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Effects of general anaesthetics on 5-HT neuronal activity in the dorsal raphe nucleus

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ABSTRACT

The ascending 5-HT system has been and continues to be the subject of much research. The majority of *in vivo* electrophysiological and neurochemical studies of 5-HT function in rodents have been conducted in animals under anaesthesia – usually chloral hydrate or urethane. However, the effects of anaesthetics, on 5-HT function have not been systematically investigated. Here we used *in vitro* electrophysiology in dorsal raphe slices, to determine the effects of anaesthetically relevant concentrations of chloral hydrate (100 μM and 1 mM), urethane (10 and 30 mM), pentobarbitone (10 and 100 μM) and ketamine (10, 100 and 300 μM) on regulators of 5-HT firing activity. We examined i) basal firing (driven by α_1 adrenoceptors), ii) the excitatory response to N-methyl-D-aspartate (NMDA), iii) the 5-HT_{1A} autoreceptor-mediated inhibitory response to 5-HT and iv) the $GABA_A$ receptor-mediated inhibitory response to 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridinyl-3-ol (THIP, gaboxadol).

Pentobarbitone selectively enhanced the response to THIP. Ketamine decreased basal firing, attenuated the response to NMDA, and enhanced responses to both 5-HT and THIP. Chloral hydrate had marginal effects on basal firing, slightly attenuated the NMDA response, and enhanced both the 5-HT and THIP responses. Urethane increased basal firing, decreased the NMDA response, increased the response to THIP, but had no effect on the 5-HT response. Our data indicate that all anaesthetics tested significantly affect the regulators of 5-HT neuronal function. These findings will aid in the interpretation of previous reports of *in vivo* studies of the 5-HT system and will allow researchers to make a rational selection of anaesthetic for future studies.

1. Introduction

The ascending 5-HT system has been the subject of a large volume of research by virtue of its role in diverse physiological functions and its implied role in several neuropsychiatric disorders. In particular there have been many hundreds of *in vivo* neurochemical (microdialysis or voltammetry) investigations of the 5-HT system as well as several hundred *in vivo* electrophysiological studies of dorsal and median raphe firing activity. The vast majority of these studies have been conducted in rats or, more recently, mice. Importantly, an overwhelming majority of the *in vivo* electrophysiological and neurochemical studies of 5-HT function have been conducted in animals maintained under anaesthesia.

The benefits of using anaesthesia in these preparations are essentially two-fold. Firstly, it is practically much easier to make measures in anaesthetized animals than in non-anaesthetised animals. In recent years the development of smaller commercially prepared probes, guide cannulae, liquid swivels and other specialist equipment have made studies in non-anaesthetized animals progressively easier. Nevertheless, the necessity for guide cannulae, wound closure, post-operative analgesia, and time to allow the animal to recover, as well as the greater difficulty of making local or intravenous drug injections or electrically stimulating specific brain nuclei, still makes these studies more practically challenging in non-anaesthetized versus anaesthetized animals. In the field of electrophysiology, although some studies in non-anaesthetized (but head restrained) cats have been reported (e.g. (Bjorvatn et al., 1998; Fornal et al., 2001)), to date to our knowledge there has been only one published report of a study conducted in non-anaesthetized rodents (Gervasoni et al., 2000). The difficulty of isolating 5-HT (serotonergic) neurones with chronically implanted electrodes and of making stable recordings in animals which are moving, have not been reliably overcome.

A second, and perhaps more important, factor which encourages the use of anaesthesia is that it ensures that the potential confounds of naturalistic and/or treatment-induced changes in

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behavioural state are avoided. It is clear from studies in the cat that 5-HT neuronal firing varies according to the sleep-wake state of the animal (Levine and Jacobs, 1992; Bjorvatn et al., 1998). Moreover the role of particular neurotransmitters in regulating 5-HT firing also varies across the sleep-wake cycle (Sakai and Crochet, 2000). Similarly, dialysate levels of 5-HT have also been shown to vary over the sleep-wake cycle (Portas et al., 1998; Penalva et al., 2003) and with various naturalistic behaviours (Linthorst et al., 2000). Thus in non-anaesthetized animals, basal firing activity or levels of neurotransmitter, as well as potentially the influence of a drug on these measures, may vary in an uncontrolled manner during the experiment, making analysis and interpretation difficult. In addition to sleep-wake associated changes, studies of drugs which cause behavioural or psychological changes, for example inducing stereotypic behaviours, altering locomotor activity, or inducing anxiety, may be complicated by indirect effects of those behavioural and psychological changes on 5-HT neurotransmission. In anaesthetized animals there are no such spontaneous or drug induced changes in behavioural state.

