Raclopride and Chlorpromazine, but not Clozapine, Increase Muscle Rigidity in the Rat: Relationship with D_2 Dopamine Receptor Occupancy

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The aim of the present study was to investigate the relationship between effects on muscle tone and D_2 receptor occupancy of two typical antipsychotic drugs, raclopride and chlorpromazine, and the atypical drug, clozapine. Increased muscle tone (i.e., muscle rigidity), was measured as increases in tonic electromyographic (EMG) activity of the antagonistic muscles of the rat hind limb. D_2 dopamine receptor occupancy was assessed in the striatum and substantia nigra, areas involved in the regulation of muscle tone. Raclopride and chlorpromazine produced dose-dependent increases in EMG activity associated with D_2 occupancy of 68%–80% in the striatum and 67%–76% in the nigra. No significant increases in EMG were observed with clozapine which showed low D_2 occupancy. The results are consistent with those from human studies showing extrapyramidal side effects were associated with striatal D_2 occupancy of >70%.
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KEY WORDS: *Antipsychotic drugs; Muscle tone; Dopamine receptors; Receptor occupancy; Rat*

The introduction of antipsychotic drugs has revolutionized the treatment of schizophrenia, but their use has been limited by the appearance of extrapyramidal side effects (EPS). These include acute dystonic reactions which appear early in treatment and are characterized by intense contractions of antagonistic muscles, and Parkinson-like side effects which include muscle rigidity and hypokinesia (Ayd 1961). It has been reported that 35% of drug noncompliance is due to the unacceptability of the side effects associated with antipsychotic drug treatment (Hoge et al. 1990) and this has stimulated a search for effective antipsychotic drugs which do not produce extrapyramidal side effects.

The likelihood of extrapyramidal side effects developing appears to be dependent on the affinity of the antipsychotic drug for D_2 dopamine receptors (Creese et al. 1976; Seeman et al. 1976) so that there is a greater incidence associated with the high-potency drugs such as haloperidol and a lesser incidence with low-potency drugs such as chlorpromazine. Support for the view that there may be an association between the appearance of extrapyramidal side effects and D_2 receptor occupancy has come from positron emission tomography (PET) studies in humans (Farde and Nördström 1992a; Farde et al. 1992b, 1992c; Nyberg et al. 1995). These studies showed that EPS were associated with high D_2 receptor occupancy of 70%–89% in the striatum obtained with conventional doses of typical antipsychotics, but showed no association with D_1 occupancy. Importantly, clozapine whose

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use is not associated with EPS, occupied only 38%–63% D_2 receptors at clinically effective doses (Farde and Nördström 1992a; Farde et al. 1992b, 1992c; Nyberg et al. 1995).

Despite being the subject of intense research interest, the mechanisms underlying drug-induced EPS remain unknown. One of the main factors contributing to this situation has been the lack of an objective, quantifiable experimental endpoint relevant to a cardinal feature of EPS, muscle rigidity, or increased muscle tone. We have developed an objective and quantifiable measure of muscle rigidity, assessed as changes in tonic electromyographic (EMG) activity in the anterior tibialis and gastrocnemius muscles of the hindlimb of conscious, unrestrained rats. We reported that tonic EMG activity is significantly increased in both muscles in an animal model of Parkinson's disease, namely following lesions of the ascending nigrostriatal dopaminergic neurons by bilateral 6-hydroxydopamine injections (Double and Crocker 1993). These EMG increases were significantly reduced by subcutaneous injection of the mixed D_1/D_2 dopamine receptor agonist, apomorphine. Further work showed that both D_1 and D_2 dopamine receptors in the substantia nigra were involved in the regulation of muscle tone (Crocker 1995; Double and Crocker 1995; Crocker 1997). These results challenged previously held views that the dopamine receptors involved were located solely in the striatum.

The aim of the present study was to investigate the effects of antipsychotic drugs on the motor system of the rat using EMG measurements as a functional endpoint, and to study their relationship with D_2 receptor occupancy in the striatum and substantia nigra. Three antipsychotic drugs, raclopride, chlorpromazine, and clozapine, were chosen because they differ clinically in their propensity to produce EPS and in their in vitro binding characteristics. Raclopride is a selective D_2 antagonist with negligible interactions with other neurotransmitter receptors (Köhler et al. 1985) and was used to establish the relationship between selective D_2 receptor blockade and changes in EMG activity. Chlorpromazine is a typical antipsychotic drug, interacting with a variety of dopamine and non-dopamine receptors and its use is associated with EPS, whereas a low incidence has been reported for raclopride (McCreadie 1992). Clozapine is the prototypic atypical antipsychotic drug, and like chlorpromazine it interacts with a variety of dopamine and non-dopamine receptors (Meltzer et al. 1989; Stockmeier et al. 1993; Hacksell et al. 1995). It is classified as an atypical antipsychotic because it is effective in the treatment of drug-resistant schizophrenia (Kane et al. 1988) and not associated with the appearance of EPS (Casey 1989).

