, and by two-way ANOVA (one within, one between) for the comparisons of the vehicle vs. clozapine effects.

RESULTS

Table 1 shows the basal levels of DA, DOPAC, and HVA expressed as picograms/20 μl (mean ± SE) and uncorrected for relative recovery.

Acute Effect of Clozapine

After the first clozapine injection, DA, DOPAC, and HVA increased in the PFC. The doses of 40 and 20 mg/kg significantly increased DA [$F(1, 6) = 28.3$, $p < 0.002$; $F(1, 6) = 6.6$, $p < 0.05$, respectively]. They also increased DOPAC [$F(1, 6) = 7.8$, $p < 0.04$; $F(1, 6) = 9.6$, $p < 0.03$] and HVA [$F(1, 6) = 26.1$, $p < 0.003$; $F(1, 6) = 6.0$, $p < 0.05$] (Fig. 1). The larger the dose, the stronger was the effect (Fig. 2). The linear equation that best fit DA data was $Y = 84 + 2.7x$, with a regression coefficient of $r = 0.99$. The statistical analysis by ANOVA showed a significant fit to a straight line [$F(1, 2) = 305.6$, $p < 0.002$]. For DOPAC, the best-fitting equation was $Y = 92 + 1.5x$, $r = 0.99$, $F(1, 2) = 75.3$, and $p < 0.01$. For HVA, the best-fitting equation was $Y = 77 + 2x$, $r = 0.99$, $F(1, 2) = 102$, and $p < 0.001$. In the NAC, on the other hand, only the highest dose of clozapine (40 mg/kg) tended to increase DA, DOPAC, and HVA, and these increases were not significant because of a large variance in the data (Fig. 3). In the STR, neither dose changed DA, DOPAC, or HVA (Fig. 4).

TABLE 1

BASAL LEVELS OF DA AND METABOLITES
(pg/20 μl; MEAN ± SEM)

	Dopamine	3,4-Dihydroxy-phenalacetic Acid	Homovanillic Acid
Prefrontal cortex	1.8 ± 0.2	339 ± 68	520 ± 89
Nucleus accumbens	9.0 ± 1.6	2186 ± 402	1249 ± 258
Striatum	18.9 ± 4.4	1645 ± 308	1750 ± 214

