Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Research Report

Predictive validity of endpoints used in electrophysiological modelling of migraine in the trigeminovascular system

Bence Farkas, Péter Kardos, Szabolcs Orosz, István Tarnawa, Csongor Csekő, György Lévay, Sándor Farkas, Balázs Lendvai, Péter Kovács*

Pharmacology and Drug Safety Research, Gedeon Richter Plc, Gyömrői út 19–21, 1103 Budapest, Hungary

ARTICLE INFO

Article history:
Accepted 28 August 2015
Available online 7 September 2015

Keywords:
Headache
Pain
Rat model
Inflammatory soup
Sensitization

ABSTRACT

The trigeminovascular system has a pivotal role in the pathomechanism of migraine. The aim of the present study was to further develop existing models of migraine making them more suitable for testing the effects of compounds with presumed antimigraine activity in anaesthetised rats. Simultaneous recording of ongoing activity of spontaneously active neurons in the trigeminocervical complex as well as their discharges evoked by electrical stimulation of the dura mater via activation of A- and C-sensory fibres were carried out. Effects of sumatriptan, propranolol and topiramate were evaluated prior to and after application of a mixture containing inflammatory mediators on the dura. Propranolol (10 mg/kg s.c) and topiramate (30 mg/kg s.c.) resulted in a tendency to decrease the level of both spontaneous and evoked activity, while sumatriptan (1 mg/kg s.c.) did not exhibit any effect on recorded parameters. Application of an inflammatory soup to the dura mater boosted up spontaneous activity, which could be significantly attenuated by propranolol and topiramate but not by sumatriptan. In addition, all compounds prevented the delayed increase of spontaneous firing. In contrast to the ongoing activity, evoked responses were not augmented by inflammatory mediators. Nevertheless, inhibitory effect of propranolol and topiramate was evident when considering A- or C-fibre responses. Findings do not support the view that electrically evoked responses are useful for the measurement of trigeminal sensitization. It is proposed however, that inhibition of enhanced firing (immediate and/or delayed) evoked by inflammatory mediators as an endpoint have higher predictive validity regarding the clinical effectiveness of compounds.

1. Introduction

Activation and/or sensitization of the trigeminovascular system (TGVS) as fundamental events in the pathomechanism of migraine are suggested by many reports based on either theoretical considerations (Tajti et al., 2011) or on data obtained in animal experiments (Burstein et al., 1998; Strassman et al., 1996) or human neuroimaging studies

Abbreviations: IS, inflammatory soup; s.c., subcutanenous; TCC, trigeminocervical complex; TGVS, trigeminovascular system

*Corresponding author. Tel.: 36 1 505 7250; fax: 36 1 889 8400.
E-mail address: b.farkas@richter.hu (B. Farkas).

http://dx.doi.org/10.1016/j.brainres.2015.08.039
0006-8993/

(Borsook et al., 2006; Stankewitz et al., 2011). Furthermore, some of the established acute medications (Burstein and Jakubowski, 2004; Cumberbatch et al., 1997, 1998) as well as preventives (Andreou and Goadsby, 2011; Shields and Goadsby, 2005, 2013) are suggested to exert their antimigraine action, at least in part, via modulation of the trigeminovascular transmission measured as influenced activity of the trigeminocervical complex (TCC) (Goadsby and Hoskin, 1996). Due to several mechanistic and phenomenological similarities to the human condition, modelling approaches based on triggering activation or sensitization of neuronal elements in the TGVS by intense chemical stimulation of the dura mater with a mixture of irritative agents, frequently called an inflammatory soup (IS) are regarded to have a high translational value by mimicking the changes in key sensory modalities associated with the disease.

Investigation of the trigeminovascular functioning at the level of the second-order neurons by electrophysiological techniques carries the advantage of direct, real-time monitoring of neuronal responses and the possibility of recording straightforward effects in response to a systemic or local pharmacological intervention. Numerous studies on monitoring of ongoing or evoked firing activity of selected units in response to various kinds of meningeal or cephalic cutaneous sensory stimulation (i.e. electrical, mechanical, thermal or chemical) in anaesthetized animals have been published (Table 1). The most relevant pearls and pitfalls of measuring migraine related changes in the TCC by the means of electrophysiology have been summarized recently (Akerman et al., 2013). Most papers published so far, however, did not investigate drug effects on spontaneous activity as well as on evoked responses in parallel. Furthermore, none of the few studies exploring both ongoing and triggered activity have attempted to differentiate between the effect of drugs on the TGVS in non-sensitized and sensitized states (see Table 1). It is to be noted that most studies applied single-unit recording from neurons with well defined cutaneous receptive field. However, baseline activity and reactivity of such individual cells could be quite diverse.