The effects of the three drugs were investigated on EMG activity and related to their D_2 receptor occupancy in the striatum and substantia nigra, using the ex vivo method of Schotte et al. (1993). Pilot experiments were conducted to establish the feasibility of the experimental approach and a preliminary report of some of these findings has been published (Hemsley and Crocker 1996, 1997).

MATERIALS AND METHODS

Animals and Drugs

Male Sprague Dawley rats (250–350 g) supplied by the Animal House of the Flinders Medical Centre, were housed in groups of five, maintained under conditions of constant temperature and humidity on a 12-hour light/dark cycle and given free access to food and water. All experiments were performed in accordance with the guidelines of the Australian National Health and Medical Research Council (NH&MRC) and were approved by the Flinders University of South Australia Animal Ethics Committee.

The morning before the rats were injected with drug or vehicle, in-dwelling electrodes were implanted in the right hindlimb as described below. One hour following complete recovery from anaesthesia, animals were connected to a Grass polygraph, allowed to habituate and a baseline EMG was recorded. Rats were divided into groups and injected either with vehicle or a dose of one of the three drugs tested. Raclopride (Astra, Sodertalje, Sweden), chlorpromazine (Sigma, Sydney, Australia), and clozapine (gift) were dissolved in isotonic saline or DMSO and injected subcutaneously in a volume of 1 ml/kg, at doses described in the text. Control rats received an injection of isotonic saline or DMSO as appro-

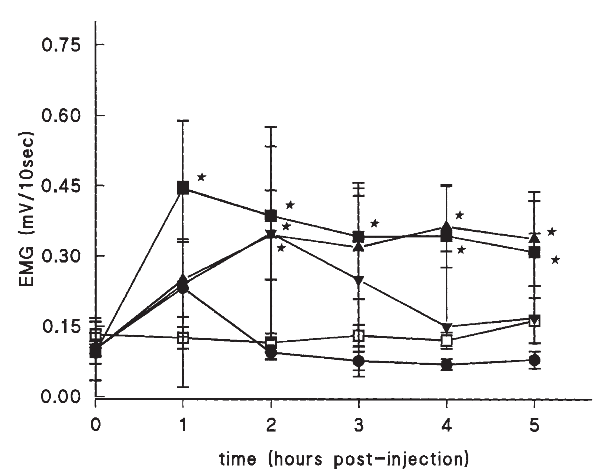

Figure 1. Effects of saline and different doses of raclopride on tonic EMG activity up to 5 hours after SC injection. For clarity of presentation only data from the gastrocnemius muscle is included. EMG activity is expressed as the mean integrated activity in mV/10s ± SEM. The doses of raclopride are 10 mg/kg ■ (5), 5 mg/kg ▲ (7), 2.5 mg/kg ▼ (8), 0.5 mg/kg ● (5), and control □ (8). Number of animals/dose shown in parentheses, *$p < .05$.

priate. EMG activity was monitored simultaneously from four rats per observation period (which always included both vehicle and drug injected rats) for up to 5 hours. Rats were killed at either 2 hours or 5 hours after injection for the determination of D_2 receptor occupancy.

EMG Measurements

The method used was that described by Double and Crocker (1995). Briefly, rats were anesthetized (sodium pentobarbitone, 45 mg/kg intraperitoneally) and placed in a stereotaxic frame. A pair of stainless steel electrodes were implanted into the gastrocnemius and anterior tibialis muscles and a fifth wire (earth) laid on the surface of the tibialis muscle. The five wires were threaded under the skin and joined to a five-pin socket attached with dental cement to the surface of the skull. Following complete recovery from anesthesia, animals were connected via a headset containing an amplifier to a Grass polygraph (Model 7D), allowed to habituate and a baseline EMG recorded. The EMG signal was amplified, filtered (10 Hz–10 kHz), rectified and integrated over 10-second periods and the resultant signal recorded at 10 Hz for 20-minute periods on a computerized recording system (CODAS, Dataq, USA). EMG is expressed as mean tonic EMG activity (mV.10 s^{-1}). Phasic activity resulting from animal movement was excluded from analysis. Movement was detected by both observation of the rats and inspection of the corresponding EMG signal which exhibited large, irregular spikes easily distinguishable from the characteristically regular nature of the tonic EMG signal.

