NeuroToxicology 54 (2016) 1–10

Contents lists available at ScienceDirect

NeuroToxicology

Full length article

Delayed application of the anesthetic propofol contrasts the neurotoxic effects of kainate on rat organotypic spinal slice cultures

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ARTICLE INFO

Article history:
Received 7 December 2015
Received in revised form 25 February 2016
Accepted 2 March 2016
Available online 3 March 2016

Keywords:
Spinal cord injury
Glutamate receptors
$GABA_A$ and glycine receptors
General anesthetic
Neuroprotection
Motoneuron

ABSTRACT

Excitotoxicity due to hyperactivation of glutamate receptors is thought to underlie acute spinal injury with subsequent strong deficit in spinal network function. Devising an efficacious protocol of neuroprotection to arrest excitotoxicity might, therefore, spare a substantial number of neurons and allow later recovery. *In vitro* preparations of the spinal cord enable detailed measurement of spinal damage evoked by the potent glutamate analogue kainate. Any clinically-relevant neuroprotective treatment should start after the initial lesion and spare networks for at least 24 h when cell damage plateaus. Using this strategy, we have observed that the gas anesthetic methoxyflurane provided strong, delayed neuroprotection. It is unclear if this beneficial effect was due to the mechanism of action by methoxyflurane, or it was the consequence of anesthetic depression. To test this hypothesis, we investigated the effect by propofol (commonly injected i.v. for general anesthesia) after kainate excitotoxicity induced on organotypic spinal slices. At 5 μM concentration, propofol significantly attenuated cell death, including neuronal losses and, especially, damage to the highly vulnerable motoneurons. The action by propofol was fully prevented when co-applied with the $GABA_A$ antagonist bicuculline, indicating that neuroprotection required intact $GABA_A$ receptor function. Although bicuculline per se was not neurotoxic, it largely enhanced the lesional effects of kainate, suggesting that $GABA_A$ receptor activity could limit excitotoxicity. Our data might offer an explanation for the beneficial clinical outcome of neurosurgery performed as soon as possible after spinal lesion: we posit that general anesthesia contributes to this outcome, regardless of the type of anesthetic used.

1. Introduction

Acute spinal cord injury (SCI) is characterized by severe deficit in motor, sensory and autonomic functions (McDonald and Sadowsky, 2002). Whether of traumatic or non-traumatic origin, the pathophysiology of SCI starts with a primary lesion followed by secondary damage (Mortazavi et al., 2015) largely contributed by massive release of glutamate, in turn triggering the generation of toxic metabolites (Schwab et al., 2006), and producing cell death via a process termed excitotoxicity (Rossignol et al., 2007).

Once SCI becomes chronic, it represents an important challenge to patients, their family and the public health system. An extensive survey of traumatic SCI in the USA has recently indicated that new cases remain at a steady level of 54 million (Jain et al., 2015) with currently poor prospects for satisfactory recovery. Notwithstanding the current focus on neuroreabilitation (Rossignol and Frigon, 2011) and neurorepair (Salewski et al., 2015), attempts to arrest the progression of secondary damage with drug administration are a priority for basic scientists and clinicians: unfortunately, little success has been achieved as shown by a large scale clinical survey (Hurlbert et al., 2013). Recent reports have, however, indicated that early neurosurgical intervention (within the first 24 h) has beneficial effects on patient clinical outcome (Dvorak et al., 2015; Fehlings et al., 2012). Because neurosurgery is usually performed under general anesthesia, it seems plausible that at least some positive contribution might come from the administration of a general anesthetic. This notion is suggested by SCI model studies (Nout et al., 2012) since general anesthesia is known to contrast excitoxicity (Kawaguchi et al., 2005). In an in vitro model of SCI (Kuzhandaivel et al., 2011) based on excitotoxicity induced by the glutamate agonist kainate that is a standard test drug to elicit neurodegeneration (Nishida et al., 2015;

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http://dx.doi.org/10.1016/j.neuro.2016.03.001
0161-813X/

Schwob et al., 1980; Wang et al., 2005), we recently observed substantial neuroprotection by the volatile anesthetic methoxyflurane (Shabbir et al., 2015a).

We wondered if a more widely employed i.v. general anesthetic may share similar properties. Thus, we investigated the neuroprotective action of propofol (2,6-disopropylphenol) commonly used for general surgery (Fan et al., 2015; Vasileiou et al., 2009; Velly et al., 2003). Propofol has been shown to be neuroprotective in brain tissue primarily via enhancement of inhibition mediated by $GABA_A$ receptors (Ito et al., 1999; Kotani et al., 2008). As a first approach to elucidate propofol effects on spinal tissue, we used organotypic slice cultures that are an advantageous model to evaluate drugs against excitotoxic SCI (Guzmán-Lenis et al., 2009; Mazzone et al., 2010; Mazzone and Nistri, 2011a,b). In this model the kainate-evoked SCI is mainly limited to neurons with special vulnerability of motoneurons, while glia is generally spared (Mazzone et al., 2010; Mazzone and Nistri, 2013). Thus, we tested propofol at the clinically-relevant concentration of 5 μM (Eckle et al., 2014; Jewett et al., 1992) and applied it 1 h after the primary insult to mimic the scenario developing after an acute injury. This low propofol concentration is believed to induce selective upregulation of $GABA_A$ receptors (Grasshoff and Antkowiak, 2004; Jewett et al. 1992; Kungys et al., 2009; Wakita et al., 2013) plus inhibition of NMDA receptors (Irifune et al., 2003; Orser et al., 1995; Yamakura et al., 1995).