Our aim was to develop an electrophysiological model, which can combine the advantages of former investigations and can be routinely applicable to test the effects of compounds with presumed antimigraine activity at the level of the second-order sensory neurons in the rat TCC. The present study focused on analyzing the utility of three different parameters, namely spontaneous activity, A- and C-fibre responses evoked by electrical stimulation. We used oligo-/multi-unit recording from cell clusters, which provides a kind of intrasubject averaging of activity of individual cells, and applied certain inclusion criteria with regards to baseline activity. In addition, we tested how the firing of trigeminal input sensitive TCC neuronal clusters responds immediately and more than an hour later to application of IS. As we performed long-term recordings and applied both drug and IS treatments, we introduced several control groups in order to be able to dissect changes induced by either the treatment or the lapse of time. Cardiovascular parameters were routinely checked for exclusion of secondary changes.

2. Results

2.1. Neuronal properties

The recording site was considered acceptable if it exhibited spontaneous firing activity *per se* and remarkable enhancement of the firing rate upon both dural and facial stimulation. Sites were located in the dorsal horn of the C1, 1000–2500 μm caudal to the obex, down to a depth of 600–1200 μm below the surface and of 1200–1800 μm lateral to the midline of the spinal cord. The overall mean firing rate recorded in the baseline period was 23.7±3.7 Hz, and the group means ranged from 18.8±2.6 Hz to 25.9±3.9 Hz (Table 2). Electrical stimulation of dural afferents evoked immediate neuronal firing; the first spikes followed the stimulation artefact with short (i.e. 4–5 ms) latency (Fig. 5B). The mean number of A-fibre mediated discharges detected in the post-stimulus time window of 4–20 ms was 8.5±0.9, while 8.4±1.1 spikes appeared in the C-fibre related time window of 20–100 ms.

2.2. Spontaneous activity

The frequency of spontaneous activity was constant (exhibited less than 10% change in average) during the baseline period in all groups. Spontaneous firing was also not significantly affected by s.c. injections, though tendencies toward an increase (in saline-injected groups) or decrease (in groups injected with active substances) were observed during the 'pre-IS' period compared to 'baseline' (Fig. 1). This means that from the beginning of the treatment period (zero min) till the last time point before dural IS challenge (30 min) the number of spontaneous discharges increased by 25±10%, 17±12% and 35±9% in those subjected to s.c. saline injection (groups: saline+buffer and saline+IS #1 / #2, respectively). On the contrary, the same parameters were −6±9%, −23±6% and −11±12% for groups treated with sumatriptan, propranolol and topiramate, respectively. In the absolute control group (without s.c. injection) this change in the ongoing activity for the same interval was 14±11%.

2.2.1. Immediate increase in firing rate evoked by IS

Application of the inflammatory soup to the dura mater resulted in rapid and marked enhancement of the continuous firing of TCC neurons ($F_{3,27}=8.41$, $P=0.00042$; $F_{3,27}=13.7$, $P=0.000012$ for saline+IS #1 and #2, respectively). The firing rate peaked at 5 to 10 min after initiation of the IS exposure and reached 239±36% ($P<0.001$) and 260±22% ($P<0.001$) of the baseline firing rate in the saline+IS #1 and saline+IS #2 groups, respectively. Then the firing rate started to decline still during the IS exposure. However, the enhanced firing rate remained sustained in both saline+IS groups for more than an hour after the removal of IS exposure, i.e. till the end of observation. Although we did not apply instrumental spike sorting, our impression from observation of spikes with different amplitudes was that the increases were attributable to both increased firing rate of active neurons as well as firing of previously silent neurons. Considering the average net augmentations (delta values) 95±24% and 81±16% were calculated for the 'early-IS' period compared to the 'pre-IS'