Quantitative Autoradiography

Two hours or 5 hours after administration of the test drug or vehicle the rats were killed by decapitation, the brains removed and sagittal sections (20 μm) cut by cryostat. Thaw mounted sections were incubated at room temperature according to the protocol of Schotte et al.

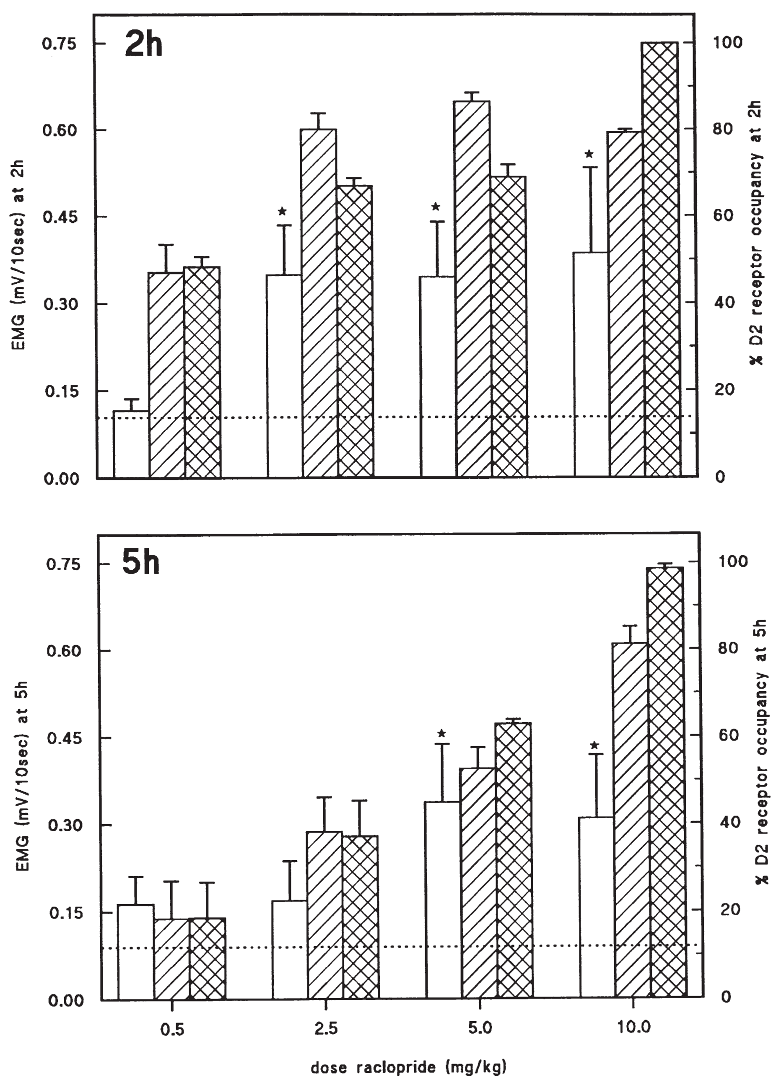

Figure 2. Effect of different doses of raclopride on tonic EMG activity (open bars) and striatal (hatched bars) and nigral (cross-hatched bars) D_2 dopamine receptor occupancy at 2 hours and 5 hours post-injection. Results are expressed as the mean integrated EMG activity in mV/10s ± SEM and D_2 occupancy as the mean percentage occupancy of the corresponding vehicle injected control group ± SEM. Number of rats at each dose as shown for Figure 1, *$p < .05$.

(1993), which incorporates a reduction in washing steps to ensure test drug occupancy is maintained through the procedure. The D_2 receptor ligand used was [^{125}I]-iodosulpiride (Amersham, Sydney, Australia) and nonspecific binding was measured in adjacent sections using sulpiride (1.7 μmol). After washing and air drying, sections were exposed to autoradiographic film (Hyperfilm, Amersham, Sydney, Australia) and the resulting images analyzed using a computerized densitometry system (MD20; Flinders Imaging, Adelaide, Australia). Standard curves were constructed using [^{125}I] standards (Amersham) and optical density values of selected brain regions converted to nCi/mg of tissue, after subtraction of nonspecific binding. Receptor concentration was calculated from the average optical density of two to three sections from each rat. Ex vivo receptor binding labeled all receptors in saline injected rats but only receptors not occupied by a test drug in the experimental groups (Schotte et al. 1993). Thus receptor ligand binding was inversely proportional to the receptor occupancy of the test drug administered in vivo.

