Propofol decreases stimulated dopamine release in the rat nucleus accumbens by a mechanism independent of dopamine D₂, GABA_A and NMDA receptors

D. Schulte¹, L. F. Callado², C. Davidson² ³, P. E. M. Phillips² ⁴, N. Roewer¹, J. Schulte am Esch¹ and J. A. Stamford²*

¹Department of Anaesthesiology, University Hospital Eppendorf, Hamburg, Germany. ²Neurotransmission Laboratory, Academic Department of Anaesthesia and Intensive Care, St Bartholomew’s and the Royal London School of Medicine and Dentistry, Royal London Hospital, Whitechapel, London E1 1BB, UK
Present addresses: ³Department of Psychiatry, Duke University Medical Center, PO Box 3870, Durham, NC 27710, USA. ⁴Department of Chemistry, University of North Carolina, Chapel Hill, NC 27599, USA
*Corresponding author: Anaesthetics Unit, Royal London Hospital, Whitechapel, London E1 1BB, UK

Although propofol (2,6-di-isopropylphenol) is a popular i.v. general anaesthetic, it has been suggested to have abuse potential. As many drugs of abuse act preferentially via release of dopamine in the limbic system, we investigated the action of propofol on stimulated dopamine release in the rat nucleus accumbens. Nucleus accumbens slices were superfused (1.6 ml min⁻¹) with artificial cerebrospinal fluid at 32°C. Dopamine release was evoked by electrical stimulation (10 pulses, 0.1 ms, 10 mA, 10 Hz, every 10 min) and monitored by fast cyclic voltammetry. Propofol 100 µmol litre⁻¹ reduced stimulated dopamine release over the 2 h after administration, relative to Intralipid controls, to mean 30 (SEM 2)% (P<0.01). The dopamine D₂ receptor antagonist metoclopramide 0.3 µmol litre⁻¹ increased dopamine release but did not block the effect of propofol (38 (3)%). The selective GABA_A antagonist bicuculline 24 µmol litre⁻¹ also failed to antagonize the action of propofol (45 (3)%). The NMDA receptor antagonist dextromethorphan 10 µmol litre⁻¹ decreased dopamine release to 57 (6)% (P<0.01) but failed to block the inhibitory effect of propofol (46 (6)%). Although propofol has been reported to bind to D₂, GABA_A and NMDA receptors, failure of metoclopramide and bicuculline to block its effects suggests that an agonist action at D₂ or GABA_A receptors does not mediate the effects of propofol on dopamine release in the rat nucleus accumbens. The lack of effect of dextromethorphan makes an NMDA receptor antagonist action unlikely.

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Propofol (2,6-di-isopropylphenol) is a popular i.v. general anaesthetic with a low incidence of nausea. Recently however evidence has begun to emerge, albeit often circumstantial, suggesting that propofol may have abuse potential.¹ Many drugs of abuse are thought to act preferentially via dopamine within the limbic system,² causing either the release of dopamine or mimicking its actions at the receptor level. Paradoxically, propofol has also been reported to bind to dopamine D₂ receptors (albeit relatively weakly),³ an action reconcilable if D₂ receptor block occurs solely presynaptically.

We have investigated the action of propofol on evoked dopamine release in rat nucleus accumbens slices, a component of the limbic system with a large dopaminergic input from the ventral tegmental area. Dopamine release was measured in ‘real time’ by fast cyclic voltammetry.⁴

Methods and results

Male Wistar rats (100–150 g) were killed by cervical dislocation and the brains were removed rapidly and chilled in ice-cold artificial cerebrospinal fluid at −1 to +1°C. A 350-µm section containing the rat nucleus accumbens was cut, transferred to the chamber and held on a stainless steel grid with a nylon mesh. The slice was superfused with oxygenated artificial cerebrospinal fluid (ACSF) 1.6 ml min⁻¹ at 32°C throughout the experiment.

A carbon fibre (8×50 µm) microelectrode was inserted
80 µm below the surface of the slice, 200 µm from a bipolar tungsten stimulating electrode (A-M Systems, Seattle, USA). Auxiliary (stainless steel wire) and reference (Ag–AgCl) electrodes were positioned at convenient locations in the slice chamber.

Dopamine release was measured using fast cyclic voltammetry (FCV) as described previously. The input voltage (1.5 cycles of a triangle waveform, -1.0 to +1.4 V vs Ag–AgCl, 480 V s⁻¹) was applied to the potentiostat every 500 ms. A sample and hold circuit monitored current at the oxidation potential for dopamine (+ 600 mV vs Ag–AgCl). Its output was displayed on a chart recorder and stored on a microcomputer using CED (Cambridge Electronic Design) ‘Chart’ and ‘Signal’ software. Electrical stimulations were applied via an NL 800 constant current isolator. Dopamine release was evoked by electrical stimulation (10 pulses, 0.1 ms, 10 mA, 10 Hz every 10 min) and monitored by FCV. Propofol has a high brain to blood concentration ratio with Seattle, USA). Auxiliary (stainless steel wire) and reference brain concentrations of 220 µg/ml and 80 µg/ml.

