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Effect of two novel CGRP-binding compounds in a closed cranial window rat model

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Abstract

We investigated the *in vivo* effects of two novel calcitonin gene-related peptide (CGRP) binding molecules in the genuine closed cranial window model in the rat. The RNA-Spiegelmer (NOX-C89) and the monoclonal CGRP antibody are CGRP scavengers and might be used as an alternative to CGRP-receptor antagonists in the treatment of migraine. Rats were anaesthetized and a closed cranial window established. Changes in dural and pial artery diameter and mean arterial blood pressure were measured simultaneously. Infusion of the RNA-Spiegelmer or the CGRP antibody alone had no effect on the arteries or the mean arterial blood pressure. We then used a bolus of 0.3 μg/kg CGRP (n=6) or electrical stimulation (25 V, 5 Hz, 1 ms pulse width and of 10 s of duration) (n=6) to induce dilatation of dural and pial arteries (mediated via CGRP-receptors). Pre-treatment with the RNA-Spiegelmer inhibited CGRP-induced vasodilatation of the dural artery (from 38±17% to 7±3%) and the pial artery (from 14±1% to 3±2%) ($P<0.05$). The RNA-Spiegelmer, however, did not significantly inhibit dilatation induced by electrical stimulation ($P>0.05$). The CGRP antibody caused a significant reduction of the dural artery diameter caused by intravenous CGRP-infusion (from 23±5% to 12±3%) ($P<0.05$), but did not inhibit dilatation caused by electrical stimulation ($P>0.05$). In conclusion, the CGRP scavengers effectively inhibited the effect of circulating CGRP but do not modify the effect of electrical stimulation and the consequent liberation of CGRP from perivascular sensory nerve fibres.

Keywords: Migraine; CGRP; Electrical stimulation; Middle cerebral artery; Middle meningeal artery; CGRP antibody; RNA-Spiegelmer

1. Introduction

Noxious stimulation of dural and cerebral arteries is known to elicit migraine pain (Ray, 1940). Calcitonin gene-related peptide (CGRP) is the most abundant peptide in trigeminal perivascular nerve fibres that innervate various pain sensitive intracranial structures (Edvinsson et al., 1998; Uddman and Edvinsson, 1989). Stimulation of sensory nerve endings causes a release of CGRP and marked dilatation of cerebral (pial) and meningeal arteries (dural) (Edvinsson et al., 1987; Jansen-Olesen et al., 2003; Williamson et al., 1997a). The levels of CGRP in external jugular venous have been shown to be increased in patients with serious and prolonged migraine attacks (Goadsby et al., 1990) while in normal migraine patients the CGRP levels were unaltered during a migraine attack as compared to outside an attack (Tvedskov et al., 2005). Intravenously CGRP provokes headache attacks (Lassen et al., 2002). The CGRP antagonist BIBN4096BS is effective in treating acute attacks of migraine (Olesen et al., 2004). CGRP therefore plays a major role in migraine pathophysiology.

Two potential migraine therapeutic agents capable of tightly and specifically binding CGRP have been developed; a CGRP-binding RNA-Spiegelmer (NOX-C89) and a CGRP monoclonal antibody (Edvinsson et al., 2007). The NOX-C89 is a single-stranded mirror-image L-oligonucleotide (Vater et al., 2003), which recognises approximately 13 amino acids at the N-terminal part of CGRP (Denekas et al., 2006). Due to the presence of L-ribose, the NOX-C89 is highly resistant to degradation by endogenous nucleases and is able to inhibit the function of the target peptide (Helmling et al., 2004; Vater and Klussmann,

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2003). When PEGylated (addition of 40 kDa polyethylene glycol), NOX-C89 has a half-life of >12 h which is due to a reduced glomerular filtration rate that is inversely proportional to the size of a molecule up to ~50 kDa. Moreover, the increased half-life entails that the resistance of the Spiegelmer to enzymatic degradation becomes more prominent (Vater and Klussmann, 2003).

In addition, a humanized monoclonal CGRP-binding antibody has been raised in rabbit for the use in CGRP-related disorders such as migraine (Edvinsson et al., 2007). In the present study, the RNA-Spiegelmer (NOX-C89) and the CGRP antibody were examined in the genuine closed cranial window model (Petersen et al., 2005a; Williamson et al., 1997a) to investigate whether they were able to inhibit CGRP-induced vasodilatation and dilatation evoked by electrical stimulation. This model allows visualization of both dural and pial arteries and measurements of mean arterial blood pressure simultaneously in the same animal (Petersen et al., 2005a).