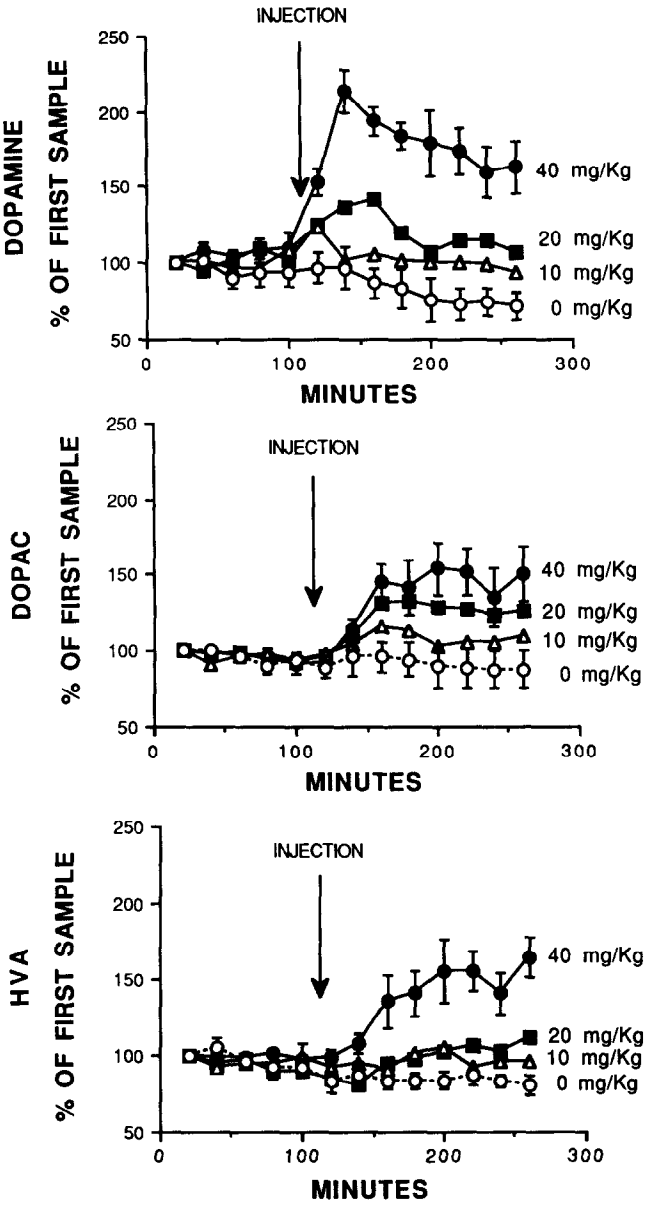

FIG. 1. Acute clozapine increased dopamine (mean ± SE) and its metabolites in the prefrontal cortex. These increases followed a dose-response relationship. Significant effects were observed at doses of 20 and 40 mg/kg.

Chronic Effect of Clozapine

For the chronic study, we added two rats to the 20-mg/kg group and two more to the vehicle group. Figures 5, 6, and 7 replot the first response (day 1) to clozapine with these rats added for direct comparison to the 30th day in the same six animals. After chronic treatment with 20 mg/kg clozapine for 29 days, there was a significant decrease of basal DA relative to day 1 [$F(1, 9) = 8.50$; $p < 0.02$], DOPAC [$F(1, 9) = 9.2$,

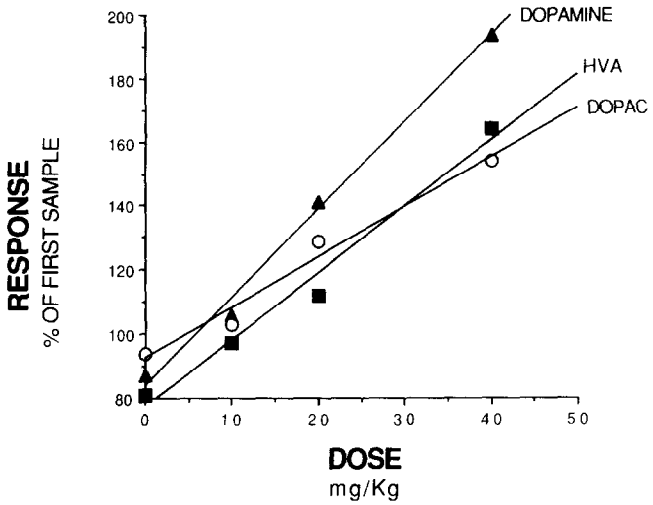

FIG. 2. Regression analysis of the effects of four doses of clozapine on the increase of dopamine (DA), 3,4-dihydroxyphenalacetic acid (DOPAC), and homovanillic acid (HVA) in the prefrontal cortex. The data for the points were taken from postinjection samples no. 3 for DA, no. 5 for DOPAC, and no. 8 for HVA (See Fig. 1).

$p < 0.02$], and HVA [$F(1, 9) = 14.64, p < 0.01$] in the PFC, but no change in the NAC or STR. The 30th injection of clozapine had no effect on any of the three regions.

Histology showed that the microdialysis probes were located in the PFC at the level of the cingulum regions I and II and the infralimbic cortex. NAC probes were in the posterior, medial region of the NAC. STR probes were in the lateral part of the caudate nucleus.

DISCUSSION

We will discuss first the initial DA level, then the acute response to clozapine, and finally the chronic response. In a previous study with the triple microdialysis technique (31), we found that the basal, initial concentration of DA, DOPAC, or HVA to be different from one region to another according to the following rank order: STR > NAC > PFC. This finding holds again in the present experiment. However, in the present experiments basal DA level was lower in the PFC and higher in the NAC than we found previously; also, DOPAC was lower than HVA in the PFC and about the same in the STR. The main difference between the present and the previous study is the calcium concentration in the Ringer's solution. In the present study, the calcium concentration was lowered to match the concentration used by O'Connor et al. (60) in a microdialysis study with clozapine. It has been shown that high calcium concentration in the perfusion fluid increases basal DA level (25,57). This would explain the lower level of DA in the PFC in the present study. The higher level of DA in the NAC might be caused by low DA in the PFC (72).

