Haloperidol and clozapine increase neural activity in the rat prefrontal cortex

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Abstract

Haloperidol and clozapine have been widely used to alleviate schizophrenic symptoms, but their physiological effects in the prefrontal cortex (PFC) are not known. Effects of haloperidol and clozapine on single unit activity were investigated in the medial PFC of anesthetized rats. Injection (intraperitoneal) of haloperidol (1 mg/kg) or clozapine (20 mg/kg) significantly elevated discharge rates of PFC neurons. Considering that hypofrontality is one characteristic of schizophrenic symptoms, these results raise the possibility that enhancement of PFC neural activity contributes to therapeutic effects of haloperidol and clozapine.

Keywords: Schizophrenia; Antipsychotic drug; Haloperidol; Clozapine; Single unit; Prefrontal cortex

Since the introduction of chlorpromazine as an effective treatment agent of schizophrenia nearly 5 decades ago, dozens of antipsychotic drugs (APDs) have been introduced. Despite the proven efficacy in the treatment of schizophrenia, precise mechanisms and sites of action of APDs have remained to be elucidated. Delineation of sites of action of APDs is probably the best strategy of elucidating the action mechanisms of APDs \cite{6}. To relate functional outcomes of APDs to their underlying actions in the nervous system, information about the effects of APDs on neural activity is especially important. Previous studies have examined the APD effects on activities of midbrain dopaminergic neurons. Results indicate that chronic administration of APDs induces depolarization block in the A9 and A10 dopaminergic neurons \cite{4,5}. In spite of its importance in schizophrenia, however, the effects of APDs on neural activity in the prefrontal cortex (PFC) have not been examined.

Several lines of evidence indicate involvement of the PFC in the pathophysiology of schizophrenia. Brain imaging studies have revealed functional \cite{1,10,14,15} as well as structural abnormalities \cite{12} in the PFC of schizophrenic patients. Furthermore, clinical response to clozapine, an atypical APD, was inversely related to prefrontal atrophy \cite{7}. These studies suggest the possibility that pathophysiology of schizophrenia involves abnormality in PFC neural activity and alterations in PFC neural activity contribute to antipsychotic actions of APDs. As an initial step toward investigating this possibility, we examined whether or not systemic injection of APDs changes spontaneous firing of single neurons in the PFC of anesthetized rats.

Single units were recorded in the medial PFC of 38 young (3-month-old) male Sprague–Dawley rats. Animal care and surgery were performed following the National Institutes of Health guidelines. Animals were mounted on a stereotaxic frame after deeply anesthetized with sodium pentobarbital (50 mg/kg). The level of anesthesia was monitored by frequently checking response to tail pinch, and sodium pentobarbital (17 mg/kg) was supplemented as necessary to maintain the depth of anesthesia. Two stainless steel screws were implanted in the skull for ground and reference leads. One or two tetrodes were mounted on a micromanipulator and lowered into the medial PFC (2.4–2.8 mm A and 0.6–0.8 mm L to bregma, 2.5–3.3 mm V from the brain surface) following craniotomy and removal of dura. Tetrodes were constructed by gently twisting four strands of polyimide-insulated nichrome wires (H.P. Reid Co., Palm Springs, CA).
Coast, FL, OD = 15 μm) and applying heat to fuse insulation together without short-circuiting the wires. The electrode tips were plated with gold to lower impedance (300–600 kOhm). Tetrodes allow both simultaneous recordings of multiple neurons and identification of each individual unit based on spike amplitude profile of each unit activity recorded across four electrode channels [13,16]. This technique, compared to other methods, has a number of advantages in recording multiple neighboring neurons with clear isolation of individual units [8]. Detailed description of recording procedures in the medial PFC has been reported [11]. Briefly, unit signals from the tetrode were recorded via a field effect transistor (FET) source-follower headstage. Output signals from the headstage were amplified 10000×, filtered between 0.6–6 kHz, digitized at 25 kHz and stored on a personal computer for future off-line analysis. Units were isolated by projecting the four channels relative amplitude data two-dimensionally, and applying boundaries to each subjectively identified unit cluster. An example of unit signals recorded with a tetrode is shown in Fig. 1A.