2. Methods

2.1. Surgical preparation

All experiments have been performed in accordance with the guidelines and regulations of the Danish Animal Experimentation Inspectorate (file: 2001/561–390) on the care and use of animals. The rats used in the experiments were maintained in cages with a 12-h light/dark cycle and free access to food and water. Due to hormonal variations in females, adult male Sprague-Dawley rats (n=12) weighting 350–400 g were used. They were anesthetized with pentobarbital (Mebumal® 50 mg/kg) intraperitonal and depth of anaesthesia was checked by suppression of the hindpaw withdrawal reflex. Anaesthesia was continuously supplemented with 20 mg/kg/min pentobarbital intravenously during the experiment. The body temperature was maintained at 37±0.5 °C throughout the experiment using an automatically regulated heating blanket system (Letica® Scientific Instruments HB101, Spain). Following anaesthesia the trachea was intubated and the animal was mechanically ventilated by a respirator (Abovent, Ago Basil, Italy) with 30/70% air mixture of O_2/ N_2O, a stroke volume of 3–3.5 ml and a stroke rate of 60–70 per min. Catheters (Portex®, Fine Bore Polythene Tubing, inner diameter 0.4 mm and outer diameter 0.8 mm, Astratech, Denmark) were placed in the left and right femoral artery for continuous measurement of mean arterial blood pressure and sampling of arterial blood for gas tension analysis. In addition, catheters were placed in the left and right vein for continuous infusion of anaesthetic and infusion of the test substance. Mean arterial blood pressure was registered by connecting the catheter to a transducer (Transducer TCM4–7, World Precision Instruments, U.S.A.) and was in the range of 81.2 mmHg to 118.7 mmHg.

2.2. Preparation of the closed cranial window and electrical stimulation

The animal was placed in a stereotactic frame. Skin covering the dorsal surface of the skull was retracted and the connective tissue and muscle removed, leaving the left parietal bone exposed. The bone was thinned, making a window (10×7 mm^2) by carefully drilling with a dental drill cooled by application of ice-cold isotonic saline. Drilling was continued until the middle meningeal artery and a pial artery were clearly visible through the intact skull. In order to induce neurogenically dilatation of the investigated arteries, a bipolar stimulation electrode (50 mm NE 200X, Harvard Apparatus, U.K.) was placed above the cortical surface of the cranial window, approximately 200 μm from the arteries. Electrical stimulation was carried out with 25 V, 5 Hz, 1 ms pulse width and of 10 s of duration (Grass Stimulator S48, Grass Instrumentation, MA, U.S.A.) (Williamson et al., 1997b). At last the cranial window was covered with mineral oil (37 °C) to avoid drying and optimise visibility (Petersen et al., 2005a). After preparation of the closed cranial window a stabilisation period of at least 1 h followed before initiating the experiment.

2.3. Video microscopy

For visualisation of the middle meningeal artery and pial artery, a video-microscope was positioned above the window. The video camera was connected to a video dimension analyser (V94, Living Systems Instrumentation©, U.S.A.), which continuously measured the diameter of the arteries. Baseline dural and pial artery diameter was in the range of 49.1–65.4 μm and 98.1–121.1 μm, respectively. During the entire experiment, changes in dimensions arising from vessel constriction or dilatation were automatically followed by rapid time resolution and displayed on a digital panel. Two scan lines perpendicular to each artery allowed measurements of both arteries at the same time in one animal. Connection of the analyser to a television monitor showed a real time image of both the middle meningeal artery and pial artery displayed on the screen. Mean arterial blood pressure was measured continuously and in parallel with measurements of pial and dural diameter in the same animal. In addition, the model allows measurement of local cerebral blood flow flux, but due to the experimental set-up which includes electrical stimulation, local cerebral blood flow flux measurement was not possible.

All data including the changes in diameter of the arteries and mean arterial blood pressure were continuously and simultaneously collected and analysed by Perisoft® (version 1; Perimed AB, Sweden). After the experiment the animal was killed by an overdose of pentobarbital.

2.4. Data treatment and statistical analysis

The effectiveness of the test substances was based on measurements of three parameters: The changes in the diameter of the dural and pial artery and changes in mean arterial blood pressure. Due to the experimental set-up it was impractical to standardise the dural and pial vessel measurement; therefore, the vessel diameter was measured in arbitrary units. Mean arterial blood pressure was given in mmHg.

Dilatation of the vessels and changes in mean arterial blood pressure were calculated as percentage change from the baseline,