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Brain penetration of local anaesthetics in the rat: Implications for experimental neuroscience

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ABSTRACT

Multiple experimental neuroscience techniques rely on the use of general anaesthesia to minimize the discomfort associated to animal restraint and to achieve a more effective control of relevant physiological parameters. In order to minimise potential interference on brain neuronal activity, such studies are typically conducted at low anaesthetic doses. This practice is often coupled to peripheral infiltration of local anaesthetics to provide supplementary analgesia and prevent sub-threshold activation of pain pathways that may confound central measurements of brain function. However, little is known of the effect of peripheral anaesthesia on central measurements of brain activity in small laboratory animal species. In order to begin to address this question, we measured total and free brain exposure of five different local anaesthetics following subcutaneous infiltration of analgesic doses in a surgical protocol widely used in rodent neuroimaging and electrophysiology studies. Notably, all the anaesthetics exhibited detectable total and free brain concentrations at all the time points examined. Lidocaine and mepivacaine showed the highest free brain exposures (>525 ng/g), followed by bupivacaine and ropivacaine (>70 ng/g). The ester-type local anaesthetic tetracaine produced the lowest free brain exposure (<8.6 ng/g). Our data suggest that peripheral administration of local anaesthetics in small laboratory animals could result in pharmacologically active brain exposures that might influence and confound central measurements of brain function. The use of the ester-type anaesthetic tetracaine produced considerably lower brain exposure, and may represent a viable experimental option when local anaesthesia is required.

1. Introduction

Recent developments in experimental neuroscience have opened the possibility to investigate multiple and complementary aspects of brain function in humans and laboratory animals Thus, it is now possible to describe neuronal activity at various levels of integration, ranging from action potential recording from single neurons up to higher level responses reflecting local coordinated activity (i.e. electroencephalography, EEG), or the activation of entire brain circuits by using non-invasive neuroimaging techniques such as fMRI (functional Magnetic Resonance Imaging), or PET (positron emission tomography). However, the application of investigational methods in small laboratory animals is often complicated by the need to avoid motion artefacts and to minimize the stress and discomfort associated to restraining procedure. To complicate the picture, invasive surgical procedures are often required to achieve control of physiological parameters, allow administration of pharmacological agents, or insertion of probes to investigate complementary aspects of brain function. As a result, a large number of neuroimaging or electrophysiology studies in rodents are performed under general anaesthesia.

An implicit but pivotal assumption for this type of studies is that anaesthetic-induced perturbations of tonic neuronal activity must be minimised by using as low doses of anaesthetic as possible. It is therefore common practice to complement general anaesthesia with peripheral administration of local anaesthetics at sites of surgery, with the aim to provide supplementary peripheral analgesia both intra- and post-operatively, and to prevent sub-threshold pain pathways activation that may confound central measurements of brain function (Peeters et al., 2001; Marota et al., 2000; Gozzi et al., 2006; Kelland et al., 1990a,b; Ruskin et al., 1999; Bonhaus et al., 1986; Craig, 2003; King et al., 2005; Febo et al., 2004).

Local anaesthetic agents have been devised to reversibly prevent transmission of the nerve impulse by producing insensitivity to painful stimuli in the area supplied by that nerve without affecting consciousness (Lagan and McLure, 2004; Covino, 1986a,b). These

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agents exert their action by blocking the inward Na^+ current at the sodium ionophore during depolarization, thus preventing propagation of axonal action potentials. However, additional mechanism may be involved, including the involvement of calcium, potassium and G-protein-regulated channels (Columb and MacLennan, 2007; Scholz, 2002; Bruhova et al., 2008; Hollmann et al., 1999, 2001; Hoenemann et al., 1998; Nietgen et al., 1997).

It has been previously shown that, upon peripheral infiltration, only a minor fraction of the injected anaesthetic (1–2%) contributes to local nerve blockade, with more than 95% of the drug entering systemic circulation (Popitz-Bergez et al., 1995). The systemic fraction can thus produce significant toxic reactions (Covino, 1986a,b; Ruetsch et al., 2001), an aspect that appears to relate to the ability of the drugs to cross the blood–brain barrier (BBB) and act on excitable membranes in the central nervous system (CNS) (Reiz and Nath, 1986; Zink and Graf, 2003; Takahashi et al., 2006). This aspect could be of great experimental relevance, as it suggests that, contrary to intuition, peripheral anaesthesia might actually produce significant central pharmacological effects.

Previous studies have addressed systemic contribution of local anaesthesia in humans or in large animals (Kohn et al., 1997; Tucker and Mather, 1979; Scott, 1986). However, the degree of brain penetration of local anaesthetics in small laboratory animal species (e.g., rodents) upon peripheral infiltration has not been systematically investigated. Given the wide use of these agents in experimental neuroscience protocols, this information could be key to identifying potential confounding contributions of local anaesthesia on central readouts of brain function, and might lead to a more rational choice of surgical and anaesthetic protocol.

