The 5-HT1A receptor selective ligands, (R)-8-OH-DPAT and (S)-UH-301, differentially affect the activity of midbrain dopamine neurons

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Summary. The effects of the selective 5-HT1A receptor agonist (R)-8-hydroxy-2(di-n-propylamino)tetralin [(R)-8-OH-DPAT] and the novel 5-HT1A antagonist (S)-5-fluoro-8-hydroxy-2-(dipropylamino)-tetralin [(S)-UH-301] were studied with regard to the firing pattern of single mesencephalic dopamine (DA) neurons with extracellular recording techniques in chloral hydrate anesthetized male rats. Neuronal activity was studied with respect to firing rate, burst firing and regularity of firing. In the ventral tegmental area (VTA) low doses of (R)-8-OH-DPAT (2-32 μg/kg i.v.) caused an increase in all three parameters. The effect on firing rate of DA neurons was more pronounced in the parabrachial pigmentosus nucleus than in the paranigral nucleus, the two major subdivisions of VTA. In the substantia nigra zona compacta (SN-ZC), (R)-8-OH-DPAT (2-256 μg/kg i.v.) had no effect on firing rate and regularity of firing and only slightly increased burst firing. High doses of (R)-8-OH-DPAT (512-1024 μg/kg i.v.) decreased the activity of DA cells in both areas, an effect that was prevented by pretreatment with the selective D2 receptor antagonist raclopride. (S)-UH-301 (100-800 μg/kg i.v.) decreased both firing rate and burst firing without affecting regularity of DA neurons in the VTA. In the SN-ZC, (S)-UH-301 decreased the firing rate but failed to affect burst firing and regularity of firing. These effects of (S)-UH-301 were blocked by raclopride pretreatment. Local application by pneumatic ejection of 8-OH-DPAT excited the DA cells in both areas, an effect that was prevented by pretreatment with the selective DA D2 receptor antagonist raclopride. (S)-UH-301 (100-800 μg/kg i.v.) decreased both firing rate and burst firing without affecting regularity of DA neurons in the VTA. In the SN-ZC, (S)-UH-301 decreased the firing rate but failed to affect burst firing and regularity of firing. These results show that 5-HT1A receptor related compounds differentially affect the electrophysiological activity of central DA neurons. The DA receptor agonistic properties of these compound appear to contribute to the inhibitory effects of high doses of (R)-8-OH-DPAT and (S)-UH-301 on DA neuronal activity. Given the potential use of 5-HT1A receptor selective compounds in the treatment of anxiety and depression their effects on central DA systems involved in mood regulation and reward related processes are of considerable importance.

Key words: 5-HT1A – Dopamine – Electrophysiology – Ventral tegmental area – Substantia nigra

Introduction

There exists ample evidence supporting an interaction between the serotonergic and dopaminergic neuronal systems in the central nervous system. For example, serotonergic neurons from both the median (MRN) and dorsal (DRN) raphe nuclei innervate the substantia nigra (SN) and the adjacent ventral tegmental area (VTA; Dray et al. 1976; Fibiger and Miller 1977; Phillipson 1979b). The serotonergic terminals have been found to provide direct synaptic inputs to dopamine (DA) neurons within both nuclei (Hervé et al. 1987; Nedergaard et al. 1988). Stimulation of the MRN or the DRN produces mainly an inhibition of SN neurons, an effect which is dependent on endogenous serotonin (5-hydroxytryptamine, 5-HT), since parachlorophenylalanine (PCPA) pretreatment abolished this inhibitory response (Dray et al. 1976; Fibiger and Miller 1977). The serotonergic terminals have been found to provide direct synaptic inputs to dopamine (DA) neurons within both nuclei (Hervé et al. 1987; Nedergaard et al. 1988). Stimulation of the MRN or the DRN produces mainly an inhibition of SN neurons, an effect which is dependent on endogenous serotonin (5-hydroxytryptamine, 5-HT), since parachlorophenylalanine (PCPA) pretreatment abolished this inhibitory response (Dray et al. 1976; Fibiger and Miller 1977). However, recent reports have found that DA neurons in the substantia nigra respond differentially after stimulation of the DRN (Kelland et al. 1990; Trent and Tepper 1991). Microiontophoretic application of 5-HT in vivo has been shown to have either inhibitory or no effect on nigral cell activity (Aghajanian and Bunney 1974; Dray et al. 1976; Collingridge and Davies 1981). In contrast, Nedergaard and coworkers (1991) have recently demonstrated that local administration of 5-HT excites DA neurons in SN-ZC in vitro. The 5-HT receptor subtype denoted 5-HT1A is located mainly in limbic areas, such as the hippocampus, some nuclei of amygdala and the lateral septum (Pazos and Palacios 1985; Miquel et al. 1991; Pompeiano et al. 1992). 5-HT1A receptors are also found in high densities in the DRN and MN located on the cell bodies and dendrites...
of 5-HT neurons (Vergé et al. 1985). 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) is a directly acting, centrally active, 5-HT agonist (Arvidsson et al. 1981; Hjorth et al. 1982) that selectively binds to the 5-HT₁A receptor (Middlemiss and Fozard 1983). The (R)-enantiomer of 8-OH-DPAT has been found to be more efficacious at the 5-HT₁A receptor than the (S)-enantiomer. Specifically, (R)-8-OH-DPAT appears to act as a full agonist at postsynaptic 5-HT₁A receptors, whereas (S)-8-OH-DPAT exhibits only a partial agonistic effect (Cornfield et al. 1991).

