Brain Research 1642 (2016) 364–375

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/brainres

Research report

Towards optimized anesthesia protocols for stereotactic surgery in rats: Analgesic, stress and general health effects of injectable anesthetics. A comparison of a recommended complete reversal anesthesia with traditional chloral hydrate monoanesthesia

Christin Hüske [a], Svenja Esther Sander [b,*], Melanie Hamann [c], Olivia Kershaw [d], Franziska Richter [a], Angelika Richter [a]

[a] Institute of Pharmacology, Pharmacy and Toxicology, Veterinary Faculty, University Leipzig, Germany
[b] Institute of Pharmacology and Toxicology, Department of Veterinary Medicine, Freie Universität Berlin, Germany
[c] Institute of Pharmacology and Toxicology, Department of Veterinary Medicine, Justus-Liebig-Universität Gießen, Germany
[d] Institute of Veterinary Pathology, Department of Veterinary Medicine, Freie Universität Berlin, Germany

ARTICLE INFO

Article history:
Received 13 January 2016
Received in revised form
17 March 2016
Accepted 7 April 2016
Available online 8 April 2016

Keywords:
Refinement
Injectable anesthetic
Chloral hydrate
Complete reversal anesthesia

ABSTRACT

Although injectable anesthetics are still widely used in laboratory rodents, scientific data concerning pain and distress during and after stereotactic surgery are rare. However, optimal anesthesia protocols have a high impact on the quality of the derived data. We therefore investigated the suitability of recommended injectable anesthesia with a traditionally used monoanesthesia for stereotactic surgery in view of optimization and refinement in rats. The influence of the recommended complete reversal anesthesia (MMF; 0.15 mg/kg medetomidine, 2 mg/kg midazolam, 0.005 mg/kg fentanyl; i.m.) with or without reversal and of chloral hydrate (430 mg/kg, 3.6%, i.p.) on various physiological, biochemical and behavioral parameters (before, during, after surgery) was analyzed. Isoflurane was also included in stress parameter analysis. In all groups, depth of anesthesia was sufficient for stereotactic surgery with no animal losses. MMF caused transient exophthalmos, myositis at the injection site and increased early postoperative pain scores. Reversal induced agitation, restlessness and hypothermia. Even the low concentrated chloral hydrate led to peritonitis and multifocal liver necrosis, corresponding to increased stress hormone levels and loss in body weight. Increased stress response was also exerted by isoflurane anesthesia. Pronounced systemic toxicity of chloral hydrate strongly questions its further use in rodent anesthesia. In view of undesired effects of MMF and isoflurane, thorough consideration of anesthesia protocols for particular research projects is indispensable. Reversal should be restricted to emergency situations. Our data support further refinement of the current protocols and the importance of sham operated controls.

1. Introduction

Stereotactic surgeries are essential in *in vivo* research in neuroscience, as they offer access to specific regions of the brain for manipulations, injections or implantations. These surgeries require a prolonged anesthesia protocol allowing sufficient time to define anatomical landmarks, for opening the skull of the animal and for implantation of e.g. chronic guide cannulas (see Hamann et al. (2008), Richter et al. (2008), Sander and Richter (2007) for detailed protocols). There are two general protocols for anesthesia: single or combined injectable anesthetics or inhalation anesthesia. The aim is to achieve narcosis with as little pain and distress as possible (Charbonneau et al., 2010) in order to fulfill animal welfare requirements but also to reduce post-anesthetic impact on the CNS and therefore on the measurements obtained in the study. Although inhalation anesthesia gains in popularity because of its high safety margin (Stokes et al., 2009; Wolfensohn and Lloyd, 2013), injectable anesthetics still offer several advantages: the administration does not require special equipment and avoids the

Abbreviations: A, adrenaline; CH, chloral hydrate; HR, heart rate; iCs, immunoreactive corticosterone; LORR, loss of righting reflex; MMF, complete reversal anesthesia; MMF+R, complete reversal anesthesia with reversal; NA, noradrenaline; RR, respiratory rate; spO_2, saturation level; ST, surgical tolerance

