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Propofol decreases stimulated dopamine release in the rat nucleus accumbens by a mechanism independent of dopamine D_2, $GABA_A$ and NMDA receptors

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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 D_2 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 D_2, $GABA_A$ and NMDA receptors, failure of metoclopramide and bicuculline to block its effects suggests that an agonist action at D_2 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 D_2 receptors (albeit relatively weakly),[3] an action reconcilable if D_2 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.[4] 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.

After six stable consecutive dopamine release events were obtained, propofol 100 μmol $litre^{-1}$ (Zeneca) or an equivalent amount of its diluent (Intralipid; Pharmacia Laboratories) was added to the ACSF for 2 h. Some slices were pretreated with metoclopramide 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). Metoclopramide 0.3 μmol $litre^{-1}$, the dopamine D_2 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[5] with brain concentrations of 220 μmol g^{-1} recorded in the rat for plasma propofol concentrations of 29 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 D_2 receptors, increasing dopamine release and thus explaining its apparent abuse potential. We have recently demonstrated that ketamine markedly increases dopamine release in the rat nucleus accumbens.[4]

Propofol *decreased* dopamine release, an effect suggesting an *agonist* action at D_2 receptors. Certainly, propofol binds to D_2 receptors, albeit weakly.[3] However, metoclopramide did not block the propofol response at a concentration that rapidly increased dopamine release and has been shown in this laboratory to block autoreceptor stimulation. Thus propofol, at this concentration, has no detectable D_2 agonist or antagonist activity on presynaptic D_2 receptors in the rat nucleus accumbens. Furthermore, Appadu, Strange and Lambert[3] showed that propofol, at typical serum concentrations, occupies no more than 15% of D_2 dopamine receptors.

Propofol is known to potentiate GABA-mediated synaptic inhibition and this might underlie its effect on dopamine release. However, failure of bicuculline, the specific $GABA_A$ receptor antagonist, to block propofol suggests that a $GABA_A$ agonist action does not mediate the observed effects.

Propofol causes inhibition of NMDA receptors *in vitro*[6] and this may mediate the hallucinations observed after propofol anaesthesia. However, although dextromethorphan itself decreased dopamine release, it was unable to block the action of propofol at a concentration known to block NMDA-mediated responses *in vitro*.[7]

The absence of involvement of D_2, $GABA_A$ and NMDA receptors may suggest that the action of propofol is mediated via less specific mechanisms, such as calcium and sodium channels. Voltage-sensitive calcium channels can perhaps be excluded. Although propofol can block L- and T-type calcium channels,[8,9] we have shown that forebrain dopamine release is mainly under the control of N- and P/Q-type calcium channels (Phillips and Stamford, unpublished data). More likely is the involvement of sodium channels. Propofol has been shown to inhibit glutamate release evoked by 4-aminopyridine and veratridine but not by potassium.[10] It is possible that the same phenomenon may apply to dopamine release.

In summary, we found that propofol inhibited dopamine release in the rat nucleus accumbens in a manner dependent neither on D_2, $GABA_A$ nor NMDA receptors. We suggest