The effect of 8-OH-DPAT on DA neuronal activity in the substantia nigra zona compacta (SN-ZC) appears somewhat equivocal. Thus, in previous studies 8-OH-DPAT has been shown to exert either a slight excitatory effect in low doses and inhibitory effect in high doses (Sinton and Fallon 1988), or only excite slowly firing cells without affecting fast cells (Kelland et al. 1990), or having no effect at all (Lum and Piercey 1988).

A newly synthesized compound, (S)-5-fluoro-8-hydroxy-2-(dipropylamino)tetralin [(S)-UH-301], with high affinity for the 5-HT₁A receptor (Hiller et al. 1990), has been found to counteract various effects induced by 8-OH-DPAT. For example, (S)-UH-301, without producing any effect by itself, reverses the (R)-8-OH-DPAT-induced reductions in brain 5-HT synthesis and release (Björk et al. 1991; Nomikos et al. 1992). In addition, (S)-UH-301 blocks the 5-HT-produced inhibition of forskolin-stimulated adenylate cyclase activity in hippocampal membranes (Björk et al. 1991). Thus, (S)-UH-301 appears to be a selective 5-HT₁A receptor antagonist at both pre- and postsynaptic levels.

In the present study we used extracellular recording techniques to examine the effects of systemically and locally administered (R)-8-OH-DPAT and (S)-UH-301 on the firing pattern of DA neurons in the VTA and SN-ZC with regard to three parameters of neuronal functions: firing rate, burst firing and regularity of firing. The importance of analyzing the firing pattern and not only the firing rate has recently been emphasized. Both within the nigrostriatal and the mesolimbic DA system, stimulation of the medial forebrain bundle in a bursting pattern induced a much larger increase in DA concentration in the terminal areas as compared to regularly spaced stimulation with the same average frequency (Gonon and Buda 1985; Gonon 1988). In fact, DA systems may influence the areas they innervate primarily through changing their pattern of firing (Bunney 1992). In the present study the effects of (R)-8-OH-DPAT and (S)-UH-301 were also investigated after treatment with the selective D₂ receptor antagonist raclopride to eliminate putative D₂ receptor mediated effects, since both drugs show affinity for this receptor (Van Wijngaarden et al. 1990; Hiller et al. 1990).

Materials and methods

Animals and preparation. Standard electrophysiological methods as previously described (Grenhoff et al. 1988) were used. Male Sprague-Dawley rats weighing between 230 and 360 g (Alab, Sollentuna, Sweden) were anesthetized with chloral hydrate (400 mg/kg i.p.) with additional doses administered i.p. to maintain surgical anaesthesia throughout the experiment. A jugular vein catheter for intravenous (i.v.) administration of drugs was inserted. Rectal temperature was kept at 37–38°C by means of an electric heating pad. The coordinates for the recording electrode were 2.5–3.5 mm anterior and 1.4–2.9 mm lateral to lambda for the SN-ZC, and 2.5–3.5 mm anterior and 0.5–0.8 mm lateral to lambda for the VTA (Paxinos and Watson 1986).