Statistical Analysis

Means were analyzed using analysis of variance techniques, followed by post hoc testing of the differences between means using Dunnett's test. The curve describing the relationship between EMG and D_2 occupancy, shown in Figure 7, was derived using a Levenberg-Marquardt algorithm to fit a weighted logistic function using Kaleidagraph Synergy Software.

RESULTS

Effects of Raclopride on EMG Activity and D_2 Receptor Occupancy

Following injection of raclopride (0.5–10 mg/kg) significant increases in EMG activity of both the gastrocnemius (Figure 1) and anterior tibialis were obtained with doses of 2.5 mg/kg and greater at 2 hour post-injection compared with saline controls. The EMG activity in control animals was 0.09 ± 0.02 mV/10s in the tibialis and 0.11 ± 0.02 mV/10s in the gastrocnemius muscles. At 2 hours following 2.5 mg/kg raclopride EMG activity was significantly increased to 0.39 ± 0.08 mV/10s in the tibialis and 0.35 ± 0.09 mV/10s in the gastrocnemius muscles, associated with D_2 receptor occupancy of 80% of striatal and 67% of nigral receptors (Figure 2). At 5 hours EMG had decreased to levels not significantly different from control values and D_2 occupancy was reduced to 38% in the striatum and 37% in the nigra. Following injection of 5 mg/kg significant increases in EMG activity were maintained from 2 hours to 5 hours post-injection associated with D_2 occupancies at 2 hours of 87% and 69% in striatum and nigra, respectively, which fell to 53% and 63% at 5 hours. After 10 mg/kg EMG was significantly increased from 1–5 hours post-injection and D_2 occupancy was >80% in striatum and nigra at both 2 and 5 hours post-injection (Figure 2). The lowest dose tested (0.5 mg/kg) produced no significant increase in EMG activity and occupancies of 47% and 48% in the striatum and nigra 2 hours post-injection.

Effects of Chlorpromazine on EMG Activity and D_2 Receptor Occupancy

Rats injected with chlorpromazine (dose range; 0.1–10.0 mg/kg) showed dose-related increases in EMG activity at 2 hours post-injection, which were significantly different from saline controls at doses of 1 mg/kg and higher (Figure 3). Two hours following injection of 1 mg/kg chlorpromazine, EMG activity was significantly increased to 0.31 ± 0.09 mV/10s in the tibialis and 0.36 ± 0.09 mV/10s in the gastrocnemius. These increases were associated with D_2 occupancy of 68% in the striatum and 76% in the nigra at 2 hours post-injection, falling to 36% and 53% respectively at 5 hours post-injection when no significant increases in EMG activity were observed (Figure 4). Doses of 2 and 10 mg/kg produced significant EMG increases at 2 hours when D_2 occupancies of 78%–79% were found in the striatum and 72%–77% in the substantia nigra. Significant EMG increases were maintained at 5 hours following both doses although D_2 occupancy in the striatum was reduced to 60% in the group receiving 2 mg/kg whereas nigral

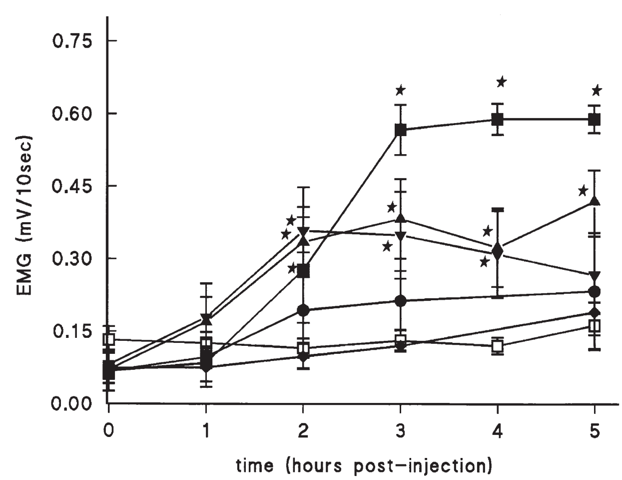

Figure 3. Effects of saline and different doses of chlorpromazine on tonic EMG activity up to 5 hours after SC injection. For clarity of presentation only data from the gastrocnemius muscle is included. EMG activity is expressed as the mean integrated activity in mV/10s ± SEM. The doses of chlorpromazine are 10 mg/kg ■ (4), 2 mg/kg ▲ (10), 1 mg/kg ▼ (10), 0.5 mg/kg ● (5), 0.1 mg/kg ◆ (3), and control □ (8). Number of animals/dose shown in parentheses, *$p < .05$.

