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Anesthetic actions of thiopental remain largely unaffected during cholinergic overstimulation in cultured cortical networks

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HIGHLIGHTS

- The anesthetic impact of thiopental was assessed in slice cultures derived from the mouse neocortex.
- *In vitro* simulation of cholinergic crisis caused significant neuronal overactivity.
- Drug potency and efficacy of thiopental remained virtually stable during cholinergic overstimulation.

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ABSTRACT

In case of military or terrorist use of organophosphorus (OP) compounds victims are likely to suffer from not only intoxication but physical trauma as well. Appropriate emergency care may therefore include general anesthesia to allow life-saving surgical intervention. Since there is evidence that drug potency and efficacy of several anesthetics are attenuated by high concentrations of acetylcholine in the CNS, this study was designed to evaluate the anesthetic actions of thiopental during cholinergic overstimulation. Making use of organotypic slice cultures derived from the mouse neocortex, drug effects were assessed by extracellular voltage recordings of network activity at basal cholinergic tone and during simulated cholinergic crisis (high cholinergic tone). The latter was achieved by inhibition of acetylcholinesterases *via* soman and an ambient acetylcholine concentration of 10 μM. The induction of cholinergic crisis *in vitro* increased the network activity of cortical neurons significantly. Surprisingly, differences in network activity between basal and high cholinergic tone became less pronounced with rising concentrations of thiopental and drug potency and efficacy were almost equivalent. These results clearly distinguish thiopental from previously tested general anesthetics and make it a promising candidate for *in vivo* studies to identify suitable anesthetics for victims of OP intoxication.

1. Introduction

Although the mechanism of organophosphorus (OP) compound poisoning is known, namely inducing acute toxicological effects by irreversible inhibition of acetylcholinesterases, the treatment of patients suffering from OP intoxication continues to pose a therapeutic problem. The resulting accumulation of acetylcholine in the synaptic cleft causes cholinergic overstimulation in the central and peripheral nervous system and eventually leads to parasympathetic overactivity and neuromuscular dysfunction (de Jong, 2003). In order to avoid critical conditions like cardiac arrest or respiratory failure, a therapy with antidote drugs (atropine and oximes) has become the prevalent medical standard (Thiermann et al., 2013; Worek and Thiermann, 2013). Yet, in scenarios of military or terrorist use of chemical weapons, victims are likely to suffer from not only intoxication but collateral physical trauma as well (Ben Abraham et al., 2002; Cosar and Kenar, 2006; White, 2002). Emergency care for such patients may therefore require induction and maintenance of general anesthesia to set the course for mechanical ventilation or life-saving surgical intervention

Abbreviations: ACSF, artificial cerebrospinal fluid; IV, intravenous; ME, Smeasure of effect size; OP, organophosphorus.

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(Cosar and Kenar, 2006). Unfortunately this circumstance raises an inconvenient problem. As most anesthetics in clinical use induce sedation, amnesia, and hypnosis by decreasing the activity and excitability of neurons in the cerebral cortex (Antkowiak, 1999; Grasshoff et al., 2005; Hentschke et al., 2005; Rudolph and Antkowiak, 2004) their impact is most likely attenuated by elevated neuronal activity during cholinergic crisis.

In fact, literature provides direct evidence that anti-cholinesterases antagonize anesthesia in humans and experimental animals. For instance, physostigmine reversed propofol- and sevoflurane-induced hypnosis (Meuret et al., 2000; Plourde et al., 2003) and increased the dose of propofol required to produce unconsciousness (Fassoulaki et al., 1997). In the same way, the administration of neostigmine to rats under isoflurane anesthesia produced spontaneous limb and orofacial movements, indicating the return of consciousness (Hudetz et al., 2003). Similar evidence can be found in a number of *in vitro* studies, showing that neuronal inhibition induced by isoflurane, sevoflurane, and etomidate is reduced by elevated levels of acetylcholine (Drexler et al., 2010a; Grasshoff et al., 2007a,b). These findings beg the question whether dosing regimens to guarantee adequate anesthetic depth are applicable for victims of OP intoxication. A theoretical approach to this problem would be the increase of drug doses to reach the desired effect. However, this is no appropriate solution considering the list of potential side effects that can easily aggravate the condition of patients with existing hemodynamic or cardiopulmonary instability (Ben Abraham et al., 2002; White, 2002). Instead, an anesthetic drug that remains largely unaffected by elevated cholinergic tone would be of great benefit, but the question of which anesthetic can meet this requirement remains yet unanswered.

In the present study we examined the effect of the ultra short-acting barbiturate thiopental, introduced as intravenous (IV) anesthetic by Lundy in 1934 (Russo and Bressolle, 1998). For this purpose, we opted for an *in vitro* system of organotypic slice cultures derived from the mouse neocortex, which proved itself as valuable tool to investigate the drug effect of anesthetics in the past (Drexler et al., 2010b). The anesthetic impact of thiopental was assessed by measuring the spontaneous action potential activity of cortical neurons in presence and absence of the drug. To evaluate the potential loss of drug effect in a state of cholinergic crisis, the neurotransmitter acetylcholine was applied and slice cultures were treated with the cholinesterase inhibitor soman (pinacolyl methylphosphonofluoridate).

