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Stimulation of rat cranial dura mater with potassium chloride causes CGRP release into the cerebrospinal fluid and increases medullary blood flow

Mária Dux [a], Christine Will [b], Mirjam Eberhardt [c], Michael J.M. Fischer [b], Karl Messlinger [b,*]

[a] Department of Physiology, University of Szeged. Dóm tér 10, H-6720 Szeged, Hungary
[b] Institute of Physiology and Pathophysiology, Friedrich-Alexander University Erlangen-Nürnberg, Universitätsstrasse 17, D-91054 Erlangen, Germany
[c] Department of Anaesthesia and Critical Care Medicine, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany

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ABSTRACT

Primary headaches may be accompanied by increased intracranial blood flow induced by the release of the potent vasodilator calcitonin gene-related peptide (CGRP) from activated meningeal afferents. We aimed to record meningeal and medullary blood flow simultaneously and to localize the sites of CGRP release in rodent preparations *in vivo* and *ex vivo*.

Blood flow in the exposed rat parietal dura mater and the medulla oblongata was recorded by laser Doppler flowmetry, while the dura was stimulated by topical application of 60 mM potassium chloride (KCl). Samples of jugular venous plasma and cerebrospinal fluid (CSF) collected from the cisterna magna were analysed for CGRP concentrations using an enzyme immunoassay. In a hemisected rat skull preparation lined with dura mater the CGRP releasing effect of KCl superfusion was examined.

Superfusion of the dura mater with KCl decreased meningeal blood flow unless alpha-adrenoceptors were blocked by phentolamine, whereas the medullary blood flow was increased. The same treatment caused increased CGRP concentrations in jugular plasma and CSF and induced significant CGRP release in the hemisected rat skull preparation. Anaesthesia of the trigeminal ganglion by injection of lidocaine reduced increases in medullary blood flow and CGRP concentration in the CSF upon meningeal KCl application.

CGRP release evoked by depolarisation of meningeal afferents is accompanied by increased blood flow in the medulla oblongata but not the dura mater. This discrepancy can be explained by the smooth muscle depolarising effect of KCl and the activation of sympathetic vasoconstrictor mechanisms. The medullary blood flow response is most likely mediated by CGRP released from activated central terminals of trigeminal afferents. Increased blood supply of the medulla oblongata and CGRP release into the CSF may also occur in headaches accompanying vigorous activation of meningeal afferents.

1. Introduction

The cranial dura mater and cerebral arteries are innervated by unmyelinated and thinly myelinated afferent nerve fibers arising from the trigeminal ganglion (Andres et al., 1987; Steiger and Meakin, 1984). An exclusively nociceptive function is attributed to these afferents, based on classical intraoperative studies showing that stimulation of meningeal structures evokes no other sensation than headaches (Feindel et al., 1960; Ray and Wolff, 1940). A considerable proportion of meningeal afferents contains neuropeptides like calcitonin gene-related peptide (CGRP) (Keller and Marfurt, 1991; Messlinger et al., 1995; O'Connor and van der Kooy, 1988) and CGRP release is an established method to quantify meningeal afferent activation *in vitro* and *in vivo* (Bellamy et al., 2006; Ebersberger et al., 1999; Goadsby et al., 1988). CGRP is a potent vasodilator of intracranial arteries inducing increases in meningeal blood flow (Escott et al., 1995; Jansen-Olesen et al., 1996; Messlinger et al., 1995). Thus recordings of meningeal vasodilatation and blood flow have been used to examine the effects of chemical mediators presumably involved in meningeal nociception (Akerman et al., 2002; Dux et al., 2002; Escott et al., 1995) and to test substances designed for headache therapy (Messlinger et al., 1997; Petersen et al., 2005; Tröltzsch et al., 2007). CGRP can also be released from the trigeminal ganglion (Eberhardt et al., 2009) and from central terminals of trigeminal afferents within the spinal trigeminal nucleus (Amrutkar et al., 2011; Jansen-Olesen et al., 2014; Kageneck et al., 2014; Offenhauser et al., 2005), where it acts most likely as pro-nociceptive mediator (Meng et al., 2009; Storer et al., 2004).