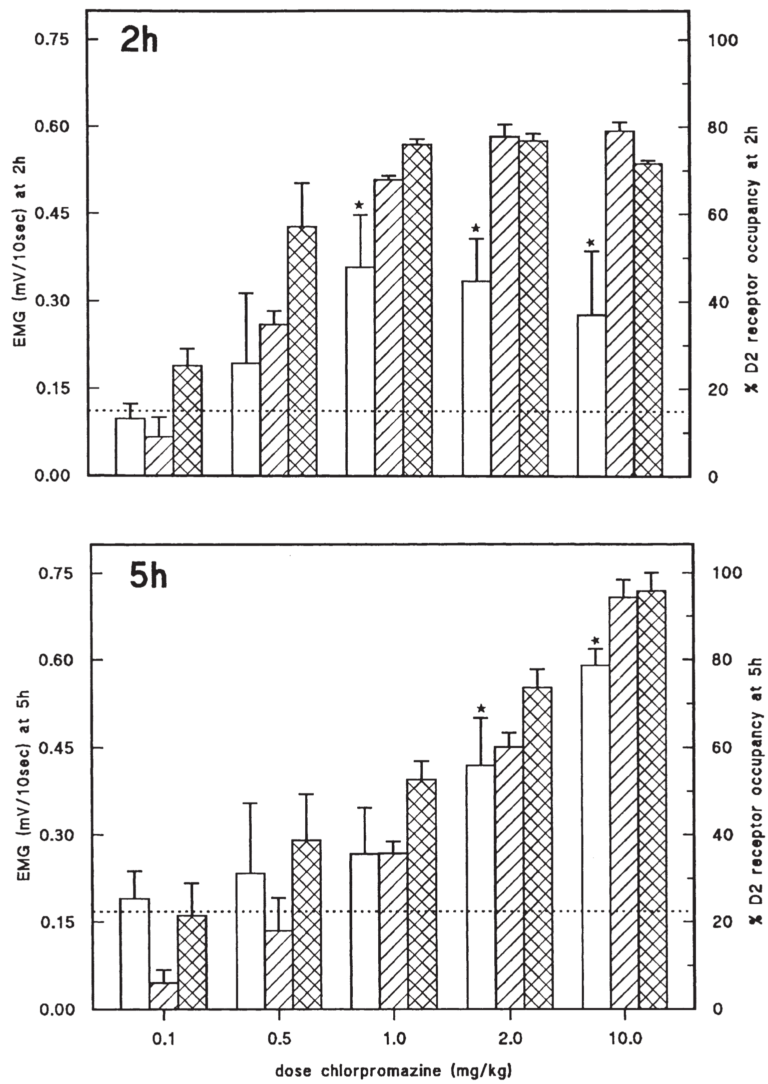

Figure 4. Effects of different doses of chlorpromazine on tonic EMG activity (open bars) and striatal (hatched bars) and nigral (cross-hatched bars) D_2 dopamine receptor occupancy at 2 hours and 5 hours post-injection. Results are expressed as the mean integrated EMG activity in mV/10s ± SEM and D_2 occupancy as the mean percentage occupancy of the corresponding vehicle injected control group ± SEM. Number of rats at each dose as shown for Figure 3, $^*p < .05$.

occupancy (74%) was relatively unaffected compared with 2-hour values (Figure 4).

Effects of Clozapine on EMG Activity and D_2 Receptor Occupancy

No significant increases in EMG activity were observed in rats injected with clozapine (2.5, 10, or 40 mg/kg) compared with saline controls (Figure 5). The EMG activity observed 2 hours following 40 mg/kg clozapine was 0.15 ± 0.03 mV/10s in the tibialis and 0.07 ± 0.03 mV/10s in the gastrocnemius muscles, associated with D_2 receptor occupancy in the striatum and substantia nigra of 54% and 63%, respectively (Figure 6).

DISCUSSION

This experimental study is the first to our knowledge which links increases in tonic EMG activity, an objective, quantifiable measure of muscle rigidity, induced by antipsychotic drugs to their level of D_2 dopamine receptor occupancy in the rat brain. It thus complements the findings from PET studies in humans (Farde and Nördström 1992a; Farde et al. 1992b, 1992c) and occupancy studies in experimental animals (Leysen et al. 1993; Schotte et al. 1993; Sumiyoshi et al. 1993, 1995). The current findings show that there are dose-related increases in EMG activity which are associated with dose-related increases in D_2 dopamine receptor occupancy in the striatum and substantia nigra.

