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Activation of dopamine D_3 autoreceptors inhibits firing of ventral tegmental dopaminergic neurones in vivo

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Abstract

The firing rate of dopaminergic neurones in the ventral tegmental area of anaesthetised rats was dose-dependently (0.31–5.0 μg/kg i.v.) inhibited by the dopamine D_3 receptor agonist, (+)-7-OH-DPAT (7-hydroxy-2-(di-*n*-propylamino)tetralin). The selective dopamine D_3 receptor antagonist, (+)-S 14297 ((+)-[7-(*N*,*N*-dipropylamino)-5,6,7,8-tetrahydro-naphtho(2,3*b*)dihydro,2,3-furane]), dose-dependently (16–125 μg/kg i.v.) inhibited the action of (+)-7-OH-DPAT (5 μg/kg i.v.); its inactive distomer, (−)-S 17777 (125 μg/kg i.v.), was ineffective. Alone, (+)-S 14297 (125 μg/kg i.v.) did not modify the firing rate. Haloperidol (16 μg/kg i.v.) fully reversed the action of (+)-7-OH-DPAT and, alone, significantly increased firing rate. These data suggest that inhibitory (dendritic) dopamine D_3 (auto)receptors control the electrical activity of ventral tegmental area-localised dopaminergic neurones.

Keywords: Dopamine D_3 receptor; Ventral tegmental area; Dopamine

Neuroanatomical studies have revealed mRNA encoding dopamine D_3 receptors in the ventral tegmental area, the origin of dopaminergic projections to the limbic system and cortex (Bouthenet et al., 1991). Further (+)-7-OH-DPAT (7-hydroxy-2-(di-*n*-propylamino)tetralin), a preferential agonist at dopamine D_3 versus D_2 receptors (Chio et al., 1993) inhibits the release of dopamine in limbic structures, an action markedly attenuated by (+)-S 14297 ((+)-[7-(*N*,*N*-dipropylamino)-5,6,7,8-tetrahydronaphto(2,3*b*)dihydro, 2,3-furane]), a selective antagonist at dopamine D_3 versus D_1, D_2, D_4 and D_5 receptors (Millan et al., 1994; Rivet et al., 1994; unpublished observation). Such studies have not, however, established whether putative dopamine D_3 (auto)receptors, localised on the dendrites of dopaminergic neurones, control the electrical activity of these pathways. Herein, we addressed this question.

Male Wistar rats of 300 ± 25 g were anaesthetised with chloral hydrate (400.0 mg/kg i.p.), a tungsten electrode lowered into the ventral tegmental area and dopaminergic neurones identified and recorded as described by Wang (1981), i.e., type I cells showing a slow, rhythmic pattern of firing and a characteristic wave-form. Drugs were dissolved in sterile water and injected i.v. into the cannulated femoral vein. Cumulative dose-response curves were performed for (+)-7-OH-DPAT with consecutive injections every 2 min. After an injection of 5 μg/kg (+)-7-OH-DPAT (a just maximally effective dose), cumulative dose-response curves were performed for reversal of its actions by (+)-S 14297. By administration of a single dose (125 μg/kg), the action of (+)-S 14297 was, further, compared to that of its inactive distomer, (−)-S 17777 (125 μg/kg) and to that of haloperidol (16 μg/kg). Electrode positions were verified by histology.

The firing rate was rapidly, dose-dependently and completely inhibited by (+)-7-OH-DPAT (Fig. 1). The action of (+)-7-OH-DPAT, which showed some spontaneous reversal over the period of antagonist administration, was dose-dependently (though only submaximally) reversed by (+)-S 14297 (Fig. 1). Administered at a single dose, (+)-S 14297 also attenuated the action of (+)-7-OH-DPAT without affecting the firing rate alone. This effect was stereospecific in that its