Acute clozapine administration increased dopamine turnover in the PFC and had little or no effect in the NAC or STR. The fact that the increase in DA turnover in the PFC was dose dependent suggests that this is a reliable pharmacologic phenomenon produced by neural feedback in response to DA receptor blockade. In addition, the same rats that showed increased dopamine turnover in the PFC did not show it in the NAC or STR. This buttresses the argument that clozapine's actions on the PFC are selective. The increase in dopamine turnover is explained as resulting from the interruption of a negative feedback loop between the PFC and the ventral tegmental area (65). The existence of ultrashort feedback loops has also been postulated (27,81). Such negative feedback in normal conditions decreases DA cell firing and DA release and metabolism, presumably by GABAergic inhibition of the DA neurons or by autoreceptor activation in the ventral tegmental area. When the DA receptors are blocked the DA cells become disinhibited, thereby increasing DA release and subsequent metabolism to DOPAC and HVA. According to this explanation, clozapine should have a high affinity for postsynaptic mesocortical DA receptors but low affinity for nigrostriatal or mesolimbic DA receptors. There is some evidence that typical and atypical neuroleptics bind differently to DA receptors. Experiments have shown that DA receptors exist in two

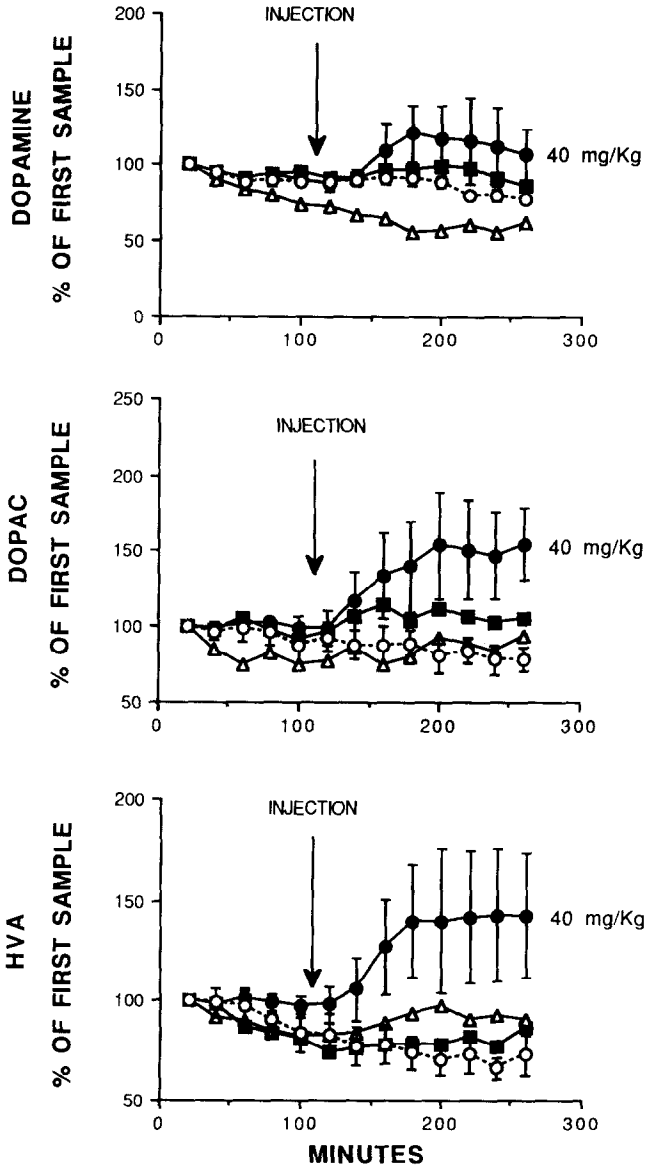

FIG. 3. The highest acute dose of clozapine (i.e., 40 mg/kg) increased dopamine turnover in the nucleus accumbens; 20 mg/kg had no effect. Abbreviations as in Fig. 2.

