Population Response of Midbrain Dopaminergic Neurons to Neuroleptics: Further Studies on Time Course and Nondopaminergic Neuronal Influences

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In the present study, we examined the effects of the cholecystokinin receptor antagonist, proglumide, on the depolarization-induced inactivation of A9 and A10 dopaminergic neurons produced by repeated administration of a classical antipsychotic drug (dopamine receptor antagonist). In addition, we studied the nature of the effects of acute (1–48 hr) and long-term (7 month) treatment with the butyrophenone neuroleptic haloperidol on both the basal firing rate and population response of dopamine-containing neurons in these 2 regions. Acute oral administration of haloperidol (0.5 mg/kg) results, within 1 hr of administration, in an increase in both the firing rate and number of spontaneously active dopamine neurons encountered in both A9 and A10 regions. These effects of a single treatment persist for a minimum of 6 hr and, with respect to firing rate, are not completely normalized for at least 24 hr. In contrast, 7 month continuous treatment with haloperidol reduces the number of spontaneously active DA neurons encountered in both regions in a manner similar to that observed at 21 d. This effect is inferred to be due to the induction of depolarization-induced inactivation of these neurons, since the acute administration of the normally hyperpolarizing, direct-acting dopamine receptor agonist apomorphine (64 μ g/kg) immediately reverses this reduced number of cells per track to near control levels. This effect appears to be dependent on the continued presence of haloperidol since, when animals treated for 7 months are sampled 14 d after the cessation of drug administration, spontaneous activity is no different from that observed in age-matched controls. Cholecystokinin, which has been shown to have a complex tonic influence on dopamine neurons in both A9 and A10, may be involved in the depolarization-induced inactivation caused by antipsychotic drugs. Thus, while the acute administration of the selective cholecystokinin receptor antagonist, proglumide, alters neither the firing rate, population response, nor degree of burst activity in these 2 regions, both the degree of bursting and number of cells per track are significantly elevated fol-

lowing 21 d oral administration. Furthermore, low doses of proglumide immediately reverse the depolarization inactivation of A9 and A10 dopamine-containing neurons produced by repeated haloperidol treatment. These data suggest that endogenous cholecystokinin may influence dopamine cell activity and play a role in the neuroleptic- and depolarization-induced inactivation of midbrain dopamine neurons.

Several studies have now demonstrated that chronic, but not acute, administration of antipsychotic drugs (APDs) produces a decrease in the number of spontaneously active dopamine (DA)-containing neurons within the midbrain (Bunney and Grace, 1978; Chiodo and Bunney, 1983a; White and Wang, 1983a, b). In these studies it has been repeatedly demonstrated that classical APDs (which are known to induce extrapyramidal side effects) inactivate DA-containing neurons within both the substantia nigra (A9) and the adjacent ventral tegmental area (A10). Atypical APDs (those associated with a low incidence of side effects) inactivate only A10 DA neurons. These effects are thought to model both the clinical efficacy and neurological side effect potential of these compounds in man. Thus, APDs with a low neurological side effect potential (e.g., clozapine and mesoridazine) inactivate A10 DA neurons and not those in the A9 area, while typical APDs [i.e., haloperidol (HAL) and chlorpromazine] decrease the number of active DA neurons observed in both regions (Chiodo and Bunney, 1983a; White and Wang, 1983a, b; Chiodo and Bunney, 1984).

Recent work has begun to investigate which mechanisms and neuronal systems are involved in the development of depolarization-caused inactivation of midbrain DA neurons. It is hoped that such studies will discover important ways to prevent the induction of depolarization inactivation of A9 neurons so that new drugs can then be developed that do not produce this effect and thus have a reduced incidence of the associated side effects. Research to date has yielded a variety of findings. For example, it has been found that neurochemical lesions of forebrain structures that are innervated by midbrain DA-containing neurons (i.e., nucleus accumbens, caudate nucleus) prevent the induction of depolarization inactivation with repeated APD administration (Bunney and Grace, 1978; White and Wang, 1983a, b). In addition, it has been suggested that feedback pathways from forebrain structures are important for the maintenance of the APD-induced inactivation of A9, but not A10, DA-containing neurons (Chiodo and Bunney, 1983a). It has also been shown that the coadministration of either an anticholinergic agent or an α_1 -noradrenergic antagonist simultaneously with a typical

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Figure 1. Effect of acute haloperidol administration on the mean ($\pm$ SEM) number of spontaneously firing DA cells per track and firing rate (spikes per sec) observed in both A9 and A10 areas. In the *top panel*, the numbers in parentheses represent the number of neurons sampled, and in the *bottom panel*, the animals sampled. * $p < 0.05$; ** $p < 0.01$, relative to untreated controls; 2-tailed Dunnett test.

