Annals of Anatomy xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Annals of Anatomy

journal homepage: www.elsevier.de/aanat

Research article

The mesenterially perfused rat small intestine: A versatile approach for pharmacological testings

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ARTICLE INFO

Article history:
Received 22 December 2013
Received in revised form 27 February 2014
Accepted 28 February 2014
Available online xxx

Keywords:
Gastrointestinal motility
Small intestine
Methods
Organ perfusion
Pentobarbital
Propofol
Ketamine

ABSTRACT

Pharmaceutical compounds enter the body via several major natural gateways; i.e. the lung, the skin and the gastrointestinal tract. Drug application during surgical operations can lead to severe impairment of gastrointestinal motility, which can contribute to a paralytic ileus. Here we investigated an ex vivo perfused small intestine model that allows us to ascertain the influence of pharmaceuticals upon the gut.

Corresponding segments from the proximal jejunum of adult rats were used. Their mesenteric arteries and veins were cannulated and the jejunal segment excised. The individual segments were placed in a custom designed perfusion chamber and perfusion performed through the intestinal lumen as well as the mesenteric superior artery. Three test drugs, which are commonly used in anesthesiology; i.e. pentobarbital, propofol and ketamine were administered via the blood vessels. Their effects upon gastrointestinal motility patterns were evaluated by optical measurements. Longitudinal and pendular movements were distinguishable and separately analyzed.

Pharmacological effects of the individual substances could be investigated. Propofol (50–200 μg/ml) was found to decrease intestinal motility, especially longitudinal movements in a dose dependent manner. Pentobarbital decreased intestinal motility only at high concentrations, above 2.5 mg/ml. A dose of 2.5 mg/ml lead to an increase in longitudinal- and pendular movements in comparison to control, while ketamine (2.5–10 mg/ml) did not alter intestinal motility at all. Histological examination of the perfused segments revealed only minor changes in tissue morphology after perfusion.

The perfusion approach shown here allows for the identification of compounds which interfere with gut motility in a highly sophisticated way. It is suitable for characterization of drug and dose specific changes in motility patterns and can be used in drug development and preclinical studies.

1. Introduction

Anything that is ingested and resorbed in the gut has the potential to influence it locally and systemically. Pharmaceuticals which are administered per inhalation or intravenously can also interfere with the gastrointestinal tract (GIT). It is known that anesthesia can cause severe side effects in the GIT, e.g. the repression of gastrointestinal (GI) motility leading to post-operative nausea and vomiting (PONV) or to serious complications in clinical practice like aspiration pneumonia (Greif et al., 1999). It can be evoked by surgical intervention, sedatives or anesthetics. GI motility disturbances can lead to postoperative sickness, which might be accompanied by nausea, vomiting, repeated cramps and abdominal pain. Intestinal motility disturbances can decrease the immune resistance and lead to an increased risk of infection (Fruhwald et al., 2006). Therefore, it is important to know which pharmaceuticals affect GI motility in general, and in particular, anesthetics.

The aim of this study was to establish an ex vivo model enabling prediction and quantification of pharmaceutical effects on GI motility. For example, narcotics that affect central nervous system (CNS) or peripheral nervous system (PNS) function may also affect the nervous system of the gut, the enteric nervous system (ENS), which mainly operates via the same neurotransmitters and neuronal mechanisms. The ENS spans the entire GIT and works independently of the brain. It consists of the submucosal and myenteric plexuses, the latter of which is located between the longitudinal- and circular muscle layers where it coordinates peristalsis as well as secretion. The submucosal plexus is located

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http://dx.doi.org/10.1016/j.aanat.2014.02.008
0940-9602/

between the circular muscular and mucosal layers. It coordinates secretion as well as neuro-immune interactions (Fruhwald et al., 2006). Enteric neurons can be classified according to their function. Intrinsic neurons as well as primary afferent neurons (IPAN), interneurons, motor neurons, secretomotor neurons and vasodilator neurons can be distinguished as the main types in mammals (Costa et al., 1996; Hagl et al., 2012). IPANs sense physiological stimuli, e.g. movements of intestinal villi, stimulation of the mucosa, contractions of the enteric muscles or changes in chymal composition (Furness et al., 2004). Communication between enteric neurons is facilitated by interneurons spanning millions of synaptic connections through the ENS (Costa and Henning, 2000). They are responsible for triggering intestinal movements through motoneurons which innervate effector systems in the GIT. Activation of motoneurons can lead to excitation or inhibition of the effectors depending on the neurotransmitters involved. Through that mechanism, motility is directly controlled by the ENS. Acetylcholine and Substance P are the most important excitatory transmitters, nitric oxide and vasoactive intestinal peptide are the most important inhibitory ones (Fruhwald et al., 2006). Pharmacological interactions or loss of the inhibitory or excitatory system can lead to hypermotility or atonia. Both conditions can affect the patient's health and wellbeing.

Although the ENS possesses structures which are functionally analogous to the blood–brain barrier (Gershon and Bursztajn, 1978), it is likely to be exposed not only to small organic molecules that are resorbed from the intestine, but also to any other type of medication, in this study, injectable anesthetics. Substances that exhibit only weak effects on the CNS might exhibit stronger effects on the ENS due to its exposed position in the GIT. In our system, the anesthetics propofol (2,6-diisopropylphenol), pentobarbital (5-ethyl-5-(1-methylbutyl)-2,4,6(1H,3H,5H)-pyrimidinetrione) and ketamine ((RS)-2-(2-chlorophenyl)-2-(methylamino)cyclohexanone) were characterized as to their effects on GI motility.