2. Materials and methods

2.1. Experimental procedures

2.1.1. Preparation of organotypic cultures

Organotypic slice cultures of spinal cord were prepared from 13 day rat embryos in accordance with previously published procedures (Avossa et al., 2003; Gahwiler et al., 1997; Mazzone et al., 2010). The fetuses were delivered by caesarean section from timed-pregnant rat anaesthetized with slowly raising levels of CO_2. Approval for these experiments was obtained from the Scuola Internazionale Superiore di Studi Avanzati (Trieste, Italy) Ethical Committee. All efforts were made to reduce the number of animals used and to minimize animal suffering. The fetuses were decapitated and their backs, isolated from their limbs and viscera, were cut into 275 μm thick transverse slices with a chopper (McIlwain tissue chopper, Canada, USA) from which the spinal cord was punched out and fixed on a glass coverslip with reconstituted chicken plasma clotted by one drop of thrombin (200 U/ml). Coverslips were inserted into plastic tubes with 1 ml of medium contained 82% Dulbecco's Modified Eagle's medium, 8% sterile water for tissue culture, 10% fetal bovine serum, osmolarity 300 mOsm, pH 7.35. From each dissection, 30–40 slices were prepared from the thoracic as well as the lumbar segments, and kept in culture for 22 days *in vitro* (DIV) before use. The tubes were kept in a roller drum rotating (120 × g/h) at 36.5 °C in Dulbecco's Modified Eagle's medium with high glucose (DME/HIGH), penicillin, and streptomycin (purchased from Euroclone, Devon, UK). Fetal calf serum (FBS) was obtained from Invitrogen, (Carlsbad, CA, USA). Nerve growth factor (NGF) was from D.B.A. Italia (Segrate, Italy), chicken plasma from Innovative (Novi, MI, USA), and thrombin from Merck (Darmstadt, Germany).

2.1.2. Protocol to study excitotoxicity

Experimental groups were obtained from spinal cord organotypic slices (at 22 DIV) and run in parallel: control, propofol (5 μM; Fresenius Kabi, Italy) treatment, kainate (10, 50 or 100 μM; Ascent Scientific, Bristol, UK) treatment, and kainate plus propofol treatment. All experiments were terminated 24 h after the initial test since our previous report had indicated that maximum cell

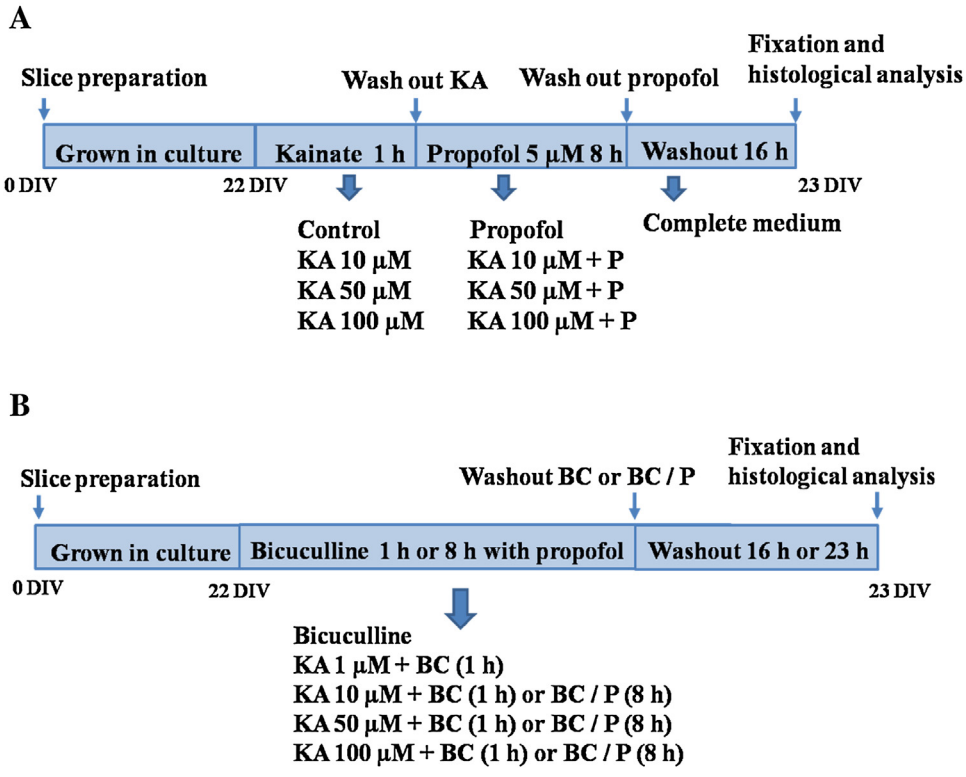

Fig. 1. Experimental protocol.
(A) Schematic diagram to illustrate experimental protocols for excitotoxic insult (KA = kainate) and the neuroprotective effect of propofol (P). (B) Schematic diagram to illustrate experimental protocols for effects of the $GABA_A$ receptor antagonist bicuculline (BC = bicuculline).