The Cyclooxygenase Inhibitors Indomethacin and Rofecoxib Reduce Regional Cerebral Blood Flow Evoked by Somatosensory Stimulation in Rats

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The present study was designed to investigate whether administration of indomethacin (IMC), a non-selective cyclooxygenase (COX-1 and COX-2) inhibitor, and Rofecoxib, a highly selective COX-2 inhibitor, affect the regulation of regional cerebral blood flow response evoked by somatosensory activation (evoked rCBF). IMC and Rofecoxib were applied intravenously (6.25 and 3 mg/kg/hr, respectively). Somatosensory activation was induced by electrical hind paw stimuli of 0.2, 1, and 5 Hz (5-sec duration, 1.5 mA). The evoked rCBF was measured in α-chloralose anesthetized rats using laser-Doppler flowmetry. Before and after drug application, the evoked rCBF showed a frequency-dependent increase in the range of 0.2–5 Hz stimulation. IMC reduced significantly (about 50%–60%) evoked rCBF in response to all frequencies of hind paw stimulation ($P < 0.05$). Rofecoxib reduced significantly (about 50%) evoked rCBF in response to 1 and 5 Hz stimulation ($P < 0.05$), but did not affect evoked rCBF at 0.2 Hz. After IMC or Rofecoxib application, the normalized evoked rCBF curves peaked earlier as compared with that before their application ($P < 0.05$), although the rise time of 0.5 sec was nearly constant regardless of the stimulus frequency. The termination time of evoked rCBF curves was changed significantly after IMC application at 0.2 Hz stimulation ($P < 0.05$), but was not affected after Rofecoxib application. Neither COX inhibitor significantly affected the baseline level of CBF. The results suggest a participation of COX products in the regulation of evoked rCBF in response to somatosensory stimulation in the brain. [Exp Biol Med Vol. 227(7):465–473, 2002]

Key words: cerebral blood flow; hind paw stimulation; somatosensory cortex; Indomethacin; Rofecoxib (MK-0966); laser-Doppler flowmetry

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The synthesis of cyclooxygenase (COX) products (prostanoids) has usually been observed in brain and/or its vasculature under pathological conditions, presumably those that are associated with activation of phospholipase A_2 and accumulation of free arachidonate (1–5). Marked increases in brain prostanoid levels are found in various intracranial afflictions, including epilepsy, craniotomy, edema formation, subarachnoid hemorrhage, and meningitis (1–9). COX products are well known factors in thrombosis (1–3, 9). However, less is known about their physiological role.

The demonstration that COX inhibitors reduce cerebral blood flow (CBF) at rest condition, and some of them markedly suppress the circulatory response to hypercapnia, suggests that prostanoid-like substances may be important modulators of cerebrovascular resistance (1–3, 10–14). It is hypothesized that the vasodilative effect of prostanoids is manifested through increasing the formation of cAMP, which relaxes smooth muscle by modulation of Ca^{2+}- and ATP-dependent K^+ channels and activation of protein kinase G (1–3, 12). However, because the COX inhibitors have no effect on the circulatory response to hypoxia, as well as some of them tend to vasodilate the blood vessels *in vitro* (1–3, 12, 14), it is assumed that prostanoids cannot be generally responsible for vascular dilation in the brain.

It was found that prostanoids are also produced by activated neurons, astrocytes, and, probably, by perivascular nerves (1, 2, 15–17). Nevertheless, their role in the coupling between neuronal activity and rCBF is obscure and less investigated. Obviously, the exact mechanism(s) of CBF modulation by COX products are very complex and remain to be clarified.

In the early 1990s, COX was demonstrated to exist as two distinct isoforms. COX-1 is constitutively expressed as a "housekeeping" enzyme in most tissues. In contrast, COX-2 can be upregulated by various agents, including cytokines and growth factors, and is expressed in many brain disorders (18). Recently, it was demonstrated that

COX-2 is induced in the brain under basal conditions as well as under neuronal activity (19), suggesting that this isoenzyme and its products may play a more complex physiological role in the brain than was expected.

The present study was designed to investigate whether COX products play a physiologically relevant role in the regulation of the rCBF response to graded neuronal stimulation. Using laser-Doppler flowmetry (LDF), we estimated the effect of Indomethacin (IMC), a well-known nonselective COX-1 and COX-2 inhibitor (1–3), and Rofecoxib, a highly selective COX-2 inhibitor (18), on the rCBF response in somatosensory cortex during electrical hind paw stimulation.

Materials and Methods

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 Research Institute for Brain and Blood Vessels (Akita, Japan).