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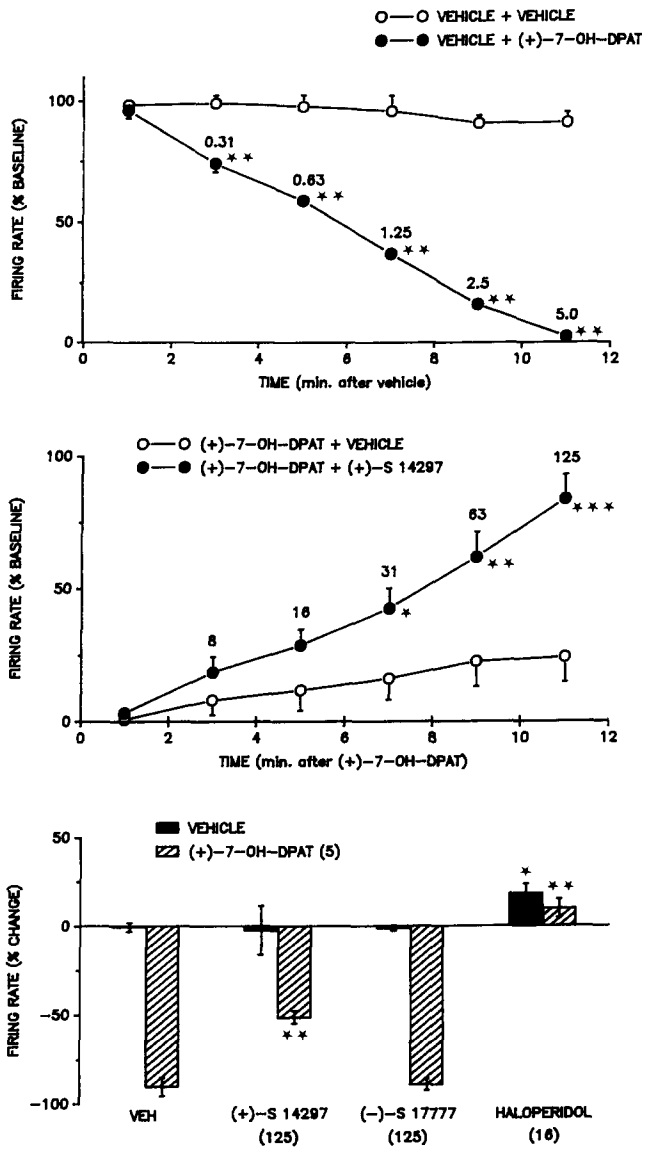

Fig. 1. Influence of dopamine D_3 receptor ligands upon the electrical activity (firing rate) of dopaminergic neurones of the ventral tegmental area of the anaesthetized rat in vivo. Data are means ± S.E.M. of firing rate (60 s bin after administration) expressed as a percentage of basal values: 3.5 ± 0.2 Hz (overall mean ± S.E.M., $n = 62$). Upper panel: Dose-dependent reduction of firing rate by cumulative doses (shown above time, in μg/kg i.v.) of (+)-7-OH-DPAT. Analysis of variance with repeated measures as follows: vehicle + vehicle, $F(5,15) = 1.1$, $P > 0.05$ and vehicle + (+)-7-OH-DPAT, $F(5,20) = 111.2$, $P < 0.001$. The inhibitory $dose_{50}$ (95% confidence limits) for (+)-7-OH-DPAT was 0.8 (0.3–2.0) μg/kg i.v. Middle panel: Dose-dependent inhibition of the action of (+)-7-OH-DPAT by cumulative doses (shown above time, in μg/kg i.v.) of (+)-S 14297. (+)-7-OH-DPAT + vehicle, $F(5,20) = 4.89$, $P = 0.004$ and (+)-7-OH-DPAT + (+)-S 14297, $F(5,30) = 26.6$, $P < 0.001$. For both panels, asterisks indicate significance of differences to corresponding vehicle values in Newman-Keuls' test, $^{*}P < 0.05$ and $^{**}P < 0.01$. Lower panel: Influence of a single dose of (+)-S 14297, (–)-S 17777 or haloperidol upon firing rate following vehicle or (+)-7-OH-DPAT. ANOVA for vehicle pretreatment, $F(3,16) = 4.04$, $P = 0.025$. ANOVA for (+)-7-OH-DPAT pretreatment, $F(3,17) = 108.9$, $P < 0.001$. Asterisks indicate significance of differences to corresponding vehicle in Dunnett's test, $^{*}P < 0.05$ and $^{**}P < 0.01$.

inactive distomer, (–)-S 17777, did not modify the action of (+)-7-OH-DPAT. Haloperidol, at a dose of 16 μg/kg i.v., completely blocked the action of (+)-7-OH-DPAT and slightly, but significantly, enhanced the firing rate alone.