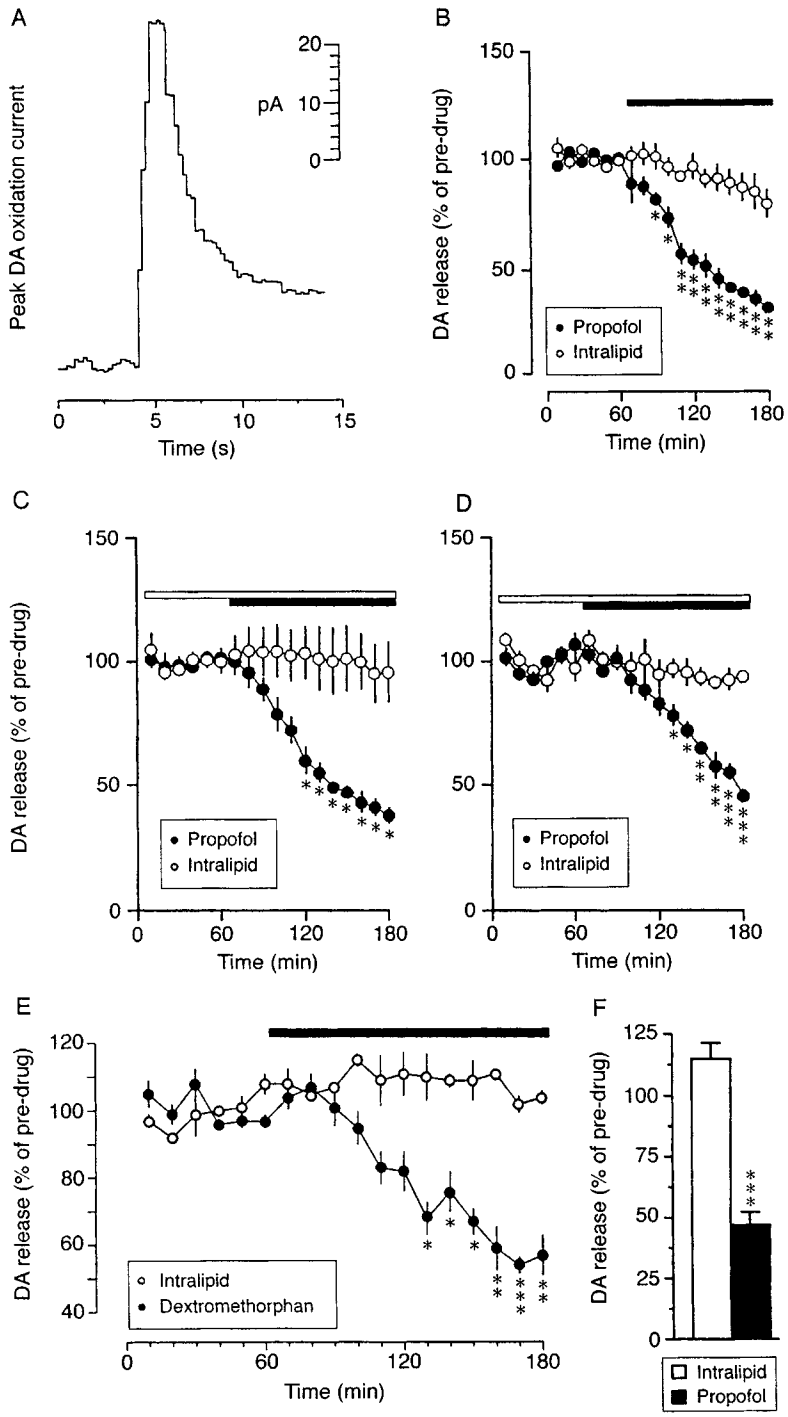

Fig 1 Propofol inhibits stimulated dopamine release. A: Sample-and-hold record of stimulated dopamine release and reuptake (as peak dopamine oxidation current) in the nucleus accumbens. The period of stimulation is shown by the filled bar. B: Effect of propofol 100 μmol litre^{-1} on stimulated dopamine 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 μmol litre^{-1} (filled bar) on stimulated dopamine release in the nucleus accumbens in the presence of metoclopramide 0.3 μmol litre^{-1} (open bar). Intralipid controls in the presence of metoclopramide are shown for comparison. D: Effect of propofol 100 μmol litre^{-1} (filled bar) on stimulated dopamine release in the nucleus accumbens in the presence of bicuculline 24 μmol litre^{-1} (open bar). Intralipid controls in the presence of bicuculline are shown for comparison. E: Effect of dextromethorphan 10 μmol 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 μmol litre^{-1} on stimulated dopamine release in the nucleus accumbens in the presence of dextromethorphan 10 μmol 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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