As noted above, anaesthetics have historically been used in countless studies of the 5-HT system and will likely be continued to be used in the future. Because of their ability to produce long-lasting stable level of surgical anaesthesia, the most commonly used anaesthetics in these studies are chloral hydrate (Gartside et al., 1997a; McQuade and Sharp, 1997; Johnson et al., 2007; Wutzler et al., 2008; Vandermaelen and Aghajanian, 1983; El Mansari and Blier, 2005; Sotty et al., 2009) and urethane (Dringenberg et al., 2003; Forster et al., 2008; Scholl et al., 2010; Lovick, 1994; Allers and Sharp, 2003; Gartside and Speers, 2011). Of course our interpretation of studies in anaesthetized animals relies on an understanding of how the anaesthetic itself modifies 5-HT function and has the potential to modify any of the factors controlling 5-HT function which are the subject of study. Hence, it is surprising that the effects of, even commonly used anaesthetics, on 5-HT function have not been systematically studied. Here we examined the effects of anaesthetics on the firing activity of 5-HT neurones in the dorsal raphe nucleus (DRN) *in vitro* slice preparation. We explored the effects of anaesthetically relevant concentrations of chloral hydrate and urethane in addition to pentobarbitone and ketamine. We studied the effects of the anaesthetics on basal firing activity (driven by α_1 adrenoceptor activation), as well as on the excitatory response to glutamate NMDA receptor activation, and on the inhibitory responses to 5-HT_{1A} receptor and $GABA_A$ receptor activation.

2. Materials and methods

2.1. Drugs and chemicals

Artificial cerebrospinal fluid (aCSF) (composition (mM): NaCl 124, $MgSO_4$ 2.4, KH_2PO_4 1.25, KCl 3.25, $NaHCO_3$ 26, $CaCl_2$ 2, D-glucose 10, pH 7.4) was prepared daily from stock solutions and distilled deionized water. Stock solutions (10 mM) of phenylephrine HCl (PE) (Sigma), 5-hydroxytryptamine (5-HT) HCl (Alfa Aesar), 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridinyl-3-ol (THIP, gaboxadol) HCl (Tocris), pentobarbitone (May and Baker), ketamine (Sigma) urethane (Sigma), were prepared daily dissolved in aCSF (except phenobarbitone dissolved in dimethylsulfoxide). A large volume of 3 μM PE in CSF was prepared and further dilutions of drugs were made in this.

2.2. Tissue

All experiments were carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986 and associated guidelines. Adult male Lister hooded rats (265–350 g) were purchased from Charles River (UK) or bred in-house. Rats were group housed under standard conditions of lighting (12 h light dark: lights on 07:00), temperature (21 °C) and humidity (40–50%) and were allowed free access to food and tap water. Rats were killed by decapitation without anaesthesia and the brain was quickly removed and immersed in oxygenated iced sucrose 'slush' (composition (mM): sucrose: 200, HEPES 10, $MgSO_4$ 7, NaH_2PO_4 1.2, KCl 2.5, $NaHCO_3$ 25, $CaCl_2$ 0.5, D-glucose 10, pH 7.4). The midbrain was fixed to the chuck of a Vibratome™ with cyanoacrylate (Superglue™) and coronal slices (350 μm thick) containing the DRN were cut. Slices were trimmed and a single slice was placed on a piece of lens cleaning tissue (Whatman 105) on an interface perfusion chamber in a constant perfusion (0.5 ml/min) of oxygenated aCSF. The perfusion and the interface chamber were warmed to 36–37 °C and the interface was kept in a stream of 95% O_2:5% CO_2 warmed and humidified by bubbling through warm water. After a period of 30–60 min the perfusion was switched to 3 μM PE and this was used for the rest of the recording session. Spare slices for use later in the day were maintained at room temperature in a Petri dish of aCSF bubbled with 95% O_2:5% CO_2.