* Correspondence to: Institute of Pharmacology and Toxicology, Department of Veterinary Medicine, Freie Universität Berlin, Koserstr. 20, 14195 Berlin, Germany.
E-mail address: svenja.sander@fu-berlin.de (S.E. Sander).

http://dx.doi.org/10.1016/j.brainres.2016.04.019
0006-8993/

risk of waste anesthetic gas leakage (Callahan et al., 2014). During stereotactic surgery animals have to be placed in a specific frame with fixation of the head, and manipulations on the skull often limit the use of standard masks for gas inhalation. Rodents are rarely intubated for inhalation anesthesia and the researcher is therefore often exposed to varying amounts of the halogenated anesthetics which have been associated with congenital abnormalities in rodents and clinical staff (Tankó et al., 2014). Therefore many researchers still resort to injectables for anesthesia. There are a number of disadvantages of injectable anesthetics such as the prolonged sleeping times, in which the animal might stay vulnerable for hypothermia and hypoglycemia (Albrecht et al., 2014). These unwanted drug effects will affect the brain and therefore the data derived from the study. Thus, injection anesthesia in rodents is quite challenging regarding adjusting a sufficient depth while avoiding potential complications (Jang et al., 2009). Based on our extensive experience with stereotactic surgeries under injectable anesthetics in rodents we here provide the neuroscience community with an in depth analysis of their analgesic properties including pain scoring, measurement of stress parameters and analyses of toxicity.

We focused on the often recommended completely reversal anesthesia (combined anesthesia) in comparison with the still often used traditional monoanesthesia with chloral hydrate (CH). For comparison purposes we also included a stress response measurement under isoflurane anesthesia, which is the currently recommended protocol for inhalation anesthesia in rodents. CH is still used for anesthesia of laboratory animals because of a long surgical tolerance (ST) and a widely-held assumption of little interactions with other drugs (Flecknell et al., 2007; Field et al., 1993). However, i.p. application of high concentrated solutions (125–275 mg/ml) induced severe peritonitis and fibrosis of serous membranes (Fleischmann et al., 1977; Dada et al., 1992), which were observed far less frequently after application of less concentrated solutions (40 mg/ml; Vachon et al., 2000). Depressing cardiovascular and respiratory as well as hypothermic and putative liver-toxic effects question the use of CH in laboratory animals (Field et al., 1993; Vachon et al., 2000). Reversal anesthetic protocols can offer a better controllability (Albrecht et al., 2014). The combination of the α_2-adrenergic agonist medetomidine, a benzodiazepine like midazolam and the opioid fentanyl (MMF) has been suggested to generate a reliable analgesia (Sinclair, 2003; Albrecht et al., 2014). The suitability of MMF in Sprague–Dawley rats, a strain often used in neuroscience research, has not been investigated so far. Vomitus, audible vocalization, restlessness and heavy excitement were reported after reversal with the specific antagonists atipamezol, flumazenil and naloxone (Hu et al., 1992). Potential disadvantages for the well-being have not been followed up yet. In contrast to CH, MMF demonstrated neuroprotective properties which might interfere with experimental setups relying on neurodegenerative processes (Ozden and Isenmann, 2004).

Regarding the assessment of pain (and distress) in animals (Murrell and Johnson, 2006), there exists no "gold-standard" due to a lack of specific indicators for pain and the subjective nature of the assessment system (Flecknell, 1994). For the purpose of achieving approximately reliable pain and distress assessment, we combined several physiological (changes in heart rate, respiration, temperature, and body weight), biochemical (levels of catecholamines and immunoreactive corticosterone), and behavioral parameters (ultrasonic vocalization, changes in posture, locomotion or activity among others) in this study. This extensive set of endpoints can be used by the neuroscience research community to assess and optimize their respective anesthesia protocols.