The present data show that the selective antagonist at rat and human dopamine D_3 versus D_2 receptors, (+)-S 14297 (Millan et al., 1994; Rivet et al., 1994) attenuates the (+)-7-OH-DPAT-induced reduction in the firing rate of ventral tegmental area dopaminergic neurones. Importantly, the action of (+)-S 14297 was stereospecific in that its inactive distomer, (–)-S 17777, did not modify the action of (+)-7-OH-DPAT. Interestingly, further, the action of (+)-7-OH-DPAT was stereospecific in that (–)-7-OH-DPAT, which has lower affinity at D_3 sites (71 nM) than (+)-7-OH-DPAT (1.8 nM) (Rivet et al., 1994), was active only at a markedly (30-fold) higher dose range (10–200 μg/kg i.v.; not shown). These findings strongly suggest a role of dopamine D_3 sites in the control of the electrical activity of ventral tegmental area-derived dopaminergic neurones. Further, the present findings are in line with the ability of (+)-S 14297 – as compared to (–)-S 17777 – to attenuate (but not abolish) the inhibitory influence of systemic (+)-7-OH-DPAT upon release of dopamine in the nucleus accumbens (Rivet et al., 1994). Interestingly, transfected D_3 receptors couple negatively and positively to Ca^{2+} and K^{+} currents, respectively, offering mechanisms whereby D_3 receptors may inhibit neuronal activity and the release of dopamine (Seabrook et al., 1994; Tang et al., 1994).

The very low (μmolar) affinity of (+)-S 14297 for D_1, D_4 and D_5 sites suggests that these are unlikely to be involved in the present findings (unpublished observation; Millan et al., 1994). Nevertheless, an action of (+)-7-OH-DPAT at dopamine D_2 in addition to D_3 sites is possible. Indeed, the relative selectivity of (+)-7-OH-DPAT at D_3 versus D_2 sites depends upon their precise affinity states and may not be as great as originally thought (Chio et al., 1993). This would explain why (+)-S 14297 achieves only *sub*-total inhibition of the influence of (+)-7-OH-DPAT upon ventral tegmental area firing rate and dopamine release whereas haloperidol, which equipotently antagonises *both* dopamine D_2 *and* D_3 sites, *completely* reverses the actions of (+)-7-OH-DPAT (Fig. 1; Rivet et al., 1994).

In contrast to haloperidol, (+)-S 14297 administered alone, did not increase the firing rate of ventral tegmental area neurones. Similarly, in distinction to haloperidol, (+)-S 14297 does not facilitate the release of dopamine in the nucleus accumbens (Rivet et al., 1994). These findings suggest that dopamine D_3 autoreceptors may show little tonic activity and that the intrinsic, excitatory action of haloperidol reflects blockade of dopamine D_2 autoreceptors (Silvia et al., 1994).

The mechanistic differences underlying this difference between dopamine D_3 and D_2 sites, for example, in terms of second messenger coupling and receptor reserve, will be of interest to determine. On the other hand, it is possible that simultaneous blockade of *both* dopamine D_3 *and* D_2 sites, as achieved with haloperidol, may be required to activate dopaminergic neurones. Selective blockade of D_3 sites by (+)-S 14297 may simply allow for dendritic dopamine to occupy dopamine D_2 autoreceptors.

In conclusion, employing the selective dopamine D_3 versus D_2 antagonist, (+)-S 14297, the present data suggest that inhibitory D_3 receptors, presumably localized dendritically in the ventral tegmental area, control the electrical activity of dopaminergic neurones. The present data do *not*, nevertheless, question the existence of ventral tegmental area-localized D_2 autoreceptors. Further, the potential existence of D_3 autoreceptors on dopaminergic *terminal* remains to be examined. These findings reinforce the concept of a functional relationship between dopamine D_3 receptors and psychiatric disorders such as drug abuse, depression and schizophrenia.

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