Eleven Sprague-Dawley male rats (387.3 ± 20.5 g, mean ± SD) and eight rats (396.4 ± 22.8 g, mean ± SD) were used to investigate the effect of IMC and Rofecoxib, respectively, on the evoked rCBF in response to hind paw stimulation. The rats were anesthetized with halothane (3% for induction and 1% during surgery) in 30% O_2 and 70% N_2O, using a face mask. Subcutaneous 2% lidocaine was used before the incision to prevent vasospasm during catheter insertion. Polyethylene catheters were used to cannulate the tail artery and the left femoral vein was used for blood pressure monitoring, blood sampling for gas analysis, and i.v. administration of anesthetic and COX inhibitor. After tracheotomy, α-chloralose (56 mg/kg, i.v.) was administered, and halothane and nitrous oxide administration was discontinued. Anesthesia was maintained with α-chloralose (44 mg/kg/hr, i.v.) and muscle relaxation was maintained with pancronium bromide (0.7 mg/kg/hr, i.v.). The body temperature was monitored with a rectal probe and maintained at about 37°C using a heating pad (MK-900; Muromachi Kikai Co., Japan).

The rat was ventilated by respirator (M-683; Harvard Apparatus, Holliston, MA) throughout the experimental period with a mixture of air and oxygen to achieve physiological arterial blood levels of O_2 and CO_2 tension (PaO_2 and $PaCO_2$, respectively). $PaCO_2$ levels were maintained in the range of 33 to 40 mmHg and PaO_2 levels were maintained in the range of 110 to 130 mmHg by regulating the stroke volume of ventilation and the fractional concentration of oxygen in the gas inspired, respectively.

The 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 × 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, measurements were performed 3 hr after the preparation of the parietal bone. The depth of anesthesia was controlled by continuous monitoring of mean arterial blood pressure (MABP) and heart rate. The rate of α-chloralose infusion was constant during all measurements after a 3-hr adapting period (Figs. 1C and 2C).

LDF Measurement and Hind Paw Stimulation. Changes in evoked rCBF were measured by LDF (TDF-LN1; Unique Medical, Tokyo, Japan). The tip diameter of the LDF probe was 0.55 mm (Probe LP-N; Unique Medical). LDF measures red blood cell behavior in the capillaries based on the Doppler effect with laser light (wavelength of 780 nm). The frequency shift of the scattered radiation is caused by moving red blood cells in the blood vessels. The sampling volume of LDF measurement was about 1 mm^3 (20). A time constant of 0.1 sec 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 maximum signal change during stimulation (15%–20% at frequency of 5 Hz, currency of 1.5 mA, duration of 5 sec), avoiding areas with large blood vessels.

Electrical hind paw stimulation was performed with two needle electrodes inserted subdermally into the right hind paw (at the planar and ankle regions, respectively) contralateral to the LDF probe. For the analysis of frequency dependence, in all rats, a current stimulus of 1.5 mA (0.1-msec pulse) was applied at a frequency of 0.2, 1, and 5 Hz with a duration of 5 sec. The order of stimulus frequencies was selected randomly; at each stimulus frequency, 20 successive pulses were applied at 60-sec intervals.

The choice of stimulation parameters was based on the previously published data. It has been reported in hemodynamic studies on rats that an increase in stimulus frequency up to approximately 5 Hz caused a linear increase in the evoked rCBF, although its further increase (above 5 Hz) led to a decrease in rCBF-response (21–24). It is well known that in the evoked rCBF-response curves, during long periods of stimulation, there is an initial peak followed by a plateau (23, 24). It is assumed that the initial peak reflects an early transient reaction to neuronal activity. In the present study, we investigated the relationship between the evoked rCBF and graded stimulus frequencies of short duration, which disrupts the biphasic response, leaving only the early transient reaction of the evoked rCBF (21, 22).

IMC and Rofecoxib Application. IMC (Sigma Chemical Co., St. Louis, MO) was first dissolved in a 50-μl sodium bicarbonate solution ($NaHCO_3$, 0.13 g/100 ml, pH 7.4) and was added to 950 μl of saline solution. It has been demonstrated that at this concentration, the solvent has no effect on CBF, cerebral oxygen consumption, local cerebral glucose utilization, arterial blood gases, MABP, or body temperature (1).

IMC was applied intravenously, a 0.5-ml single injection of 5 mg/kg body wt.; 30 min after that, infusion with