Propofol is an anesthetic frequently used in intensive care medicine. It is a water insoluble 2,6-diisopropylphenol which is administered in an oil in water emulsion. Similar to barbiturates it can induce coma and is therefore used for initiation of narcosis without exhibiting analgesic effects. In comparison to other anesthetics, induction and awakening from anesthesia are described as rather comfortable with rapid recovery (Friedberg, 1993). Propofol is well suited for total intravenous anesthesia (TIVA) in combination with a strong analgesic drug like ketamine.

Pentobarbital belongs to the barbiturate group of anesthetics. They exhibit anticonvulsive and sedative effects and are preoperatively used to induce coma. Barbiturates induce their effects through binding to the $GABA_A$ receptor in the CNS. The $GABA_A$ receptor is a ligand-gated ion channel (Sieghart, 1992). After binding of gamma-aminobutyric acid, it decreases excitability of neurons by facilitating chloride influx (Davies, 2003). Barbiturates are frequently used as sedatives as well as for the induction of narcosis similar to propofol. In the ENS, GABA receptors trigger either excitatory cholinergic or inhibitory noradrenergic neuronal activity (Krantis, 2001). This can lead to contraction or relaxation of the smooth muscles. Previous studies using pentobarbital in guinea pig ileum have shown that it can inhibit contractions (Mayer et al., 1981).

Ketamine is an anesthetic frequently used as a substitute for opiods, which are known to exhibit a negative effect on peristalsis and can lead to obstipation and promote PONV (Byers et al., 1995; Kurz and Sessler, 2003). In contrast to the previously mentioned anesthetics, propofol and pentobarbital, ketamine exhibits strong analgesic effects. It is used, often in combination with propofol ("ketafol"), for induction of general anesthesia in total intravenous anesthesia. Moreover it can be used in the induction of general anesthesia in combination with a sedative. It is used in short and very painful procedures such as treatment of burns and in emergency medicine due to the fact that it is a potent, readily available analgesic drug and is applied primarily via the intramuscular route which does not decrease blood pressure.

In intensive care medicine, anesthetics are used for narcosis. Parts of the CNS are pharmacologically switched off, dampening pain sensation, aversion reflexes, consciousness and often muscle tonus. Depending on application, inhalative and injectable anesthetics are distinguished. Injectable anesthetics include opiods, barbiturates and several individual substances such as propofol and ketamine. Previous studies have shown that barbiturates can inhibit intestinal peristalsis in vivo (Holzer and Dirnhofer, 1987). Some studies also suggest that propofol, which is frequently used as an anesthetic, can repress GI motility. In the case of ketamine, different studies revealed contradictory results (Grant et al., 1981; Schnoor et al., 2005). Therefore propofol, pentobarbital and ketamine were chosen in order to validate this new perfusion model.

2. Materials and methods

2.1. Animals

Thirteen adult Sprague-Dawley rats of both sexes weighing 250–500 g were used for the experiments. Animal experiments were reported to the responsible authorities and approved by German legislation.

The rats were sacrificed by gassing with carbon dioxide followed by cervical dislocation prior to median laparotomy. Intestinal parts not intended for perfusion were ligated and resected. A 4 cm long intestinal segment from the proximal jejunal region with an adhering intact mesenterial root was used for perfusion. The mesenteric superior artery and the mesenteric superior vein were cannulated with a BD Vasculon Plus 26GA × 19 mm intravenous catheter. The resected segment was stored on ice prior to perfusion for a maximum of 15 min.

For baseline recording in control experiments, 4 animals were used. For each substance tested, 3 animals were used.

2.2. Histology

Prior to perfusion, 1 cm of tissue was resected orally from the site where the perfused segment was resected. It was subsequently fix in 4% formaldehyde in PBS for histological evaluation. After perfusion, a consecutive piece of tissue was resected from the oral end of the perfused segment and processed likewise. Tissues were paraffin embedded and 5 μm tissue slices were used for standard hematoxylin–eosin (HE) staining to analyze the influence of perfusion upon the tissue.

2.3. Intestinal perfusion

Perfusion was performed from both the serosal and luminal side. The cannulated mesenterial root was perfused through the superior mesenteric artery. For serosal superfusion as well as luminal and superior mesenteric artery perfusion, Tyrode solution (Lammers, 2005) was utilized. Propofol (Propofol Fresenius®), pentobarbital (Narcoren®) and ketamine (Ursotamin®) were added to the solution which was perfused through the blood vessels.

Perfusion was carried out in a custom designed organ bath (6.7 cm × 5 cm × 30 cm) flushed with Tyrode solution at a flow rate of about 100 ml/min, which superfused the isolated intestinal segment. To superfuse the intestine, a Heissner submerged pump Typ P6 was utilized. The Tyrode solution was gassed with carbogen (95% O_2, 5% CO_2). The pH was constant at 7.35 ± 0.05 during perfusion.

Please cite this article in press as: Dominik, S., et al., The mesenterially perfused rat small intestine: A versatile approach for pharmacological testings. Ann. Anatomy (2014), http://dx.doi.org/10.1016/j.aanat.2014.02.008