Although CGRP has been attributed a key role in primary headaches for many years (Arulmani et al., 2004a; Edvinsson and Goadsby, 1994; Goadsby and Edvinsson, 1994; Lassen et al., 2002), its nociceptive

* Corresponding author at: Institute of Physiology and Pathophysiology, University of Erlangen-Nürnberg, Universitätsstr. 17, D-91054 Erlangen, Germany.
E-mail address: karl.messlinger@fau.de (K. Messlinger).

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function remains elusive. Increases in CGRP plasma levels have been found in acute phases of migraine and cluster headache (Bellamy et al., 2006; Goadsby et al., 1988, 1990) and also interictally in chronic migraine (Cernuda-Morollón et al., 2013). Inhibition of CGRP receptors is clearly therapeutic in migraine (Ho et al., 2008; Olesen et al., 2004) and inactivation of CGRP or CGRP receptors by four newly developed humanized monoclonal antibodies is promising in reducing the frequency of headache attacks in chronic and frequent migraine based on a variety of clinical phase-3 studies (Bigal et al., 2015; Dux and Messlinger, 2015; Giamberardino et al., 2016; Schuster and Rapoport, 2016).

The lack of understanding the role of CGRP in migraine pain may be partly due to our ignorance of its precise sites of release and action. While there is no doubt that CGRP is released when meningeal afferents are massively activated, it is unclear whether the bulk of CGRP found in plasma samples is released at peripheral or central sites in animal experiments (Goadsby et al., 1988) as well as in patients during headache attacks (Bellamy et al., 2006; Goadsby et al., 1990). CGRP released from central afferent terminals or from afferents innervating pial blood vessels can be expected to accumulate in the cerebrospinal fluid (CFS) rather than in the venous compartment due to the blood-brain barrier. Finally, blood flow increase should also occur in the medullary dorsal area, when central trigeminal afferents are activated. Release of CGRP during nociceptive processing may thereby support the blood supply of central structures for an increased metabolism. Using video imaging, our group has recently shown that local electrical stimulation of the rat cranial dura mater causes vasodilatation of pial arteries of the dorsal medulla (Will et al., 2016).

The preparation described here extends our established models of meningeal nociception which employed CGRP release from meningeal afferents in the hemisected rodent skull preparation (Ebersberger et al., 1999; Fischer and Messlinger, 2007; Gupta et al., 2010), trigeminal ganglion (Eberhardt et al., 2008) and brainstem (Amrutkar et al., 2011; Kageneck et al., 2014) and meningeal blood flow recordings in the exposed cranial dura mater in rats (Dux and Messlinger, 2001; Dux et al., 2003; Kurosawa et al., 1995). The aim of the present study was to measure CGRP concentrations in samples of jugular plasma and CSF of the cisterna cerebello-medullaris (cisterna magna) together with changes in medullary blood flow provoked by chemical depolarization of meningeal afferents as a rat model of meningeal nociception (Fig. 1A). We hypothesized that massive activation of meningeal afferents, as it is assumed to occur in severe headaches, causes release of CGRP into plasma and CSF and is accompanied by an increase in blood flow not only in the cranial dura mater but also in pial blood vessels of the medulla oblongata.

2. Methods

2.1. Experimental animals and surgery

All experimental procedures were carried out in accordance with the ethical issues of the International Association for the Study of Pain and in compliance with the guidelines for the welfare of experimental animals of the Federal Republic of Germany and the European Union (Council Directive 2010/63/EU). The experimental protocols were reviewed by an ethics committee and approved by the local District Government. Efforts were made to keep the number of animals as low as possible. Adult male Wistar rats weighing 300–400 g (10–14 weeks old) were used. The animals were anaesthetized with 5% isoflurane (Forene, Abbott, Wiesbaden, Germany) inhaled in a closed box, continued by application of 2% isoflurane through a tight mask. In most experiments for plasma CGRP measurements (see below) animals were initially anaesthetized with intraperitoneal (i.p.) application of 120–150 mg/kg thiopentone (Trapanal®, Altana, Konstanz, Germany). Femoral artery and vein were cannulated for recording of blood pressure and intravenous (i.v.) infusion of substances. In animals designated for

