SPECIAL REPORT

Evidence that 5-hydroxytryptamine release in rat dorsal raphé nucleus is controlled by 5-HT₁₅, 5-HT₁₆ and 5-HT₁₀ autoreceptors

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Electrolytically stimulated 5-hydroxytryptamine (5-HT) release was monitored in slices of dorsal raphé nucleus (DRN) by fast cyclic voltammetry. Pseudo-single pulse stimulations (5 pulses at 100 Hz) were used to enable the effect of various receptor agonists to be seen without competition from endogenously released transmitter. The selective 5-HT₁₅ receptor agonist, (+)-8-OH-DPAT (1.0 μM) decreased stimulated 5-HT release to 31 ± 3% of controls. This decrease was inhibited by the 5-HT₁₅ receptor antagonists, (+)-WAY-100135 (1.0 μM) and WAY-100635 (0.1 μM) but not by the 5-HT₁₀ antagonist, GR127935 (0.05 μM). The selective 5-HT₁₆ receptor agonist, CP-93129 (0.3 μM) decreased stimulated 5-HT release to 61 ± 4% of controls. This effect was antagonized by the 5-HT₁₀ receptor antagonist, isomaltane (0.5 μM) but not by (+)-WAY-100135. The 5-HT₁₀ agonist, sumatriptan (0.5 μM) decreased stimulated 5-HT release to 52 ± 2% of controls. This decrease was blocked by GR-127935 but not by WAY-100635. These results suggest that 5-HT release in the rat DRN is under the control of 5-HT₁₅, 5-HT₁₆ and 5-HT₁₀ autoreceptors.

Keywords: Dorsal raphé nucleus; 5-hydroxytryptamine; 5-HT₁₅ autoreceptor; 5-HT₁₆ autoreceptor; 5-HT₁₀ autoreceptor; fast cyclic voltammetry; brain slice

Introduction The dorsal raphé nucleus (DRN) is one of the primary 5-hydroxytryptamine (5-HT) cell body areas and projects to most parts of the brain. Traditionally 5-HT neurotransmission in the DRN is thought to be under 5-HT₁₅ receptor control. Stimulation of 5-HT₁₅ autoreceptors slows raphé cell firing (Sprague & Aghajanian, 1987) and decreases 5-HT release (Davidson & Stamford, 1994).

Recently however, studies in the guinea-pig have shown that 5-HT₁₅ autoreceptors can also influence 5-HT release in the DRN (Starkey & Skingle, 1994). The rat homologue of the 5-HT₁₅ receptor is the 5-HT₁₅ receptor (Hoyer et al., 1994) and we have now shown (Davidson & Stamford, 1994) that 5-HT release in the rat DRN may also be reduced by the 5-HT₁₀ agonist, CP-94253 (3-1,2,3,5,6-tetrahydro-4-pyridyl)-5-propoxy pyrrolo[3,2-b]pyridine).

The rat is not generally held to have functional 5-HT₁₀ receptors, although mRNA for the rat homologue of the 5-HT₁₀ receptor is found in the DRN (Hamblin et al., 1992) and it has been tentatively suggested that there may be functional 5-HT₁₀ receptors in the rat DRN (Pineyro et al., 1994). However these conclusions were largely based on the use of mianserin as a 5-HT₁₀ antagonist, a drug with only modest selectivity over 5-HT₁₅ receptors (Hoyer et al., 1994).

In this study we have used selective agonists and antagonists for 5-HT₁₅, 5-HT₁₆ and 5-HT₁₀ receptors to determine which autoreceptors are involved in the electrically stimulated 5-HT release in the rat DRN.

Methods Brain slices Male Wistar rats (100–150 g) were killed by cervical dislocation. Brains were rapidly removed under ice-cold artificial cerebrospinal fluid (ACSF). Coronal slices (350 μm) of DRN were taken at +4.8 mm versus the interaural line (Paxinos & Watson, 1986) and placed in a standard chamber. Slices were superfused with warmed (32°C) oxygenated (95% O₂/5% CO₂) ACSF at 1.2 ml min⁻¹ and allowed to equilibrate for 1 h before electrical stimulation.

Fast cyclic voltammetry Fast cyclic voltammetry was used to monitor 5-HT release: a triangular voltage waveform, −1.0 to +1.4 V, 480 V/s, was applied to a carbon fibre microelectrode (CFM: tip size 40 × 8 μm) twice per second. A sample and hold circuit was set to monitor current at the 5-HT oxidation potential, +0.6 V versus Ag/AgCl and its output stored on computer.

