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# Effect of cyclooxygenase-2 on the regulation of cerebral blood flow during neuronal activation in the rat

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ABSTRACT

The present study was designed to clarify the precision of the main approach for investigating the regulation of local cerebral blood flow (CBF) response to neuronal activation in the brain (neurovascular coupling). In this study, we examined the effects of NS-398, a highly selective cyclooxygenase-2 inhibitor, on the physiological variables, baseline CBF, and local CBF response during rat somatosensory neuronal activation by laser-Doppler flowmetry. Blood pressure and heart rate were significantly decreased 3 h after i.v. infusion of NS-398. Baseline CBF and local CBF during somatosensory activation gradually decreased with an increase in time of NS-398 infusion up to 3 h, although neuronal activity in the somatosensory area was almost constant during the infusion. The results suggest that cyclooxygenase-2 participates in the regulation of local CBF during neuronal activation in rats. The present study also revealed the potential side-effects of dimethylsulfoxide, a solvent of NS-398, on neurovascular coupling.



## 1. Introduction

The tight spatial and temporal coupling of neuronal activity with local cerebral blood flow (CBF), which is known as neurovascular coupling, is a hallmark of brain function (Magistretti et al., 1999; Iadecola, 2004). Functional hyperemia occurs within seconds of neuronal activation and is spatially coupled to brain areas with increased energy demand (Chaigneau et al., 2003; Lauritzen, 2005). Any damage to the neurovascular coupling mechanism is accompanied by disturbance of brain cell homeostasis and provokes brain pathology and cell death. Clarifying the key molecular targets underlying the regulation of neurovascular coupling and controlling them could present a consequential way of increasing the life of brain cells, overcoming neurodegenerative diseases, and delaying the process of senescence.

The commonly accepted hypothesis is that local vasodilation in response to neuronal activity depends on the activation of astrocytes by locally released neurotransmitters. Many biochemical substances mimic the role of triggers and key mediators of neurovascular coupling. However, the data are usually controversial, the major reason being that investigations of this life science phenomenon are based on indirect approaches. At least three biochemical pathways of astrocyte-mediated vasodilation act like triggers (Bakalova et al., 2002; Ances, 2004; Girouard and Iadecola, 2006; Phillis et al., 2006; Takano et al., 2006; Wang et al., 2006): (i) arachidonic acid cascade; (ii) nitric oxide cascade; (iii) adenosine-mediated pathway. In addition, other substances such as $Ca^{2+}$, $H^{+}$, carbon dioxide, and carbon oxide are also related to the vasodilation pathway.

In the present study, we focused on the products of the arachidonic acid cascade as one of the critical triggers or secondary regulators of neurovascular coupling in the brain. Our reason for this was based mainly on the unique lipid composition of rat brain tissue. Approximately 90% of rat brain cell membranes of synapses and astrocytes consist only of polyunsaturated lipids, composed of arachidonic acid, docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) residues (Kagan et al., 1992; Chalon et al., 2001; Yehuda et al., 2002; Solfrizzi et al., 2005). There are no other animal tissues with such lipid composition. Arachidonic acid is a precursor of prostaglandins, DHA of docosanoids, and EPA of eicosanoids. It is well known that all neurodegenerative diseases are accompanied by a significant decrease of these polyunsaturated fatty acids (PUFAs) in the brain, as well as by an imbalance in their metabolites (Kagan et al., 1992; Yehuda et al., 2002; Solfrizzi et al., 2005). Therefore, the metabolites of PUFAs could be selected as priority molecular targets in investigations of the regulatory mechanisms of neurovascular coupling. However, it is necessary to note that the reason for the unique lipid composition of brain

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membranes could be simpler than the reasons mentioned above. Neurotransmission requires a permanent dynamic of brain biomembranes, which are obligatory for continuous transport of neurotransmitters and neuromediators. Thus, brain membranes have to be flexible and permeable, which is ensured through their polyunsaturated lipid structure.

The aim of the present study is to affirm the role of the arachidonic acid cascade in the local CBF response to neuronal activity (neurovascular coupling) and to clarify the precise mechanism of this regulation. In this study, we examined the effects of NS-398, a highly selective cyclooxygenase-2 inhibitor (an inhibitor of prostaglandin synthesis), on CBF regulation during somatosensory neuronal activation in rats by laser-Doppler flowmetry. The present study also revealed the potential side-effects of dimethylsulfoxide (DMSO), a solvent of NS-398, on neurovascular coupling.

## 2. Materials and methods

### 2.1. Animal preparation

All experiments were conducted in accordance with the guidelines of the Physiological Society of Japan and were approved by the Animal Care and Use Committee of the National Institute of Radiological Sciences, Chiba, Japan.

