The Role Of SERT And OCT 3 In The Removal Of Exogenous 5-HT From The Dorsal Raphé Of Anaesthetized Rats

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Synaptic and extra-synaptic concentrations of 5-HT are tightly regulated by the 5-HT transporter (SERT) and the organic cation transporter 3 (OCT3, uptake2, see Dawes, 2009). Using the archetypical SSRI, fluoxetine, and a potent OCT3 inhibitor decynium-22 (D-22), (Hayer-Zillgen et al, 2002), we have investigated the effect of blocking these uptake systems on the clearance of exogenous applied 5-HT to the dorsal raphé. 5-HT was detected using fast cyclic voltammetry (Millar & Barnett, TG 1988).

Experiments were performed on artificially ventilated male Sprague Dawley rats (200-300g) anaesthetized with isoflurane (5% in 100% O₂) and maintained with chloral hydrate (100-200 mg⁻¹-kg⁻¹-h⁻¹). Mean arterial pressure (MAP) and heart rate (HR) were continuously monitored. The depth of anaesthesia was assessed by the stability of MAP and HR following a noxious stimulus. A multi-barrelled microelectrode incorporating a carbon fibre for voltammetric recording was positioned stereotaxically in the dorsal raphé. One barrel was used to inject 5-HT (10⁻⁵ M) at a constant pressure (5-12 psi). The pressure was adjusted until a background concentration of 80-120 nM 5-HT was detected by the voltammeter. Once this concentration had been reached the preparation was left to stabilize for 15-20 min before the test drugs were administered i.v. Fluoxetine (1 mg kg⁻¹) was dissolved in saline, while D-22 (1 mg kg⁻¹) and chlorisondamine (1 mg kg⁻¹) were dissolved in DMSO. Changes in the 5-HT concentration were compared with time-matched vehicle controls using two-way ANOVA. All values are expressed as mean ± s.e.mean. P <0.05 was considered significant. Electrode locations were confirmed histologically by the injection of pontamine sky blue (1% in saline) from one barrel of the microelectrode.

Fluoxetine (n=5) had no significant effect on the clearance of exogenous 5-HT. After 2 min in the presence of fluoxetine the concentration of 5-HT had only increased by 5.5±1.6 % compared with 1.4 ± 1.5% for saline controls (n=5). For D-22 (n=4) a significant increase in the concentration of 5-HT was detected within 1 min, reaching a maximum in 2 min of 23.5 ± 2.1% (c.f. -0.6 ± 2.0 % with DMSO alone; n=4). By 9 min the 5-HT concentration had returned to control levels. After 2 min D-22 had caused in fall in BP of 49 ± 7mmHg. However a similar fall of 44 ± 5 mmHg (n=3) caused by i.v. chlorisondamine had no significant effect on clearance of exogenous 5-HT.

The data indicates that OCT 3 is involved in the clearance of exogenous 5-HT in the dorsal raphé, even when SERT function is intact. Surprisingly fluoxetine had no effect, suggesting that SERT was not involved. However, the failure to observe any effect with fluoxetine could be due to saturation of SERT by the applied 5-HT, the dose of fluoxetine used may not effective and/or OCT 3 removed all applied 5-HT before it could be removed by SERT.

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