APD (HAL) prevents the induction of depolarization inactivation of A9 neurons (Chiodo and Bunney, 1985). The result of anticholinergic coadministration may be relevant to the clinical observation that cotreatment with an anticholinergic drug and an APD is associated with a reduction in extrapyramidal side effects (Fann and Lake, 1976; Manos et al., 1981; McEvoy, 1983).

In the present paper we describe a series of studies designed to further characterize the nature of neuroleptic-induced depolarization inactivation of DA neurons. First, we examined the time course of the effects of a single oral administration of a neuroleptic on the electrical activity of mesencephalic DA neurons. Second, the effect of long-term (7 month), repeated oral administration was also determined. This was considered important since, to date, the effects of oral neuroleptics given for only 1–21 d had been studied electrophysiologically. Finally, since it has been observed that cholecystokinin (CCK), administered either intravenously or microiontophoretically (Skirboll et al., 1981), can depolarize DA-containing neurons sufficiently to inactivate them, we examined the effects of the specific CCK antagonist proglumide (Chiodo and Bunney, 1983b; Wang et al., 1984; Bunney et al., 1985) on the depolarization inactivation of midbrain DA neurons induced by repeated HAL administration.

Materials and Methods

Animals and drug treatments. Male albino rats (Sprague-Dawley; CAMM) weighing 200–225 gm at the start of the experiment were used. Animals were housed 2 per cage on a 12 hr light:dark cycle, with free access to food and water. In the acute experiments, animals were given one of the following drugs via intragastric intubation: HAL (0.5 mg/kg; McNeil) or proglumide (PROG; 10 mg/kg; Robins). For the chronic experiments, additional groups of animals were given some of the same drugs in their drinking water for 21 d. Control animals were housed for the same period of time. All animals were weighed twice a week, and the drugs were administered in lightproof water bottles. Daily water intakes were determined and used to calculate the mean daily dosage. No differences in weight gains were observed.

Figure 2. Effects of acute, 21 d (21-d), and 7 month (7-m) treatment with haloperidol on the mean ($\pm$ SEM) number of spontaneously firing DA cells per track observed in both A9 (*top graph*) and A10 (*bottom graph*) regions. The shaded areas represent the control means ($\pm$ SEM). The bars labeled 7-m + APO present data collected from animals treated for 7 months and then given apomorphine acutely at the time of recording (64 μ g/kg; see text). The bars labeled 7-m + 14d present data collected from animals treated for 7 months with HAL and allowed a 14 d washout period. * $p < 0.01$, relative to untreated controls; 2-tailed Dunnett test. Number below each bar indicates the number of animals studied.

Electrophysiological methods. Either 1 hr after acute drug administration or on the last day of continuous treatment, animals were anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic apparatus. The experimenter was always blind with respect to the treatment condition of the animal being studied. Body temperature was monitored and maintained at 36–37°C. The scalp was reflected, and the skull overlying both the substantia nigra (A9; anterior 1750 to 2580 μ m, lateral 1700 to 2100 μ m; König and Klippel, 1981) and the ventral tegmental area (A10; anterior 1610 to 2420 μ m, lateral 100 to 500 μ m) was removed. Single-unit extracellular neuronal activity was recorded using a single-barrel micropipette (overall tip diameter, <1 μ m), which was filled with 2 M NaCl containing 2% pontamine sky blue dye (*in vitro* impedance at 135 Hz, 3–10 M Ω). Unit activity was monitored using a high-input-impedance amplifier (bandpass filter settings: 0.1–3 kHz) whose output was led into an analog oscilloscope, audiomonitor, PDP-11/34 digital computer, and window discriminator. Spontaneously active DA neurons from both A9 and A10 areas were counted by passing the recording micropipette through a stereotaxically defined block of tissue (120,000 μ m²) that could be reproducibly located from animal to animal. Twelve electrode penetrations (200 μ m apart) were made within each region. The sequence of these tracks was kept constant between animals. Only electrophysiologically identified DA neurons were sampled (see Bunney, 1979; Grace and Bunney, 1980; Grace and Bunney, 1984a–c). Computer analysis of interspike interval data was performed to determine the degree of burst pattern discharge of the DA neurons sampled (Chiodo et al., 1984). Recording sites were marked by passing –30 μ A of current through the recording electrode for a minimum of 10 min, which resulted in the deposition a 50 μ m diameter spot of sky blue dye at the pipette tip. At the end of each experiment, brains were fixed in 10% buffered formalin. Frozen sections were then cut at 50 μ m intervals and stained with a Formol-thionin solution (Donovick, 1974). A light microscope was used to locate the spot of ejected dye. Physical examination verified that all electrode tracks in the present experiment were confined to stereotaxically defined blocks of tissue located within the A9 and A10 regions.