In the absence of drugs, stimulated dopamine release was significantly (P<0.01) reduced by methoclopramide 0.3 µmol litre⁻¹ (SmithKline Beecham), bicuculline 24 µmol litre⁻¹ (Sigma) or dextromethorphan 10 µmol litre⁻¹ (RBI) from 60 min before experimentation and throughout. In separate experiments, the effect of dextromethorphan 10 µmol litre⁻¹ itself was investigated. Stock solutions of each drug were prepared in distilled water with the exception of propofol, which was dissolved in Intralipid. Subsequent dilutions were made in ACSF.

All drug effects on dopamine release were plotted against time. The effects of the drugs were compared with the appropriate time matched points in their respective vehicle controls using the Student’s t test.

Electrical stimulation (10 pulses, 0.1 ms, 10 mA, 10 Hz) in the rat nucleus accumbens evoked release of dopamine that was readily detected at an adjacent carbon fibre microelectrode. Figure 1A shows a typical stimulated dopamine release event.

In the absence of drugs, stimulated dopamine release decreased slightly over the course of the experiment (Fig. 1B). Propofol 100 µmol litre⁻¹ significantly (P<0.01) reduced stimulated dopamine release relative to Intralipid controls (Fig. 1B). Methoclopramide 0.3 µmol litre⁻¹, the dopamine D₂ receptor antagonist, significantly increased dopamine release (data not shown) but did not block the effect of propofol (Fig. 1C). As before, propofol significantly (P<0.05) reduced stimulated dopamine release. Bicuculline 24 µmol litre⁻¹, the selective GABA_A antagonist, had no effect on dopamine release alone and also failed to antagonize the action of propofol (Fig. 1D).

Dextromethorphan 10 µmol litre⁻¹ significantly reduced dopamine release over a similar time frame to propofol (Fig. 1E). However, propofol still significantly reduced dopamine release in the presence of dextromethorphan (Fig. 1F).

**Comment**

Propofol has a high brain to blood concentration ratio with plasma propofol concentrations of 220 µmol litre⁻¹. We used a concentration of propofol (100 µmol litre⁻¹) that, while below peak brain concentrations achieved, nonetheless represented a typical brain concentration after i.v. propofol anaesthesia in the rat. It is worth remembering that propofol is highly protein bound and thus the concentration at the active site(s) may be considerably lower.

The experiments were predicated upon the hypothesis that propofol might block presynaptic D₂ receptors, increasing dopamine release and thus explaining its apparent abuse potential. We have recently demonstrated that ketamine applied via an NL 800 constant current isolator. Dopamine release was evoked by electrical stimulation (10 pulses, 0.1 ms, 10 mA, 10 Hz every 10 min) and monitored by FCV. Propofol has a high brain to blood concentration ratio with Seattle, USA). Auxiliary (stainless steel wire) and reference brain concentrations of 220 µg/ml and 80 µg/ml.

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Propofol inhibits stimulated dopamine release. A: Sample-and-hold record of stimulated dopamine release and reuptake (as peak dopamine oxidation current) in the nucleus accumbens. The period of stimulation is shown by the filled bar. B: Effect of propofol 100 \( \mu \text{mol litre}^{-1} \) on stimulated dopamine release in the nucleus accumbens. Intralipid controls are shown for comparison. The filled bar shows the period of drug or vehicle administration. C: Effect of propofol 100 \( \mu \text{mol litre}^{-1} \) (filled bar) on stimulated dopamine release in the nucleus accumbens in the presence of metoclopramide 0.3 \( \mu \text{mol litre}^{-1} \) (open bar). Intralipid controls in the presence of metoclopramide are shown for comparison. D: Effect of propofol 100 \( \mu \text{mol litre}^{-1} \) (filled bar) on stimulated dopamine release in the nucleus accumbens in the presence of bicuculline 24 \( \mu \text{mol litre}^{-1} \) (open bar). Intralipid controls in the presence of bicuculline are shown for comparison. E: Effect of dextromethorphan 10 \( \mu \text{mol litre}^{-1} \) on stimulated dopamine release in the nucleus accumbens. Intralipid controls are shown for comparison. The filled bar shows the period of drug administration. F: Effect of propofol 100 \( \mu \text{mol litre}^{-1} \) on stimulated dopamine release in the nucleus accumbens in the presence of dextromethorphan 10 \( \mu \text{mol litre}^{-1} \), 2 h after administration. Intralipid controls in the presence of dextromethorphan are shown for comparison. All values are mean (SEM), \( n=4 \). *\( P<0.05 \), **\( P<0.01 \), ***\( P<0.001 \) vs time-matched controls (Student’s \( t \) test).
that propofol may be acting not through receptor mechanisms but by block of ion channels.

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References
1 Zacny JP, Lichtor JL, Thompson W, Apfelbaum JL. Propofol at a subanesthetic dose may have abuse potential in healthy volunteers. Anesth Analg 1993; 77: 544–52
6 Orser BA, Bertlik M, Wang LY, MacDonald JF. Inhibition by propofol (2.6 di-isopropylphenol) of the N-methyl-D-aspartate subtype of glutamate receptor in cultured hippocampal neurones. Br J Pharmacol 1995; 116: 1761–8
8 Hirota K, Lambert DG. I.v. anaesthetic agents do not interact with the verapamil binding site on L-type voltage-sensitive Ca2+ channels. Br J Anaesth 1996; 77: 385–6