2. Material and methods

2.1. Drugs and chemicals

Save for the horse serum (Life Technologies, Carlsbad, CA, USA) and the salts and glucose (Applichem, Darmstadt, Germany) to prepare the standard perfusion fluid, all drugs and substances were purchased from Sigma–Aldrich (St. Louis, MO, USA). Soman (pinacolyl methylphosphonofluoridate) was made available by the German Ministry of Defence in Bonn.

2.2. Preparation of organotypic slice cultures

All procedures were in accordance with the German law on animal experimentation and approved by the animal care committee (Eberhard-Karls-University, Tuebingen, Germany). In addition, all efforts were made to minimize animal suffering and the number of animals used.

Preparation of neocortical slice cultures was based on a technique previously described by Gähwiler (1981). In short, three day-old wild-type (C57 black 6) mice were deeply anaesthetized with isoflurane and decapitated after loss of response to noxious stimuli (tail pinch and cold thermal). Cerebral hemispheres were removed and a vibratome (WPI, Hitchin, UK) was used to cut 300 μm-thick coronal slices. Neocortical sections were excised and fixed on coverslips by coagulating 10 μl heparinized chicken plasma with 10 μl thrombin solution. Each coverslip was transferred into a plastic tube filled with 750 μl nutrient fluid, which consisted of horse serum (25%), Hanks' balanced salt solution (25%) and basal medium Eagle (50%) supplemented with glutamine and glucose. Corresponding to the roller tube technique by Gähwiler (1981) the tissue was further maintained in a roller drum at 37 °C and nutrient fluid was renewed twice a week. Subsequent to preparation and every renewal of nutrient fluid, cell cultures were incubated at 95% oxygen and 5% carbon dioxide for 1–2 h to adjust pH. To reduce proliferation of glial cells, antimitotics (10 μM 5-fluoro-2-deoxyuridine, 10 μM cytosine-*b*–D-arabino-furanoside, 10 μM uridine) were once-only added to the nutrient fluid the day following preparation. After two weeks of maturing *in vitro,* neocortical slice cultures developed spontaneous network activity, characterized by clusters of action potential firing (bursts) and intermittent periods of neuronal silence. Electrophysiological recordings were carried out between day 14 and 45 *in vitro*.

2.3. Electrophysiology

To perform extracellular voltage recordings of neocortical network activity, a coverslip was placed in a heated bathchamber (volume: 1.5 ml, temperature: 34 °C) (Luigs und Neumann, Ratingen, Germany) and perfused with artificial cerebrospinal fluid (ACSF) at a flow rate of approximately 1 ml min^{-1}. The ACSF consisted of 120 mM NaCl, 3.5 mM KCl, 1.13 mM NaH_2PO_4, 1 mM $MgCl_2$, 26 mM $NaHCO_3$, 1.2 mM $CaCl_2$, and 11 mM D-glucose and was bubbled with 95% oxygen and 5% carbon dioxide to adjust pH at 7.4. Under optical control (inverted microscope at low magnification), ACSF-filled glass electrodes (resistance 2–5 MΩ) were advanced into the tissue until extracellular single- or multiunit spike activity could be clearly discriminated from baseline noise. Bandpass filtered (1 Hz–5 kHz) signals were acquired on a personal computer via DigiData 1400 interface and AxoScope 7 software (Molecular Devices, Sunnyvale, CA, USA) and saved on hard disc for offline analysis.

2.4. Preparation and application of test solutions

All drug-containing solutions were prepared from stock solutions on a daily base. Thiopental was dissolved in equilibrated ACSF and filled into gastight glass syringes (Hamilton, Reno, NV, USA). Drug-containing solutions were applied to the bath-chamber by a syringe pump (Havard Apparatus, Holliston, MA, USA) and Teflon tubing (Lee, Westbrook, CT, USA). To ensure steady state conditions, recordings during anesthetic treatment were carried out 12 min after switching the perfusion from standard ACSF to drug containing ACSF. In order to simulate the condition of a cholinergic crisis the perfusion fluid contained a combination of thiopental and the neurotransmitter acetylcholine, and cholinesterases were irreversibly blocked by soman to avoid hydrolysis of acetylcholine in the course of the experiment. For that purpose, the slice cultures were incubated in ACSF containing 10 μM soman for 18 min right before they were transferred to the recording chamber. The selected acetylcholine concentration (10 μM) was derived from data of a study performed by Tonduli et al. and can be assumed to occur in the brain during severe intoxication (Tonduli et al., 1999). Details on the calculation of the selected ACh value are presented in a previous work (Grasshoff et al., 2007b).