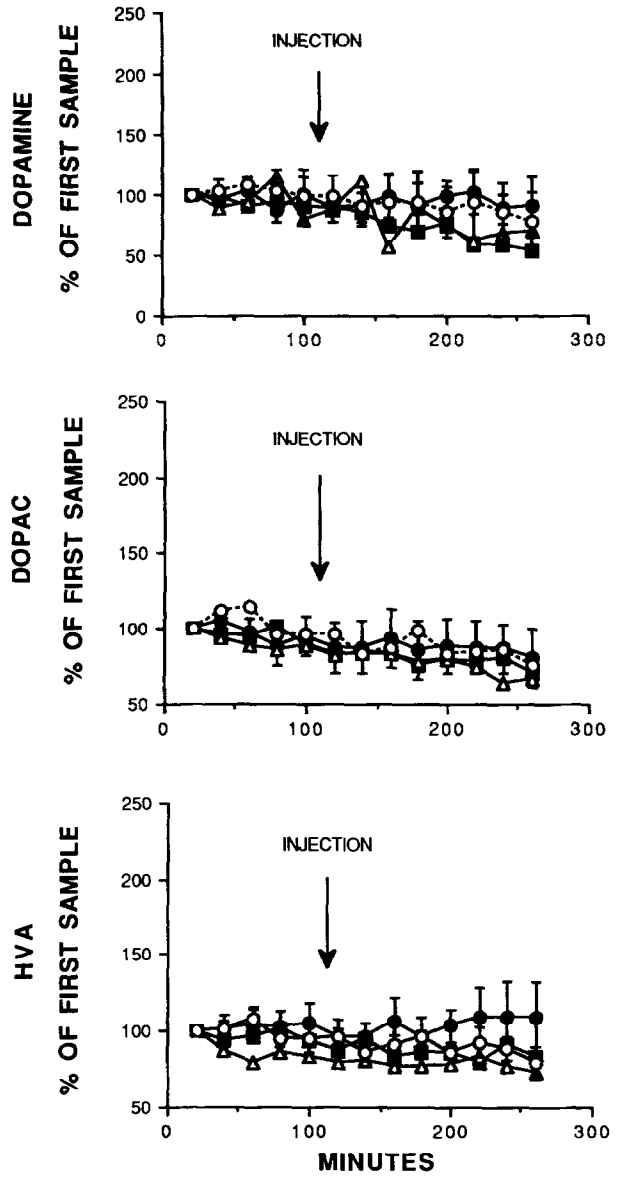

FIG. 4. Acute clozapine at three different doses had no effect of dopamine turnover in the striatum. Abbreviations as in Fig. 2.

or more different states or conformations. The D_1 receptor exists as adenylatecyclase-coupled and uncoupled forms. Typical neuroleptics have more affinity for the uncoupled form, and atypical neuroleptics such as clozapine have higher affinity for the coupled form (3). Regional differences for the binding of neuroleptics have been reported. A study has shown that atypical neuroleptics such as clozapine and sulpiride are effective inhibitors of spiroperidol binding in the septum, olfactory tubercle, and hippocampus but are ineffective on the STR (45). Moreover, recent cloning studies have shown the existence of a D_1 subfamily of receptors and a D_2 subfamily (71). The D_2 subfamily consists, so far, of the classical D_2 plus the D_3 and D_4 receptors. Interestingly, clozapine exhibits 10-fold higher affinity than any other neuroleptic (71) for D_4 receptors. The messenger RNA for synthesis of human D_4 receptors has its highest expression at the prefrontal cortex, midbrain, amygdala, and medulla (79). If the clozapine effect in the present experiment was due to interaction with D_4 receptors, this could explain why this drug preferentially increases DA turnover in the prefrontal cortex, although this is not necessarily the case in rats. Finally, in addition to its affinity for DA receptors, clozapine has affinity for cholinergic (54), $\alpha 1$ adrenergic (47,51,56,75), and 5-HT_2 (30,55) receptors. Interaction with one or more of these receptors might make clozapine more effective in regions such as the PFC than in the STR.