In the present study we defined the optimized protocol for stereotactic surgery as follows: (1) induction of a surgical tolerance (ST) of at least 60 min for a stereotactic surgery, (2) a smooth activation of stress response, (3) going along with acceptable, stable vital parameters including a low mortality and (4) producing no tissue irritations or other painful side effects.

2. Results

2.1. Anesthesia, reflexes and ultrasonic vocalization

An overview of the number of animals reaching LORR (loss of righting reflex), ST (surgical tolerance), number of animals undergoing surgery, relevant times and the death rate for the injection anesthetic protocols are given in Table 1. With the anesthesia protocols investigated in this study all animals reached unconsciousness. However, we determined that ST is not long enough by one injection with both of the anesthetic protocols, so additional dosing was taken into account. Although latency to LORR was significantly prolonged in rats of the CH group in comparison to MMF and MMF+R treated animals (One way ANOVA, $F(2,30)=10.7$, $p<0.001$; see Table 1 for post-hoc test results), surgical tolerance was achieved earlier in this group compared to rats of the MMF+R group (latency to ST) (One Way ANOVA, $F(2,29)=8.2$, $p<0.05$; Table 1). Two CH-treated rats lost ST before the first incision, while five rats injected with MMF were not able to be operated due to a complete lack (one rat) of ST or a too short one (4 rats). In accordance to the protocol, these animals

Table 1
Parameter for anesthesia.

Parameters	Experimental group ($n=11$, including 6♂, 5♀)		
	CH	**MMF**	**MMF+R**
Animals with LORR: *n* (%)	**11** (100)	**11** (100)	**11** (100)
Latency to LORR [min]			
♂	**2.24 ± 0.12**[a,b]	**1.42 ± 0.12**	**1.63 ± 0.16**
♀	2.33 ± 0.14	1.24 ± 0.10	1.64 ± 0.21
	2.12 ± 0.17	1.64 ± 0.19	1.62 ± 0.28
Animals with ST: *n* (%)	**11** (100)	**10** (91)	**11** (100)
Latency to ST [min]			
	5.66 ± 0.24[b]	**6.44 ± 0.47**	**8.73 ± 0.81**
♂	5.48 ± 0.36	6.86 ± 0.68	10.25 ± 1.13
♀	5.87 ± 0.38	6.02 ± 0.65	6.90 ± 0.42*
Operated animals: *n* (%)	**9** (82)	**6** (55)	[**11** (100)]
Duration 1. ST [min]			
	49.14 ± 4.48	**47.83 ± 7.05**	**44.77 ± 5.27**
♂	47.56 ± 6.49	36.72 ± 10.97	34.26 ± 6.96
♀	51.03 ± 6.70	58.95 ± 6.45	57.38 ± 2.64*
Duration of anesthesia [min]			
	155.66 ± 8.21[b]	**182.23 ± 20.58**[c]	**71.40 ± 0.73**[d]
♂	137.63 ± 8.12	128.89 ± 7.56	71.89 ± 1.25
♀	177.29 ± 7.59*	246.96 ± 17.96*	70.82 ± 0.67
Death rate: *n*	**0**	**0**	**0**

Comparison of efficiency to induce LORR (loss of righting reflex) and ST (surgical tolerance: loss of pain reflex), time-related parameters of anesthesia, animals operated and death rate using chloral hydrate anesthesia (CH), complete reversible anesthesia (MMF) and complete reversible anesthesia with reversal (MMF+R). Depth and quality were characterized by use of reflex tests, observation and responses to stimuli. As indicated by brackets [..], these data cannot be compared with other groups, because of earlier repeated dosage. Significant differences are indicated as followed.

[a] $p<0.05$ CH vs. MMF.

[b] $p<0.05$ CH vs. MMF+R.

[c] $p<0.05$ MMF vs. MMF+R (One Way ANOVA, Holm–Sidak method). As expected; the duration of anesthesia was significantly shorter after reversal (MMF+R) than in the other groups.

[d] $p<0.05$

* Significant differences between male and female rats are indicated by asterisks. (student's *t*-test, $p<0.05$).