Here we measured the brain exposure of five commonly used local anaesthetics at various time points following peripheral infiltration of analgesic doses (Lagan and McLure, 2004) (Fig. 1 and Table 1), using a representative surgical protocol that is widely used in experimental neuroscience to obtain stable physiological conditions in rodent neuroimaging and electrophysiology measurements (Gozzi et al., 2008; Julien et al., 2004; Kelland et al., 1990a,b). The compounds chosen (lidocaine, mepivacaine, bupivacaine, ropivacaine and tetrcaione) are characterised by different potency, onset and duration of action (Fig. 1 and Table 1) reflecting different physicochemical (i.e. lipid solubility, amide, vs. ester-type scaffolds) and pharmacokinetic (i.e. protein binding, pK_i) properties (Lagan and McLure, 2004; Covino, 1986a,b; Hall and Clarke, 1991; Englesson, 1975; Scott, 1975).

Lidocaine is a widely used amide-anaesthetic able to deliver a profound block with a rapid speed of onset due to its low pK_a (7.7). Its protein binding is relatively low (64%) so its duration of action is predictably short (Covino, 1986a,b). Mepivacaine is a structural variant of lidocaine with a longer analgesic effects, and lower toxicity and vasodilatatory effects (Ruetsch et al., 2001; Willatts and Reynolds, 1985; Kasaba et al., 2003; Tagariello et al., 2001). Bupivacaine is a long-acting, highly lipophilic compound characterised by a high protein binding (95%) and intermediate onset of anaesthesia (pK_a 8.1). Bupivacaine is also a substrate of the BBB P-glycoprotein (P-gp) efflux pump (Funao et al., 2003) a feature that has been shown to limit CNS penetration of several psychoactive compounds (Doran et al., 2005). Ropivacaine is a chemical analogue of bupivacaine characterised by a lower lipid solubility and systemic toxicity. It is slightly less potent and shorter acting than bupivacaine but has greater vasoconstrictor activity (McLure, 1996; Hansen, 2004). Finally, tetracaine is an old-generation ester anaesthetic characterised by very high potency and prolonged anaesthetic effect (Lagan and McLure, 2004). As ester anaesthetics are rapidly hydrolysed in plasma by non-specific plasma esterases (McLure and Rubin, 2005), we hypothesised that this aspect could limit the CNS exposure of this drug with respect to more stable compounds such as the amide-type anaesthetics tested in this study.

2. Materials and methods

2.1. Chemicals

Commercial solution of lidocaine 2% (Fort Dodge Veterinaria, Spain), bupivacaine 0.25% (w/v, Marcaina®, AstraZeneca, Milan, Italy), ropivacaine 1% (w/v, Naropin®, AstraZeneca, Milan, Italy) were used throughout the study. The latter was diluted in saline solution to obtain a 0.25% (w/v). Mepivacaine hydro-chloride and tetracaine hydro-chloride were provided by Sigma–Aldrich (St. Louis, USA) and dissolved in saline to yield 1% and 0.1% (w/v) solutions, respectively. D(+)-Tubocurarine chloride hydrate (Sigma–Aldrich, Milano, Italy) was dissolved at 0.25 g/ml in heparinized 25 UI/ml physiologic solution.

Phenilmethanesulfonyl fluoride (PMSF), a specific esterase inhibitor, was obtained from Sigma–Aldrich Chemie (GmbH, Steinheim, Germany).

2.2. Animals

Specific opportunistic pathogens-free male *Sprague–Dawley rats* (weight: 301–368 g) from Charles River Italia (Calco, Lecco, Italy) were used for the study. The animals were housed in stable groups of five individuals in polycarbonate cages with sawdust litter. During the pre-experimental period, food and filtered water from the normal domestic supply were provided *ad libitum*. Animals were maintained on a light:dark cycle of 12 h:12 h (6 a.m. to 6 p.m. of artificial light). The room temperature was within the range 20–22 °C and relative humidity within the range 45–65%. After arrival, animals were acclimatized for at least 5 days before being used in the experiments.

All the experiments were carried out in accordance with Italian regulation governing animal welfare and protection (which acknowledges the European Directive 86/609/EEC) and according to internal GlaxoSmithKline Committee on Animal Research & Ethics (CARE) review. All the efforts were made to minimize the number of animals used and their distress.

2.3. Animal preparation

The surgical protocol used here has been previously described in greater detail (Gozzi et al., 2008). Briefly, 45 subjects were randomly assigned to 5 groups (9 animals/group/local anaesthetic). Anaesthesia was induced with isofluorane (5% in oxygen/nitrogen 1:2). The animals were then placed on interactive heating pads, connected through rectal probes, and isoflurane level was decreased to 2.5%. Prior to surgical incision, each animal received a subcutaneous (SC) infiltration) of local anaesthetic at each surgical site (neck and femoral area) of a volume of 0.1 ml/point (0.2 ml/rat) of local anaesthetic. The time of injection of the local anaesthetic was considered −40 min with respect with subsequent brain exposure measurements. The neck and femoral area were shaved and the skin disinfected.

2.4. Tracheostomy

A midline skin incision was made along the length of the neck and, after separating the two halves of the sternhyoid muscle, the trachea was exposed. This was incised in the sub laryngeal region and a G14 cannula was inserted into it, secured with a suture and connected to a pump for artificial ventilation.