When at least one well isolated and stable unit was obtained, baseline unit discharges were recorded for 20 min and unit signals were recorded for additional 45 min after injecting (i.p.) haloperidol (1 mg/kg), clozapine (20 mg/kg) or vehicle (tartaric acid, pH 5.0). When the recording was complete, an electrolytic current (50 μA 10 s) was applied through one of the recording electrodes to make a marking lesion and the animals were perfused with 10% formalin-saline. The brain was then removed, left in formalin-saline for 3–7 days, and transferred to a 10% formalin-saline/30% sucrose solution for about 3 days until it sank to the bottom. Forty micron coronal sections were cut on a sliding microtome and stained with cresyl violet. Tracks and lesion sites were identified under a light microscope.

Effects of haloperidol and clozapine were measured by comparing unit discharges during the 10 min period immediately before the drug injection (baseline) and the 10 min period during which drug effect was stable (from 20 to 30 min after drug injection). Drug effects on firing rate and firing pattern, i.e. inter-spike interval (ISI) distribution, were examined in this study. Because short ISIs contribute more significantly to temporal summation of postsynaptic neurons, only ISIs in the range of tens of milliseconds (burst firing) were considered. ISIs were divided in bins of 10 ms and an index of excessive firing was calculated for each bin as the following:

$$\text{Index of excessive firing} = \log_{10}\left(\frac{O_i}{E_i}\right),$$

where $O_i$ and $E_i$ represent the observed and the expected numbers of ISIs in the $i$th bin, respectively. The expected number of ISIs was calculated assuming that each spike train is an independent Poisson process with a given average firing rate. The result indicates that ISIs up to 30 ms showed higher levels of occurrences than the expected (Fig. 3A). Based on this observation, analysis of burst firing was confined to ISIs within 30 ms. The index of burst firing was determined in the same way as the index of excessive firing except that the ISIs between 1 and 30 ms were included in the calculation:

$$\text{Index of burst firing} = \log_{10}\left(\frac{O_{30}}{E_{30}}\right),$$

where $O_{30}$ and $E_{30}$, respectively, represent the observed and the expected numbers of ISIs (assuming that each spike train is an independent Poisson process) that are shorter than 30 ms. ISIs that are shorter than 1 ms were omitted from the calculation due to the limited temporal resolution of successive spike detection with the current recording method. Non-parametric Wilcoxon signed ranks test was used to determine statistical significance of the treatments for all indices. Data are expressed as mean ± SEM.

A total of 128 well-isolated units were recorded in this study. Light microscopic examinations clearly revealed electrode tracks and electrolytic lesion sites, (i.e. recording

Fig. 1. An example of tetrode recording and histological verification. (A) An example of multiple single unit recording with a tetrode. The abscissa and ordinate of the scatter plot represent peak amplitudes of spike signals recorded by channels 4 and 3 of the four tetrode channels, respectively. Each point is a spike signal that crossed the experimenter-defined threshold. In this example, four units were recorded simultaneously. On the right, average spike waveforms recorded by each tetrode channel (Ch1–4) for each cluster (single unit) are shown. Each row and column represent a cluster and a tetrode channel, respectively. Calibration: 1 ms and 0.5 mV (B) Histological verification of recording sites. The arrow indicates an electrode track. Unit recordings were made in the prelimbic cortex (PL) of the medial PFC. AP: 2.7 mm. Calibration: 1 mm.
locations) in the medial PFC. Recordings were made in the prelimbic cortex (Fig. 1B). Up to six single units were recorded simultaneously in this study. The PFC units showed variable discharge characteristics. The mean discharge rates during the baseline period distributed widely from 0.01 to 9.35 Hz. The overall average firing rate was 0.53 ± 0.09 Hz. This is considerably lower compared to deep layer PFC units recorded in behaving rats (2.07 ± 0.16) [11]. One outstanding feature was that there were a number of extremely low firing (<0.1 Hz) units, and yet many of the low firing neurons showed burst firing. Deep layer medial PFC units in behaving animals were classified into regular spiking and fast spiking cells in the previous study [11]. Classification of the current PFC units based on discharge characteristics was less obvious, however. Therefore, no attempt was made to classify PFC units recorded in the present study.