The results for the selective D_2 antagonist raclopride show an association between increased EMG activity and D_2 receptor occupancy which is similar to that reported for raclopride from PET studies (Farde et al. 1988; Nördström et al. 1993). PET studies have shown the level of striatal D_2 occupancy associated with EPS to be 70%–89% for a number of typical antipsychotic drugs (Farde and Nördström 1992a; Farde et al. 1992b, 1992c; Nyberg et al. 1995). In the current study doses of

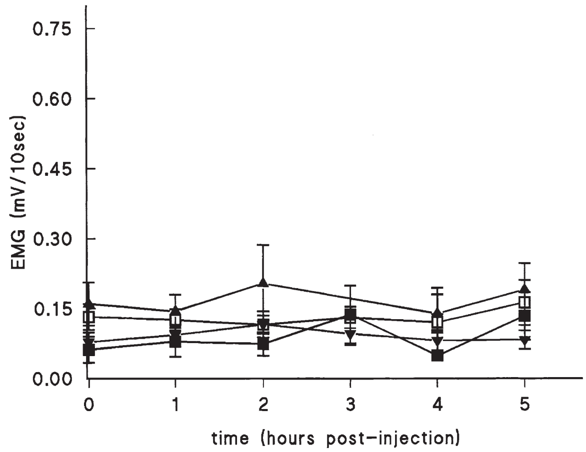

Figure 5. Effects of DMSO and different doses of clozapine on tonic EMG activity up to 5 hours after SC injection. For clarity of presentation only data from the gastrocnemius muscle is included. EMG activity is expressed as the mean integrated activity in mV/10s ± SEM. The doses of clozapine are 40 mg/kg ■ (5), 10 mg/kg ▲ (7), 2.5 mg/kg ▼ (4), and control □ (8). Number of animals/dose shown in parentheses, *$p < .05$.

2.5–10 mg/kg raclopride produced significant increases in EMG activity at 2 hours post-injection ranging from 0.35–0.39 mV/10s, suggesting this was the maximum response elicited by raclopride. At the lowest of these doses (2.5 mg/kg) the maximum response was associated with D_2 occupancy of 80% in the striatum and 67% in the nigra. At 5 hours post-injection receptor occupancy had fallen to 38% and 37% in both striatum and nigra when a fall in EMG activity to values not significantly different from controls was observed. The maintenance of the maximum EMG response at 5 hours observed in the group receiving 10 mg/kg was associated with maintenance of D_2 occupancies of >81% in the striatum and nigra.

These findings are similar to those obtained with chlorpromazine where a maximum EMG response of 0.30–0.36 mV/10s was elicited at 2 hours by doses of 1–10 mg/kg associated with D_2 occupancies of >68% in the striatum and >76% in the nigra. At 5 hours following 1 mg/kg chlorpromazine the EMG response was reduced to levels not significantly different from controls when D_2 occupancy was 36% and 57%, respectively. On the other hand the EMG response was maintained at 5 hours following the 10-mg/kg dose when occupancy was >90% in striatum and nigra.

The relationship between EMG increases and striatal and nigral D_2 occupancies 2 hours following injection of raclopride and chlorpromazine is shown in Figure 7. The ED_{50} for striatal D_2 occupancy was 57.6% which was similar to that of 49.5% for nigral occupancy. The E_{max} was 0.27 and 0.26 mV/10s above baseline EMG for striatal and nigral occupancy respectively, indicating a similar relationship between EMG and occupancy in striatum and nigra. Overall the results for both drugs suggest that onset of the EMG response is dependent on a threshold D_2 occupancy of >68% in striatum and >69% in the nigra. This threshold is very similar to that we reported in a study using the irreversible dopamine receptor antagonist, N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) to inactivate dopamine receptors throughout the brain (Hemsley and Crocker 1998), in which maximum increases in EMG activity were associated with a loss of 74% striatal and 67% nigral D_2 receptors.

