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Clozapine antagonizes the induction of striatal Fos expression by typical neuroleptics

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Abstract

Previous studies have shown that the atypical neuroleptic clozapine is less potent at inducing Fos expression in the dorsolateral striatum than are typical neuroleptics. We report here that pretreatment with clozapine (5–20 mg/kg) actually attenuates the striatal Fos expression induced by the typical neuroleptics haloperidol and raclopride. These results suggest clozapine has pharmacological properties which actively antagonize the effects of dopamine D_2 receptor blockade on striatal immediate-early gene expression.

Keywords: Atypical neuroleptic; c-Fos; Striatum

Although all known antipsychotic drugs are able to block dopamine receptors, atypical neuroleptics such as clozapine are associated with a much lower incidence of extrapyramidal side effects than are typical neuroleptics, such as haloperidol. The extrapyramidal effects of neuroleptics appear to result from actions in the dorsal striatum and several lines of evidence suggest that atypicals exert less of an effect in this region than do typical neuroleptics (Ashby and Wang, 1996). Atypical neuroleptics, for example, are much less effective than typical neuroleptics at inducing expression of the immediate early gene c-*fos* in the dorsal striatum (Robertson and Fibiger, 1992). Two general types of explanations of these effects are possible. What might be termed 'passive theories' of 'atypicality' would suggest that the relative inability of clozapine to induce Fos-like immunoreactivity in the dorsal striatum results from an absence of some property, such as high affinity for dopamine D_2 receptors, which is possessed by typical neuroleptics. Passive theories would thus predict that coadministration of clozapine and a typical neuroleptic would result in striatal Fos expression at least as pronounced as that seen after administration of the typical alone. In contrast, what might be termed 'active theories' of atypicality would suggest that clozapine has some property, not shared by typical neuroleptics, which actively suppresses Fos expression in the dorsal striatum. This class of theory would predict that coadministration of appropriate doses of a typical and an atypical neuroleptic would result in less striatal Fos expression than that seen after the typical neuroleptic alone. In the current experiments we attempted to differentiate between these possibilities by examining the effects of clozapine pretreatment on the striatal Fos expression induced by typical neuroleptics.

In the first study, groups of 9–10 adult, male, Sprague–Dawley derived rats were injected with either saline or clozapine (20 mg/kg, i.p.) 15 min before being injected with haloperidol (1.0 mg/kg s.c.). Animals were perfused 90 min later under deep pentobarbital anesthesia and sections through the striatum were processed for Fos-like immunoreactivity using standard methods employing a sheep anti-Fos serum (Genosys, The Woodlands, TX, USA; OA-11-824) as the primary antibody which was visualized using a Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA, USA). Immunoreactive cells were counted in 1×1 mm fields in the dorsolateral striatum using a Quantimet image analysis system. Clozapine pretreatment significantly reduced the number of labeled cells from a mean of 346 ± 36 in control animals to a mean of 240 ± 29 ($t(17) = 2.248$; $P < 0.04$). In order to confirm this result we ran additional groups of rats who received