Acute results in the present experiment agree with those of Chen et al. (16) in freely moving rats, but are different from a microdialysis study in anesthetized rats in which O'Connor et al. (60) found that acute clozapine increased dopamine turnover in the STR. Two major differences might account for the discrepancy: they used general anesthesia and different probe locations. Their result showed that clozapine was less effective in one part of the STR than another, which means that not all the regions of the STR react to clozapine (60). Our probes were located in a region of the STR that is lateral and caudal to the head of the caudate, and the probe was sampling from both the dorsal and ventral regions. In a study in awake rats, Ichikawa and Meltzer (38) found that acute injections of clozapine increased dopamine and DOPAC in striatal and accumbens dialyzates. Again, the conditions in that experiment and ours were different. Their study was done in rats 24 h after implantation of U-shaped probes. The present experi-

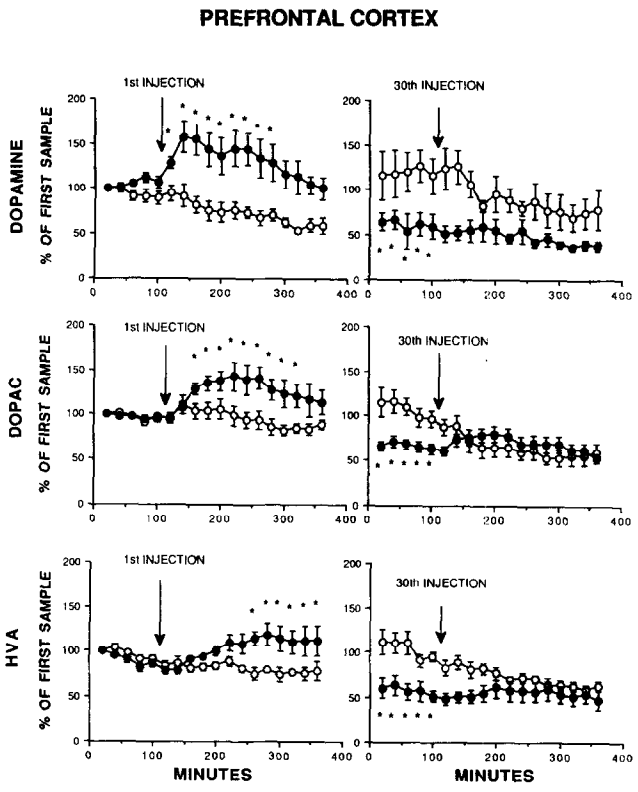

FIG. 5. Each of the six panels shows extracellular dopamine before, during, and after clozapine injection. In the prefrontal cortex, acute administration of 20 mg/kg of clozapine (left column, ●) significantly increased dopamine turnover, but chronic administration (right column, ●) decreased it relative to day 1 in the clozapine group (*$i <$ 0.02). Note that the 30th injection of clozapine had no effect on dopamine turnover. ○, Data of the control group. Abbreviations as in Fig. 2.