Extracellular recording procedures with systemic administration of drugs. Extracellular recording electrodes were pulled from Omegadot glass capillaries and filled with 2% Pontamine Sky Blue (PSB) in 2 M NaCl, yielding an impedance of 2–3 MΩ. Typical DA neurons were found 7.0–8.0 mm from brain surface in the SN-ZC and 7.5–8.5 mm in the VTA. Cells were identified as dopaminergic on the basis of established electrophysiological characteristics (Wang 1981; Grace and Bunney 1983), i.e. a triphasic action potential of more than 2 ms duration and a basal firing rate of 1–10 Hz. Only one cell was used in each animal. At the end of the experiment the recording site was marked with PSB and the brain was dissected out, cut in 50 μm sections and stained with neutral red. All cells included in this study were found in the SN-ZC or the VTA.

Extracellular action potentials were amplified, discriminated and monitored on an oscilloscope and an audiomonitor. Discriminated spikes were fed into a IBM PS/2 computer (via Cambridge Electronic Design 1401 interface and Spike2 software) and continuous rate recordings were generated on a pen chart recorder (Gould RS 3200). Analysis of the temporal pattern of firing of DA neurons was performed off-line with the Spike2 software. Firing rate, burst firing and variation coefficient were calculated over periods of 300-500 consecutive intervals between spikes. The onset of a burst was defined by an interval less than 80 ms, and a burst termination at the next interval exceeding 160 ms (Grace and Bunney 1984). Burst firing was quantified as the percentage ratio between spikes in bursts and the total number of spikes. Variation coefficient was defined as the percentage ratio between the standard deviation and the mean value of the inter-spike intervals (Werner and Mountcastle 1963).

All drugs were administered i.v. and for dose-response curves, drugs were given in exponentially increasing doses at 3 min intervals. Drug effects were assessed by comparison of a period of 300–500 inter-spike intervals recorded immediately before drug administration (control values) to a period of the same number of intervals recorded during maximal drug effect at each dose level. Two different groups of rats were used for the dose-response experiment with (R)-8-OH-DPAT, one group received 2–32 μg/kg of the drug and the second 64–1024 μg/kg. The use of two different groups was considered necessary because of the long duration of the experiment and the necessity of accuracy in assessment of the administered doses. In some experiments, (R)-8-OH-DPAT (256–1024 μg/kg, i.v.) or (S)-UH-301 (400–800 μg/kg, i.v.) was given 10 min after administration of raclopride (0.5 mg/kg, i.v.).

Extracellular recording procedures with pressure ejection of drugs. Male Sprague-Dawley rats (OFA strain, IFPA CREDO, France) weighing 290–350 g were prepared in a similar way as described above and the same coordinates for the VTA and the SN-ZC were used. Intracerebral drug applications were performed by pneumatic microejections combined with extracellular unit recordings (Akaoka et al. 1992). Briefly, glass tubings of calibrated narrow internal diameter were pulled and broken to 40–60 μm external tip diameter. A recording glass electrode was bent approximately 11 mm from the tip and positioned immediately next to the ejection barrel. The tip of the recording electrode was positioned 60 μm beyond the ejection pipette before they were secured together by a photopolymerizable resin. DA neurons were identified as described above.

(±)-8-OH-DPAT (10 mM, pH = 7) and (S)-UH-301 (10 mM, pH = 7) were dissolved in culture medium buffered with phosphate and containing calcium and magnesium ions (pH = 7; Dulbecco's saline buffer). Ejection pipettes were filled by negative pressure through the tip. Pneumatic ejections were performed by applying single or repeated pressure pulses (typically 50–200 ms duration at 0.5–2 Hz frequency for 5 to 15 s) via a PPM-2 pressure pump (Medical System Corp.) driven by nitrogen. Ejected volume could be measured in the nanoliter range.