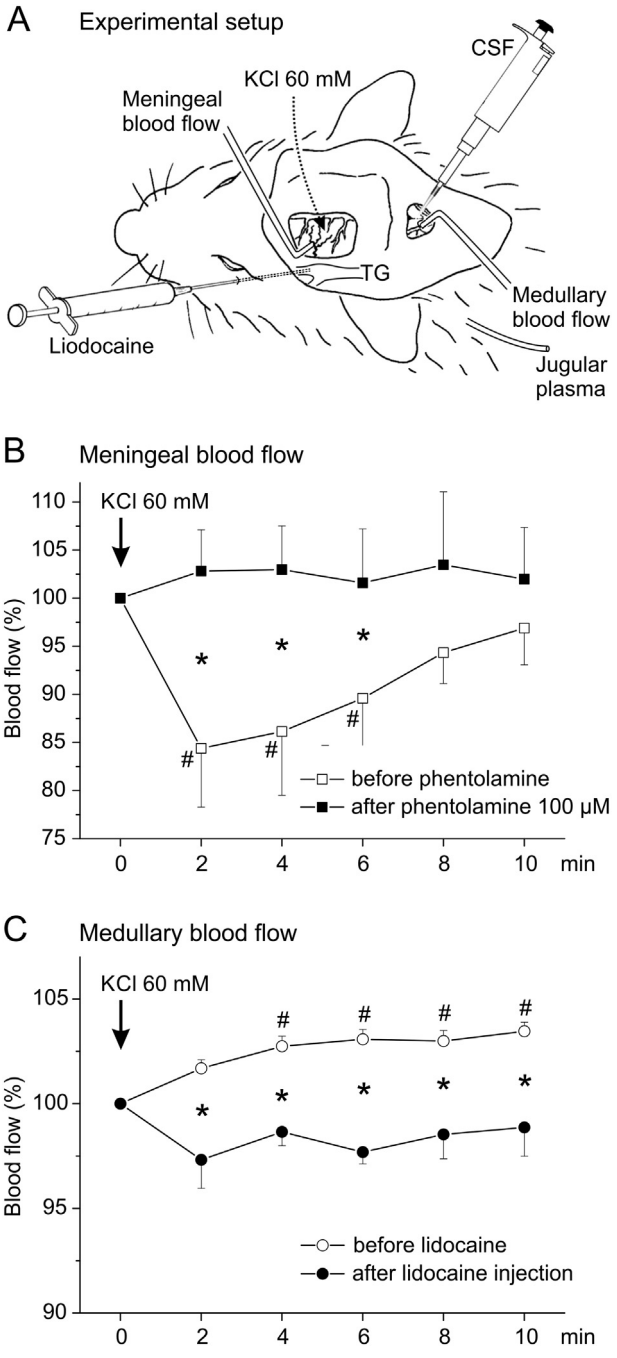

Fig. 1. Experimental setup (A) and changes in meningeal and medullary blood flow (normalized to baseline) during topical application of potassium chloride (KCl 60 mM) onto the dura mater (B, C). A: Laser Doppler L-type needle probes were positioned upon arterial vessels in the exposed dura mater and the medulla oblongata. Collection of cerebrospinal fluid (CSF) from the surface of the medulla and of jugular venous blood was combined with flow measurements and with injection of lidocaine into the trigeminal ganglion (TG) in some series of experiments. B: Response of dural blood vessels to KCl before and after phentolamine hydrochloride (100 µM) measured at intervals of 2 min; # different to baseline (ANOVA), * different to the effect of KCl prior to blocking alpha-adrenoceptors (*t*-test; n = 10). C: Effect of trigeminal ganglion anaesthesia on medullary blood flow induced by KCl (60 mM); # different to baseline (ANOVA),* different to the effect of KCl prior to ganglion anaesthesia (*t*-test; n = 10).

venous blood sampling, a plastic catheter was introduced in cranial direction into the jugular vein of the side ipsilateral to the trepanation (see below) and flushed with saline containing 10 U/ml heparin (Ratiopharm, Ulm, Germany). The opening of the catheter was placed close to the bifurcation of the jugular vein. The animals were tracheotomized and artificially ventilated with a mixture of oxygen-

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