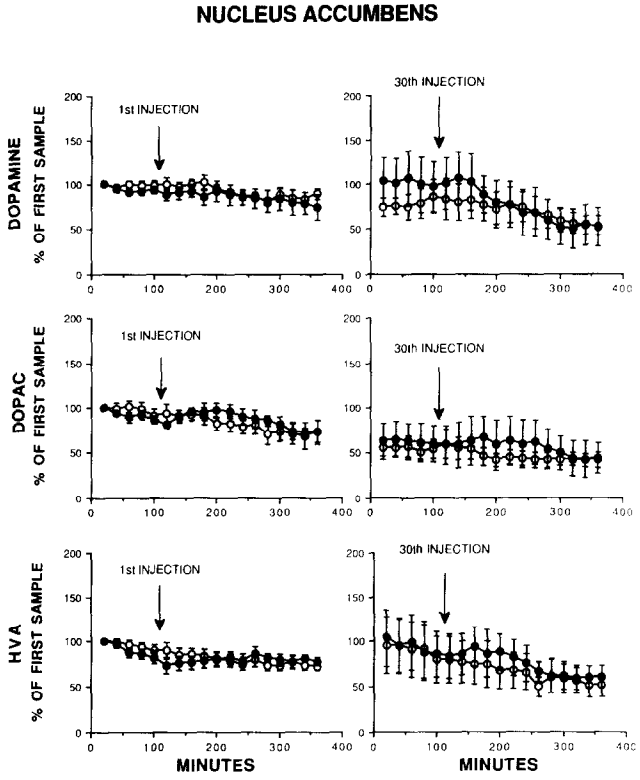

FIG. 6. In the nucleus accumbens, both acute (left column, ●) and chronic (right column, ●) administration of clozapine had no effect on dopamine, 3,4-dihydroxyphenalacetic acid (DOPAC), or homovanillic acid (HVA). ○, Data of the control group.

ments were carried out in rats fully recovered from surgery and bearing guide shafts for the insertion of narrow probes. In addition, the probes in Ichikawa and Meltzer's study could have been in a different location than the ones in our study. The studies of O'Connor et al. (60) and Ichikawa and Meltzer (38) suggest that clozapine does block striatal and accumbens DA receptors, whereas the present study suggests that the lack of extrapyramidal side-effects with clozapine treatment could be attributed in part to a lack of blocking action on striatal or accumbens dopamine receptors. Recent experiments with local infusion by reverse dialysis showed a large effect for clozapine in the PFC, whereas haloperidol was more effective in the STR (63). Studies on immediate early gene activation by clozapine add support to our contention that the main locus of clozapine action is the PFC. A clozapine dose of 20 mg/kg had no detectable effect on c-fos expression in the striatum of rats (66). In the NAC, there were regional differences with the anterior NAC showing large amounts of Fos; the posterior accumbens had low Fos reactivity, particularly in the core of the nucleus. In the PFC, clozapine produced a significant, dose-dependent increase in Fos-positive neurons. The present results complement the c-fos expression studies. Acute administration of clozapine increased DA in a dose-dependent fashion only in the PFC, with mixed results in the NAC and negative results in the striatum.

The chronic effects of clozapine are particularly interesting and clinically relevant. Basal DA and its metabolites were lowered, suggesting decreased DA turnover in the PFC, but not in the NAC or STR. The fact that this effect was anatomically selective provides an important control for the effects of the microdialysis procedure itself. In particular, it is very unlikely that probe reinsertion causes this selective decrease in DA turnover in the PFC. Multiple probe insertions can be a problem in some cases (14), but in the present design if probe reinsertion were a causal agent, it should have decreased DA turnover in the control group or in the STR or NAC of the experimental groups. Second, we have shown that the percent response to haloperidol was not affected by reinsertion 30 days after chronic treatment with haloperidol, even though basal levels were lower in the PFC (33). Moreover, probe reinsertion with no drug treatment did not affect DA levels (31). Similarly, Georgieva et al. (28) reinserted microdialysis probes at the 15th and 17th day after a first insertion in the striatum and observed no change in the DA level, suggesting that the time elapsed between reinsertions was critical. The longer the time, the smaller the difference was in DOPAC and HVA after reinsertion. Therefore, the results confirm and extend two other microdialysis reports (37,40) in which no effect of long-term administration of clozapine on basal levels was observed in the NAC or STR. On the other hand, these results contrast with the microdialysis study of Chen et al. (17) in awake rats and in vivo voltammetry studies in anesthetized rats that had shown a decrease in dopamine turnover in the NAC but not in the STR after chronic administration of clozapine (9,46). It is possible that general anesthesia changes the response to neuroleptics. It is known that general anesthesia causes stress and can change chemical balances in the brain.