There is a trend suggesting that the relationship between EMG and occupancy may be different in terms of the offset of drug effects. Thus maintenance of a maximal EMG response at 5 hours was seen when D_2 occupancy was reduced to 53% and 63% in striatum and nigra respectively, following injection of 5 mg/kg raclopride, and to 60% and 74% after 2 mg/kg chlorpromazine. However when striatal D_2 occupancy was reduced below a threshold of ~38% at 5 hours post-injection, a fall in EMG activity to values not significantly different from controls was observed for both drugs. This might mean that the D_2 occupancy threshold for the 5-hour EMG response is less than the ~70% reported above and 70%–89% from PET studies. Alternatively, the apparent change in the relationship between occupancy and offset of drug effects may relate to changes in receptor-mediated mechanisms which might involve immediate early gene expression (Robertson and Fibiger 1992) or sensitization to D_2 receptor blockade (Barnes et al. 1990).

No significant increases in EMG activity were ever found with clozapine up to a dose of 40 mg/kg, which far exceeds the range used clinically (Kane et al. 1988), and occupied only 54% of striatal and 63% of nigral receptors, values similar to those reported by Sumiyoshi et al. (1995) who utilized an in vivo receptor binding technique. In PET studies the low incidence of EPS associated with the use of clozapine has been attributed to its reduced occupancy of striatal D_2 receptors, which ranged from 38%–63%, that is, less than the >70% occupancy threshold obtained with the typical antipsychotic drugs (Farde and Nördström 1992a; Farde et al. 1992b, 1992c; Nördström et al. 1993; Nyberg et al. 1995). A similar relationship between EPS and D_2 occupancy was also found in a SPECT study (Scherer et al. 1994). The findings of the current study support the view that clozapine did not produce increased EMG activity because its maximum D_2 occupancy was below the occupancy threshold associated with the onset of EMG increases observed with raclopride and chlorpromazine. However, clozapine also interacts strongly with muscarinic and $5HT_2$ receptors and has been shown to exhibit high $5HT_2$ occupancy at the doses we used (Leysen et al. 1993; Schotte et al. 1993), so that a role of non-dopamine receptors in the regulation of EMG activity cannot be ruled out and is the subject of ongoing studies.

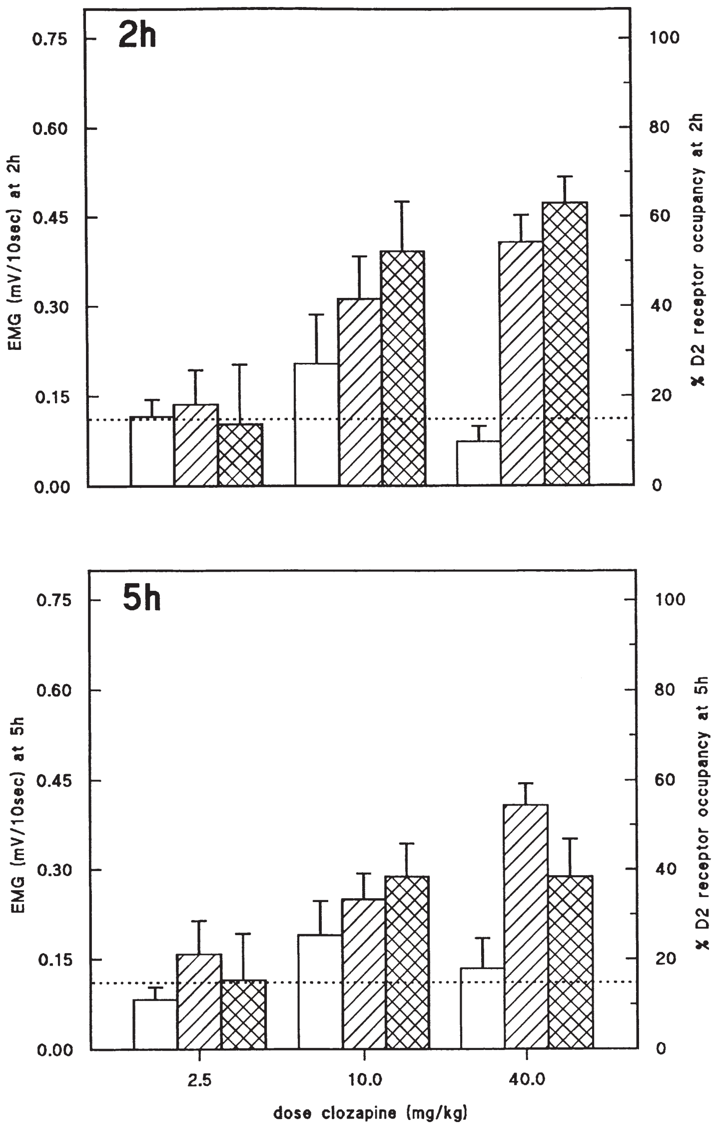

Figure 6. Effects of different doses of clozapine on tonic EMG activity (open bars) and striatal (hatched bars) and nigral (cross-hatched bars) D_2 dopamine receptor occupancy at 2 hours and 5 hours post-injection. Results are expressed as the mean integrated EMG activity in mV/10s ± SEM and D_2 occupancy as the mean percentage occupancy of the corresponding vehicle injected control group ± SEM. Number of rats at each dose as shown for Figure 3, $^{*}p < .05$.