Twenty-two Sprague–Dawley male rats (385.4 ± 3.2 g) were used to investigate the effect of NS-398 (selective inhibitor of cyclooxygenase-2 isoenzyme, COX-2) and DMSO (a solvent of NS-398) on the CBF response. The NS-398-treated group consisted of thirteen rats, and the DMSO-treated group, used as a control, consisted of nine rats. The rats were anesthetized with isoflurane (4.0% for induction and 1.5% during surgery) in 15% $O_2$ and 85% air using a face mask. Subcutaneous 2% lidocaine was used before incision to prevent vasospasm during catheter insertion. Polyethylene catheters were used to cannulate the tail artery and the left femoral vein for blood pressure monitoring, blood sampling for gas analysis, and i.v. administration of anesthetic and NS-398 (or DMSO). After tracheotomy, α-chloralose (50 mg/kg, i.v.) was injected, and isoflurane administration was discontinued. Anesthesia was maintained with α-chloralose (45 mg/(kg h), i.v.) and muscle relaxation with pancronium bromide (0.7 mg/(kg h), i.v.). Body temperature was monitored with a rectal probe and maintained at about 37.0 °C using a heating pad (ATC-210, Unique Medical, Japan). During the experiment, the rats were ventilated by respirator (SN-480-n, Shinano, Japan) with a mixture of air and oxygen to achieve physiological arterial blood levels of $PaO_2$ and $PaCO_2$ ($PaCO_2$ of 33–40 mmHg and $PaO_2$ of 110–130 mmHg) by regulating the stroke volume of ventilation and the fractional concentration of oxygen in the inspired gas, respectively.

Each rat was fixed in a stereotactic frame, and the parietal bone was thinned to translucency over the left somatosensory cortex using a dental drill (an area of 3 mm × 3 mm, centered at 2.5 mm caudal and 2.5 mm lateral to the bregma). To ensure a stable physiological condition of the animal, the measurements were performed for 3 h after preparation of the parietal bone. The depth of anesthesia was controlled by continuous monitoring of mean arterial blood pressure (MABP) and heart rate during the stabilization period (Fig. 1). The rate of α-chloralose infusion was constant during the measurements after a 3-h adaptation period.

### 2.2. LDF measurement and hind paw stimulation

The increase in local CBF response during hind paw stimulation (evoked CBF) was measured by LDF (FLO-C1, OMEGA FLO, Japan). The tip diameter of the LDF probe was 0.55 mm (Probe NS, OMEGA FLO). LDF was used to measure red blood cell behavior in capillaries based on the Doppler-effect with laser light (wavelength of 780 nm). The frequency-shift of scattered radiation was caused by the movement of red blood cells in the blood vessels. The sampling volume of LDF measurement was about 1 $mm^3$ (Solfrizzi et al., 2005). A time constant of 0.1 s was used to detect the LDF signal.

The LDF probe was positioned over the thinned skull (over the somatosensory area of the hind paw) perpendicular to the brain surface. It was attached to the thinned parietal bone and then finely positioned using a micromanipulator to obtain the

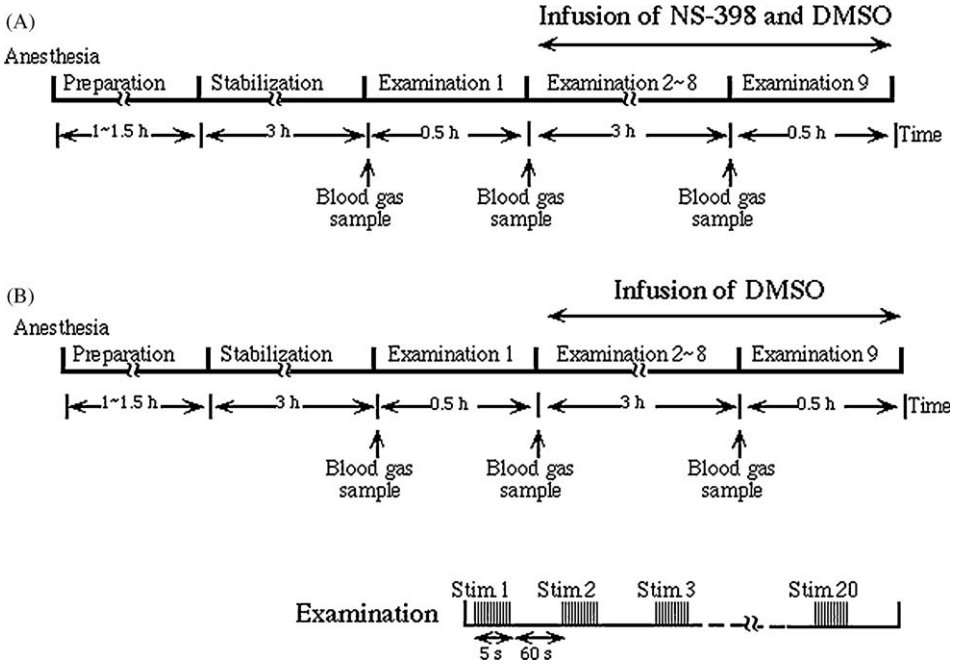


**Fig. 1.** Experimental protocol of i.v. administration of NS-398 dissolved in DMSO (A) and that of only DMSO (B). The experiment was started about 3 h after the preparation of the animal. The evoked CBF response was examined before and after drug administration. At each examination, 20 successive pulses of 5 Hz (5-s duration, 1.5 mA) were applied at 60 s intervals.