Only neurons with firing rates higher than 0.1 Hz during the baseline period were included in the analysis (n = 38 for haloperidol and n = 45 for clozapine) to improve reliability of statistical comparisons. However, inclusion of low rate units (<0.1 Hz; n = 28 in haloperidol and n = 17 in clozapine experiments) in the analysis yielded the same conclusions. Vehicle injection did not change discharge rates of PFC neurons. The average firing rates before and after vehicle (tartaric acid) injection were 0.66 ± 0.22 and 0.68 ± 0.22 Hz, respectively, (n = 5), which were not significantly different (P > 0.05). On the other hand, both haloperidol and clozapine injection altered discharge rates of most PFC neurons. Examples are shown in Fig. 2A and firing rates of all neurons before and after the drug treatment are shown in Fig. 2B. Points along the 45° line are the units that did not change firing rates after the drug treatment. Points above and below the 45° line are the units that increased and decreased firing rates after the drug treatment, respectively. As shown, most (34 out of 45 for clozapine and 25 out of 38 for haloperidol) points were found above the 45° line for both clozapine and haloperidol data. On average, firing rate increased from 0.84 ± 0.13 to 1.21 ± 0.15 following clozapine injection. Likewise, haloperidol injection enhanced average firing rate from 0.75 ± 0.24 to 1.24 ± 0.55. The enhancements of firing rates were statistically significant (P < 0.01 for clozapine and P < 0.05 for haloperidol).

Both clozapine and haloperidol decreased the degree of burst firing in significant manners. Clozapine and haloperidol reduced the degree of burst firing from 0.69 ± 0.06 to 0.50 ± 0.06 (n = 45, P < 0.01) and from 0.67 ± 0.07 to 0.57 ± 0.07 (n = 32, P < 0.05), respectively. Six neurons in the haloperidol experiment, that showed no incidence of ISI within 30ms either during the baseline or after the drug treatment, were excluded from the burst firing analysis. A close examination of the data revealed that there exists an inverse relationship between average firing rate and the index of burst firing. Significant negative correlation was observed between the index of burst firing and logarithm of average firing rate whether the data were considered together (r = −0.34; P < 0.01, Fig. 3) or separately according to four different conditions (before and after clozapine or haloperidol treatment; data not shown). Because decreased burst firing following the drug treatment can be explained by changes in the average firing rate, it would be reasonable to conclude that clozapine and haloperidol enhance firing rates of PFC neurons without changing burst firing characteristics.

Cautions should be made when interpreting the present results. First, experiments were performed with anesthetized rats. The APDs may exert different effects in behaving rats. Second, the present study does not provide information about chronic effects of APDs. Since it often takes 2–3 weeks of treatment before APDs exert their therapeutic effects, information about chronic as well as acute effects of APDs on PFC neural activity is necessary. Even with these caveats, the present study raises the possibility that enhancement of PFC neural activity contributes to therapeu-
that clozapine and haloperidol alter activities of other brain areas that in turn enhance PFC neural activity. For example, a recent study has shown that both haloperidol and clozapine enhance firing rates of ventral tegmental area (VTA) dopaminergic neurons that project to the medial PFC [9]. In the present study, the APDs were injected not locally but systemically because one purpose of this study was to obtain information related to the effects of APD intake by humans. It is thus impossible to conjecture whether the observed changes in PFC neural activity are due to local or secondary actions of the APDs. Further studies employing local injections are needed to elucidate exact action mechanisms of each drug.

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