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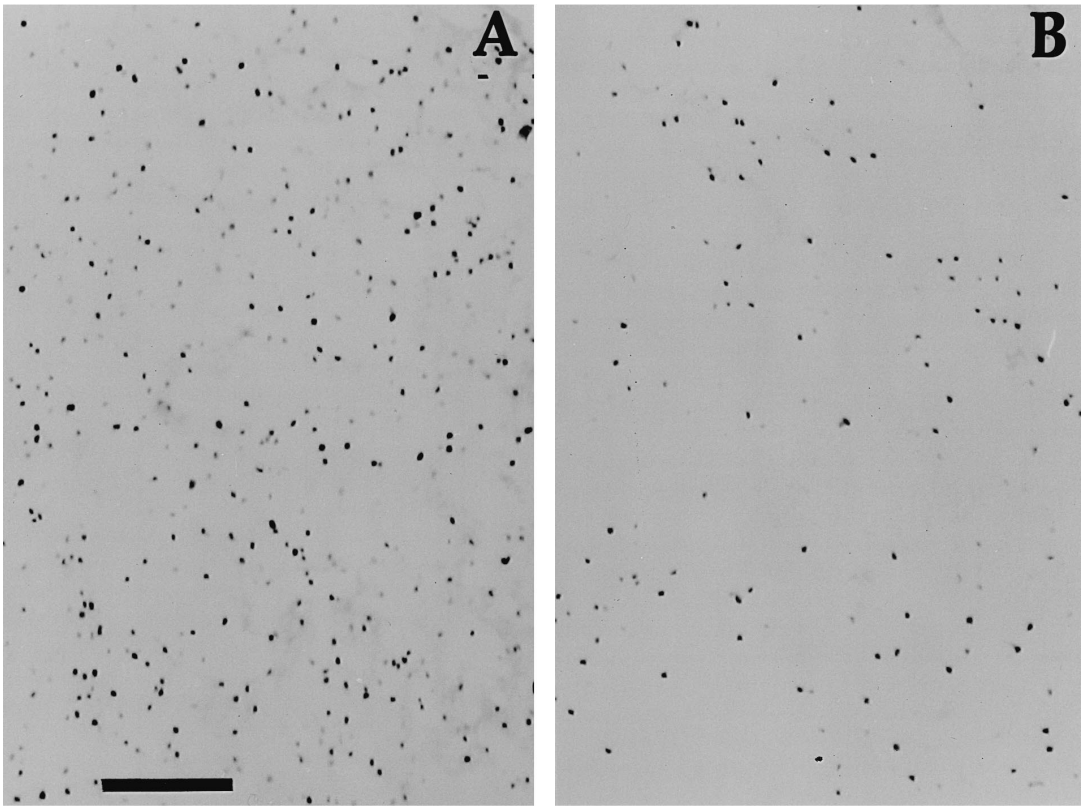

Fig. 1. Photomicrographs of typical fields in the dorsolateral striatum showing Fos-like immunoreactivity following injections of (A) vehicle followed by raclopride (1.0 mg/kg) or (B) clozapine (20 mg/kg) followed by raclopride. Scale bar equals 100 μm.

haloperidol (1.0 mg/kg) 15 min following injections of either saline or of clozapine at doses of 5 or 20 mg/kg. In these subjects clozapine again produced a significant, dose-dependent, suppression of haloperidol induced Fos-like immunoreactivity ($F(2,10) = 5.39$; $P < 0.03$). Mean numbers of labeled cells for rats receiving saline or the low or high doses of clozapine were 351 ± 30, 251 ± 33 and 212 ± 7, respectively. Finally, we used analogous methods to examine the effects of clozapine pretreatment (20 mg/kg) on the striatal response induced by the selective dopamine D_2 antagonist raclopride (1.0 mg/kg) and again observed a significant suppression of Fos expression. Mean cell counts were 633 ± 79 and 274 ± 15 for vehicle and clozapine pretreated subjects, respectively ($t(9) = 4.87$; $P < 0.001$) (Fig. 1).

Several authors have shown that clozapine is able to antagonize some of the behavioral and electrophysiological effects of typical neuroleptics (e.g., Haracz et al., 1993; Sayers et al., 1976); the current studies demonstrate that clozapine is also able to inhibit the effects of typical neuroleptics on Fos expression in the dorsal striatum. The possession of active inhibitory properties may provide at least a partial explanation for the relative inability of clozapine to induce Fos-like immunoreactivity in the dorsal striatum when given by itself. It is possible that the inhibitory effects of clozapine may be related to its antimuscarinic properties, but the available evidence on this point is inconsistent; although cholinergic blockade has been reported to inhibit neuroleptic induced striatal Fos expression (Guo et al., 1992), this type of effect has not been observed in all studies (Gerfen and Kitai, 1997; MacGibbon et al., 1995). The inhibitory effects of clozapine might be related to its antihistaminergic effects (Ashby and Wang, 1996), to a combination of actions at multiple receptors or, perhaps, to its unique binding properties at the D_2 receptor (Malmberg et al., 1993). Further studies will be necessary to investigate these questions as well as to determine whether an ability to inhibit Fos expression in the dorsal striatum is unique to clozapine or whether it is a general property of atypical neuroleptics.

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