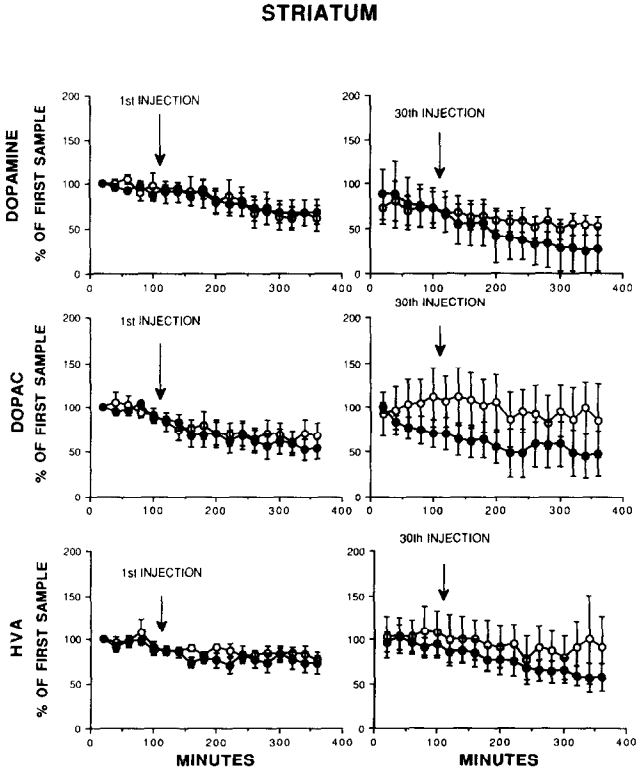

FIG. 7. Both acute (left column, ●) and chronic (right column, ●) administration of clozapine had no effect on dopamine, 3,4-dihydroxyphenalacetic acid (DOPAC), or homovanillic acid (HVA) in the striatum. ○, Data of the control group.

Anesthesia is reported to increase serotonin and DA turnover. It also changes the response to haloperidol (41,76).

Microdialysis in the PFC of awake rats revealed a decrease in basal extracellular DA when the clozapine was given subcutaneously in our study (31), but given IP, Chen et al. (16) found no effect. When clozapine was given orally, Youngren et al. (83) reported an increase, but they did the microdialysis with anesthesia. The route of administration may be a relevant variable in addition to those already mentioned, such as anesthesia and probe placement. There are also large differences in the calcium concentration used for probe perfusion [e.g., 3.37 mM in the Chen study (17), 2 mM in this study, and 1.2 mM in the Youngren (83) study]. The present results point to the mesocortical DA system as a site of clozapine antipsychotic action when given chronically.

The present results with chronic clozapine can be compared to haloperidol. With both drugs, our triple microdialysis studies show the strongest chronic effect on the PFC (31,33). Chronic administration of haloperidol did not affect the DA and DOPAC levels in the NAC; however, the Ichicawa and Meltzer study (37) did show a decrease of DA and DOPAC in the STR and NAC, a result that has been replicated by those authors (38). However, our study and theirs were done with different doses of haloperidol; we used 0.5 mg/kg and they used 2 mg/kg. A recent microdialysis report (12) showed that chronic administration of 0.5 mg/kg of haloperidol had no effect on dopamine levels in the STR, which coincides with our results for the same dose. Therefore, it is likely that the dose of haloperidol is a critical variable for the evaluation of the effect of chronic administration.

The consequences for dopaminergic theories of schizophrenia are interesting. It was postulated that the mesolimbic system is the locus of action for the antipsychotic effects of neuroleptics (50). The present findings and those of Ichicawa and Meltzer suggest instead that the antipsychotic effect of atypical neuroleptics is due in part to their action on the PFC. This confirms early speculation of PFC involvement in schizophrenia based on cognitive and language processing. Later, Laduron et al. (44) found that the PFC was the most affected by chronic neuroleptic treatment based on brain homogenate studies. Other evidence obtained with blood flow measurement techniques have shown alterations in the normal blood flow response in the PFC of schizophrenic patients when they execute alternate delayed-response tests (80). However, on a note of caution, we still cannot rule out involvement of the NAC or some of its components (17). A reciprocal relationship between the PFC and the NAC has been shown (64,72). When amygdala stimulation increases DA turnover in the PFC, it decreases extracellular DA in the NAC (64); and conversely, amygdala lesions decrease extracellular DA in the PFC and increase it in the NAC (72). Therefore, the decrease of DA turnover in the PFC, if and when it is caused by clozapine, might tend to increase DA turnover in the NAC, thereby counteracting any abnormal tendency toward low DA turnover in the NAC which might be involved in affective disorders.

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