2.3. Electrophysiological recordings

Recordings were made from presumed 5-HT neurones in the medio-ventral portion of the DRN using glass microelectrodes pulled from 1.5 mm capillary glass (Clark Electromedical or WPI) filled with 2 M NaCl. Electrodes were lowered into and through the slice using a micromanipulator (Prior or Narashige). A silver wire in contact with the perfusion, served as a reference electrode. The recording electrode was connected to a headstage and AC amplifier (×1000 with 150 Hz–14.5 KHz band pass filter), and thereafter to a digital oscilloscope (Gould), and Spike processor (Digitimer). The spike processor was used to discriminate action potentials based on their amplitude and generate transistor–transistor logic (TTL) pulses which were then fed via an interface (micro1401, CED, UK) to a personal computer. Spike 2 version 4 (CED, UK) was used to collect and analyse the data.

2.4. Drug application

Drug solutions (in 3 μM PE/aCSF) were applied via the perfusion. Agonists (NMDA, 5-HT, and THIP) were applied in 2 min pulses initially in the absence of, and then in the presence of the anaesthetics (pentobarbitone, ketamine, chloral hydrate, urethane). Anaesthetics were applied for 5 min before and then continuously during the reapplication of the agonists. Effects of the anaesthetics were found to be established and stable after this period. The majority of neurones/slices were tested with more than one agonist and with one anaesthetic applied at increasing concentrations. Concentrations of 5-HT (5, 10, 25 or 50 μM) and THIP (10 or 25 μM) were chosen to give a submaximal response in each individual cell i.e. if the first concentration of THIP tested produced 100% inhibition a lower concentration was then tested and used in conjunction with the anaesthetic. NMDA was tested at 30 μM in all neurones. In some neurones a second anaesthetic was tested after a period of washout—in these cases we ensured that (altered) agonist responses had returned to control values (see example Figs. 2, 4 and 7).

2.5. Anaesthetic concentrations

2.5.1. Pentobarbitone

Anaesthetic doses of pentobarbitone range from 25 to 50 mg/kg (Flecknell, 1996). After 40 mg/kg brain levels have been reported to be approximately 100 μM (Roberts et al., 1970; Yanai et al., 1986) or 200 μM (Ossenberg et al., 1975; Petrenko et al., 2004) whilst after 25 mg/kg pentobarbitone, brain levels have been reported to be 12–15 μg/g (or approx 55–70 μM) (Baekeland and Greene, 1958; Kato and Chiesara, 1962). Here we used 10 and 100 μM pentobarbitone.

2.5.2. Ketamine

Ketamine induces surgical anaesthesia at 75–100 mg/kg i.p. (Flecknell, 1996). In mice after 100 mg/kg brain levels have been reported to be around 50 μg/g (approx 180 μM) (Irifune et al., 1992; Petrenko et al., 2004). Cessation of hypnosis or return of the righting reflex in rodents has been shown to occur at brain levels below around 100 μM (Cohen et al., 1973; Marietta et al., 1976) or 150 μM (Blednov and Simpson, 1999). Here we examined the effects of 10, 100 and 300 μM which span the anaesthetic range.

2.5.3. Chloral hydrate

Immediately at loss of righting reflex, brain chloral hydrate concentrations have been reported to be approximately 280 μg/g brain (approx 1.7 mM) although levels fell rapidly thereafter to less than 100 μg/g brain (approx 0.6 mM) (Mackay and Cooper, 1962). These levels are similar to those reported by Krieglstein and Stock, who found brain levels of 0.7 μmol/g tissue (equating to 0.7 mM) after an anaesthetic dose of the drug (Krieglstein and Stock, 1973). Here we tested concentrations of 100 μM and 1 mM chloral hydrate which cover the range expected during anaesthesia.

2.5.4. Urethane

Brain levels of urethane after anaesthetic doses (1–1.5 g/kg) have been measured and found to be around 1200 μg/ml (≅13 mM) ((Bauquier and Golder, 2010) and Bauquier, personal communication). Here we tested concentrations of 10 and 30 mM to span the concentration range expected during surgical anaesthesia.