Statistics. Comparisons were made between populations of DA neurons sampled in both control and drug-treated (acute or chronic) animals. Overall statistical significance was determined by an analysis of variance. Post hoc comparisons of individual group differences relative

Figure 3. Effects of both acute (A-PROG) and chronic (C-PROG) administration of the CCK receptor antagonist proglumide at 2 doses (1 and 10 mg/kg; oral) on the mean ($\pm$ SEM) number of spontaneously firing DA cells per track, spontaneous firing rates (spikes per sec), and degree of bursting (burst number). The number within each bar indicates either the number of animals sampled (cells per track) or the number of cells recorded (spikes per sec and burst number). * p < 0.05; ** p < 0.01, relative to untreated controls; 2-tailed Dunnett test.

to control were conducted using a 2-tailed Dunnett test. When pre- and postcomparisons were made on a single group, a paired t test was employed.

Results

Time course of HAL effects

In the present experiments, animals were administered HAL (0.5 mg/kg) once and sampled electrophysiologically either 1, 3, 6, 24, or 48 hr later. In the 1, 3, and 6 hr groups there was a significant increase in the number of active DA neurons in

both A9 and A10, as compared to control animals, while the 24 and 48 hr groups were not different (except for a slight but significant elevation in firing rate in A10; Fig. 1). The firing rates of DA neurons in both regions were also significantly increased in the 1, 3, and 6 hr groups (Fig. 1). In order to examine the effects of continuous oral HAL treatment, the number of cells per track in A9 and A10 were counted in additional animals after either 21 d or 7 months (210 d) of continuous drug administration. As previously reported (see the introduction), 21 d of continuous APD administration significantly decreased the number of cells per track in both A9 and A10 (Fig. 2). In animals that received HAL for 7 months, the number of spontaneously active DA neurons in both regions was also significantly less than that observed in controls, but indistinguishable from the decrease seen at 21 d (Fig. 2). This decrease in the number of DA cells per track could be reversed by the systemic administration of the DA receptor agonist, apomorphine (APO; 64 μ g/kg, i.v.; Fig. 2). In this experiment, 1 side of the animal was sampled first, then analogous areas on the other side were recorded after the administration of APO. In a final group of animals treated with HAL for 7 months, HAL was withdrawn and the animals were recorded 14 d later. In this group, the number of DA neurons in both A9 and A10 was no different from control values (Fig. 2).

Effects of PROG on the population response of midbrain DA neurons

In animals treated acutely with PROG (1 or 10 mg/kg), there was no effect on the number of cells per track, firing rate, and degree of bursting of DA neurons in either the A9 or A10 area (Fig. 3). After 21 d of repeated administration at the same doses of PROG, there was a dose-dependent increase in cells per track in both A9 and A10 regions, as well as an increase in the degree of bursting in both regions (Fig. 3). However, there was no change in the firing rate in either region. In additional animals that had been treated with HAL for 21 d, the effects of acute PROG administration on day 21 was examined. In this experiment, both areas were sampled on 1 side of the brain (either left or right) and the other side was sampled immediately after the administration of either PROG (0.4 mg/kg, i.v.) or naloxone (NAL, 1 mg/kg, i.v.). As shown in Figure 4, PROG adminis-

Figure 4. Influence of different pharmacological treatments on the depolarization inactivation produced by 21 d administration of HAL. Data are represented as the mean ($\pm$ SEM) number of spontaneously firing DA cells per track that were observed in both A9 and A10 regions. CON, control; PROG, proglumide; NAL, naloxone. The number below each bar is the number of animals sampled. * p < 0.01, relative to untreated controls; 2-tailed Dunnett test.

tration reversed the HAL-induced depolarization inactivation of both A9 and A10 DA neurons, while NAL was without effect.