Stimulation protocol The CFM was placed in the centre of the DRN and a bipolar stimulating electrode located 200 μm ventrally. Pseudo-single pulse electrical stimulations consisting of five 0.1 ms duration pulses (100 Hz, 10 mA) were applied every 5 min. Stimulations were applied until 4 stable 5-HT release events were recorded, at which point the agonist was added for 40 min. Where an antagonist was also used, it was added to the superfusate at least 80 min before the agonist to allow any effect to reach a stable plateau.

Statistical analysis Each group of drugs was analysed by one-way ANOVA, followed by post-hoc application of the Tukey-Kramer test to locate significant differences. Probability levels of P<0.05 were deemed significant.

Drugs N-[2-(4-[(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyrindinyl)cyclohexanecarboxamide trihydrochloride (WAY-100635), (+)-8-hydroxy-2-(di-n-propylamin)tetralin ((+)-8-OH-DPAT), 3-[(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyridin-5-one (CP-93129), N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2’-methyl-4’-(5-methyl-1,2,4-oxadiazol-3-yl) [1,1-biphenyl]-4-carboxamide (GR-127935), (+)-N-tert-butyl-3-[4-[2-methoxyphenyl]piperazin-1-yl]-2-phenylpropanamide ((+)-WAY-100135). sumatriptan and isomaltane were initially dissolved in distilled water to make 1 M stock solutions. A few drops of dimethyl sulphoxide were initially used to dissolve GR-127935. Further dilutions were made in ACSF.

Results The selective 5-HT₁₅ agonist, (+)-8-OH-DPAT (1.0 μM) significantly (P<0.05 to 0.001) decreased stimulated 5-HT release to 31 ± 3% of controls (Figure 1a). This
The decrease was prevented by (+)-WAY-100135 (1.0 μM) and WAY-100635 (0.1 μM). GR-27935 (0.05 μM) had no effect on the response to (+)-WAY-100135. CP-93129 (0.5 μM) also decreased stimulated 5-HT release (P < 0.05 to 0.001), to 61 ± 4% of control (Figure 1b), an effect antagonised by isamoltane (0.5 μM) but not by (+)-WAY-100135 (1.0 μM).

Figure 1c shows that sumatriptan (0.5 μM) also decreased stimulated 5-HT release to 52 ± 2% of controls (P < 0.01 to 0.001). This effect was inhibited by GR-27935 but not by WAY-100635 or isamoltane.

**Discussion**

Figure 1a shows that the selective 5-HT1A agonist, (+)-WAY-100135 (1.0 μM) inhibits 5-HT release in the DRN. The complete antagonism of (+)-8-OH-DPAT by the selective 5-HT1A antagonists (+)-WAY-100135 and WAY-100635 confirms that this effect is wholly mediated via 5-HT1A receptors.

Figure 1b shows that the selective 5-HT1B agonist, CP-93129 (Koe et al., 1992) also inhibits 5-HT release. Since the effect is blocked by the selective 5-HT1B antagonist, isamoltane but not by (+)-WAY-100135, the data suggest that there is a functional 5-HT1B autoreceptor in the rat DRN. This is directly analogous to the finding of a 5-HT1D receptor in the guinea-pig DRN by Starkey & Skingle (1994) and may suggest a parallel role for the 5-HT1B site in the rat.

Most interestingly, the selective 5-HT1D agonist, sumatriptan, also inhibits 5-HT release in the DRN and the absence of effect of (+)-WAY-100135 (at a concentration that blocks (+)-8-OH-DPAT) excludes an action via 5-HT1A receptors. Conversely, the 5-HT1D antagonist, GR-127935 shows good antagonism of sumatriptan, while isamoltane, which has a pKᵦ for 5-HT1D receptors of 4.4 compared with 7.3 at the 5-HT1B receptor (Hoyer et al., 1994), had no significant effect. Although it is possible that, at this concentration (0.5 μM), sumatriptan has a small effect at the 5-HT1B receptor (pKᵦ = 6.0: Hoyer et al., 1994), the absence of effect of isamoltane makes this unlikely. The 5-HT1B subtype is also unlikely to explain the actions of sumatriptan and GR-127935 since their affinity at this site is at least 2 orders of magnitude lower than at the 5-HT1D receptor (Starkey & Skingle, 1994).

The observation of multiple functional 5-HT1 subtypes in the DRN begs the question: where are these receptors located? The 5-HT1B receptor is often assumed to be a terminal autoreceptor and may therefore be located on terminals in the DRN from other 5-hydroxytryptaminergic nuclei (Kalen et al., 1985) or from axon collaterals within the DRN itself (Chazel & Ralston, 1987). The presence of 5-HT1D mRNA in the DRN (Hamblin et al., 1992) may suggest that DRN 5-HT1D receptors are on dendrites or recurrent collaterals. Further work is in progress to clarify these matters.

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**References**


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