Significant maximum increases in EMG activity were not seen until approximately 1–2 hours after raclopride or chlorpromazine injection, when occupancy of D_2 receptors exceeded 67%. Thus, it seems likely that the time course of the onset of the EMG increases is related to the time taken for threshold D_2 receptor occupancy by the drug. A study investigating the time course of D_2 receptor occupancy by several typical antipsychotic drugs, including chlorpromazine, found maximal occupancy of striatal D_2 receptors did not occur until ~1 hour post-injection (Sumiyoshi et al. 1995), although no functional endpoint of D_2 occupancy was measured. Further support for the concept that the time when EMG increases occurs is related to D_2 receptor occupancy comes from our recent study using EEDQ, in which the increase in EMG activity was closely associated with the time and level of dopamine receptor inactivation (Hemsley and Crocker 1998).

In the current study we measured occupancy in both striatum and substantia nigra because we have shown that D_2 receptors in the substantia nigra play a key role in the regulation of muscle tone (Crocker 1995; Double and Crocker 1995; Crocker 1997). Recently we compared the effects of inactivating dopamine receptors throughout the brain with those seen following inactivation only in the substantia nigra and showed similar increases in EMG activity, further supporting the importance of nigral dopamine mechanisms (Hemsley and Crocker 1998). Our current findings show that for each dose level of the three drugs tested, D_2 occupancy in the striatum and nigra was similar, emphasizing the fact it is impossible to draw conclusions about the importance of a particular brain region in motor control from these types of studies. This is reinforced by the similar relationship between EMG and D_2 occupancy in the striatum and nigra as shown in Figure 7 and the

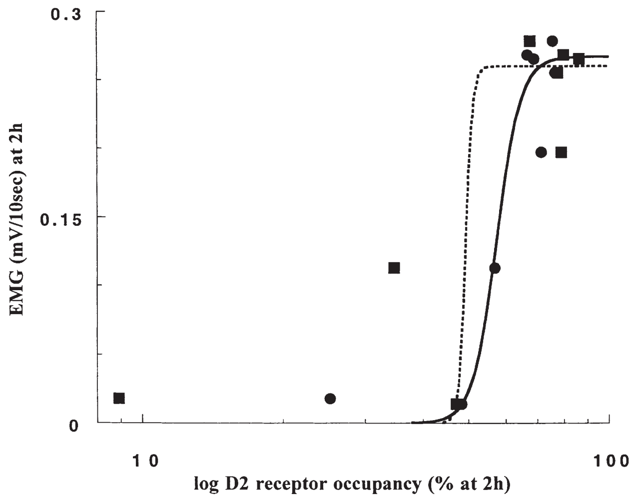

Figure 7. Relationship between tonic EMG activity and D_2 dopamine receptor occupancy in the striatum (solid line) and nigra (dotted line) 2 hours following injection of raclopride or chlorpromazine. The curve was fitted using Kaleidagraph software as described in Methods.

similar ED_{50} values for striatal and nigral occupancy. Thus it cannot be concluded from PET studies that D_2 occupancy in the striatum rather than in another region such as the nigra, is responsible for the appearance of EPS, because D_2 occupancy is likely to be similar in all brain regions containing D_2 receptors. However because of the resolution of current PET cameras and the unavailability of suitable ligands, measurements of D_2 occupancy in areas as small as the substantia nigra in humans has been precluded.

There is experimental evidence that intrastriatal injection of haloperidol in the rat increases EMG activity supporting the view that the striatum is a site of action for antipsychotic drugs (Ellenbroek et al. 1986), while findings from our laboratory indicate that nigral dopamine mechanisms are important in regulating EMG activity tone (Crocker 1995; Double and Crocker 1995; Crocker 1997; Hemsley and Crocker 1998). Nevertheless although our current findings have demonstrated a relationship between D_2 receptor occupancy in the striatum and nigra and increased EMG activity for both raclopride and chlorpromazine, the relative roles of dopamine mechanisms in the striatum and substantia nigra in mediating the effects of antipsychotic drugs remain to be elucidated.

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