Discussion

This study has examined the response of midbrain DA neurons to either a single or a continuous 7 month administration of the APD, HAL. The acute oral administration of HAL increases the basal firing rate and number of cells per track in both A9 and A10 areas within 1 hr. These effects do not return to normal until 24 hr later. This is in agreement with earlier observations that demonstrated that acute APD treatment does not produce a depolarization inactivation of midbrain DA-containing neurons (Grace and Bunney, 1979; Chiodo and Bunney, 1983a; White and Wang, 1983a, b). Animals sampled after 7 months of continuous oral HAL administration showed a decreased number of spontaneously active midbrain DA neurons owing to the apparent induction of a state of depolarization inactivation similar to that observed in animals treated for only 21 d. This effect disappeared after a 14 d drug-free period, which suggests that the continued presence of APDs in the brain is necessary to maintain depolarization inactivation. Moreover, it would appear that, while this cell per track model may be valid in indicating whether a given APD will produce extrapyramidal side effects (see Chiodo and Bunney, 1983a, 1984), the reversibility of the phenomenon after 7 months of treatment suggests that it is not part of the mechanism responsible for the irreversible tardive dyskinesia that occurs in up to one-third of the patients receiving such medications (Jeste and Wyatt, 1982).

The peptide CCK may be important both in controlling the activity of DA neurons and in mediating their response to repeated APD treatment. Thus, while acute PROG administration (at 2 doses) has no effect on either A9 or A10 DA neurons, chronic administration of this CCK receptor antagonist (Chiodo and Bunney, 1983b; Chiodo et al., 1987) increases both the number of cells per track and the degree of bursting in both regions. These data suggest that chronic but *not* acute blockade of CCK receptors is able to alter the basal functioning of midbrain DA-containing neurons. The precise mechanisms by which chronic CCK receptor blockade achieves these effects are not known and will require further investigation. One might speculate that chronic treatment with PROG leads to CCK receptor supersensitivity and that this may, in part, play a role. In animals chronically treated with APDs, the acute administration of the CCK receptor antagonist PROG (at a dose known to maximally block CCK in DA neurons; Chiodo and Bunney, 1983b) immediately reverses depolarization inactivation. These combined data suggest that CCK systems may be important in the continued expression of depolarization inactivation in midbrain DA-containing neurons.

Since a feedback pathway from the striatum that utilizes a group of peptides derived from prodynorphin has been identified (Kalsidani et al., 1982; Khachaturian et al., 1982; Vincent et al., 1982; Akil et al., 1984), this opioid system could conceivably play a role in depolarization inactivation of these cells. However, the present studies suggest that this pathway is not important, since an intravenous dose of the opiate antagonist NAL that is high enough to block both dynorphin and enkephalinergic receptors (1 mg/kg; Yoshimuri et al., 1982) did not reverse HAL-induced depolarization blockade.

The above findings add to the growing body of literature that suggests that several distinct neurotransmitter systems are im-

portant for the continued expression of APD-induced depolarization inactivation of midbrain DA neurons (see Chiodo and Bunney, 1983a, 1985; White and Wang, 1983a, b). These systems appear to include those using CCK and perhaps GABA but not opiate (e.g., dynorphin, enkephalin) neuromessengers. Further studies on the nature of these interactions may make it possible to pharmacologically prevent the expression of depolarization inactivation induced by APDs by coadministering a drug that selectively prevents the inactivation of A9 DA neurons. Under such circumstances, one could predict that extrapyramidal symptoms and reversible tardive dyskinesia would not be produced by APDs that would otherwise normally produce these side effects (Chiodo and Bunney, 1984, 1985).

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