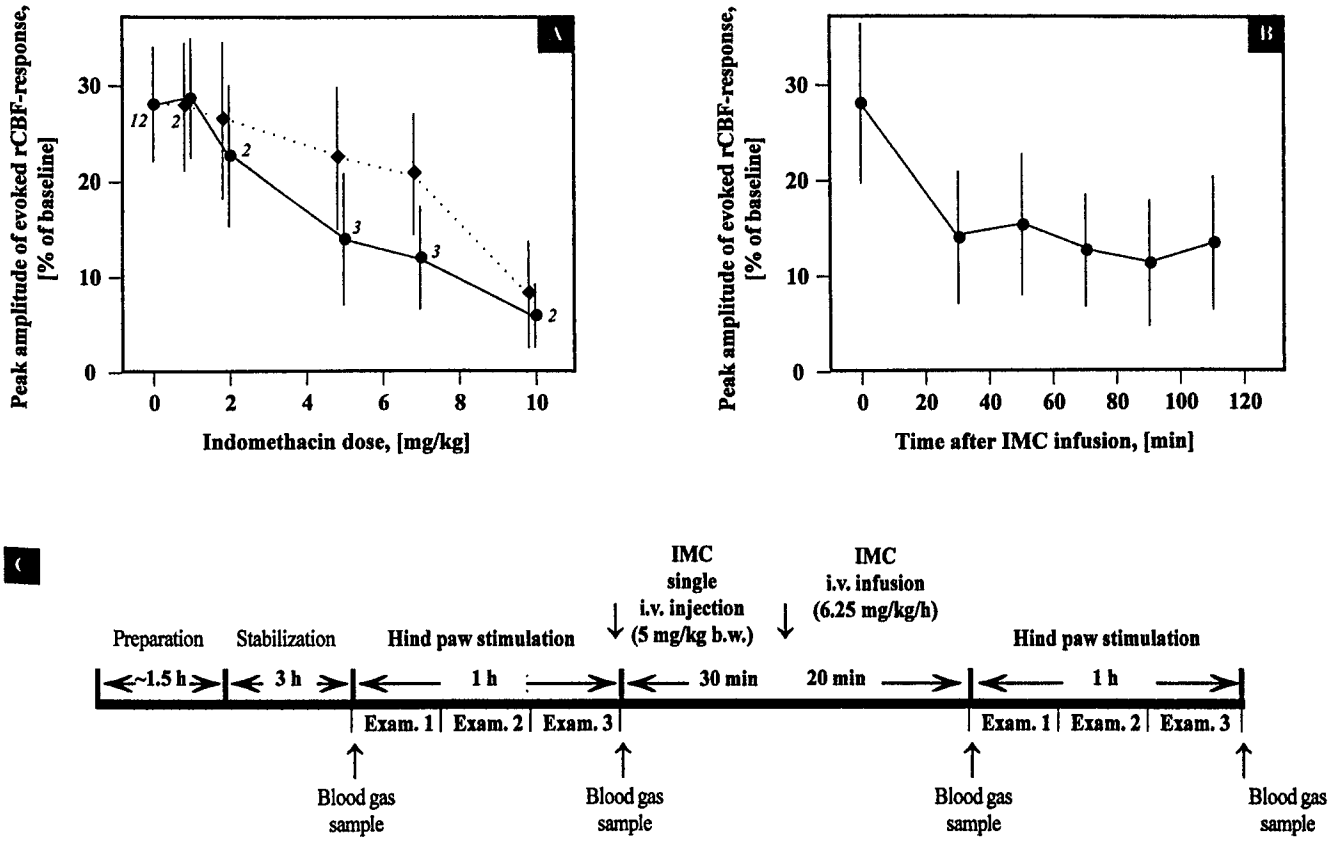

Figure 1. Experimental scheme of IMC application. (A) Dose-dependent effects of a single i.v. injection of IMC on the peak amplitude of evoked rCBF response in somatosensory cortex. Dotted line, 10 min after IMC application; solid line, 30 min after IMC application. Stimulating parameters: 1.5 mA, 5-sec duration, 5 Hz frequency. The number of rats in each group is marked on the points of dose-dependent curves. The peak amplitude was calculated as the percentage of baseline. Baseline level was considered to be 100%. (B) Time-dependent effect of continuous i.v. infusion of IMC on the peak amplitude of evoked rCBF response in somatosensory cortex. The dose and scheme of IMC application are the same as in C. Stimulating parameters: 1.5 mA, 5-sec duration, 5 Hz frequency. The peak amplitude was calculated as the percentage of baseline. Baseline level was considered to be 100%. (C) Experimental protocol. The experiment was carried out about 3 hr after the preparation of the animal. The evoked rCBF were examined before and after IMC application (5 mg/kg single i.v. injection; 30 min after that, 6.25 mg/kg/hr i.v. infusion). At each examination, 20 successive pulses of 0.2, 1, and 5 Hz frequency (5-sec duration, 1.5 mA), were applied at 60-sec intervals. The order of stimulus frequencies was selected randomly.

6.25 mg/kg body wt./hr. The rate of infusion was 1 ml/hr. The time protocol is given in Figure 1C.

Rofecoxib (MSD GmBh, Germany) was applied intravenously, a 0.5-ml single injection of 4 mg/kg body wt.; 15 min after that, infusion with 3 mg/kg body wt./hr. The rate of infusion was 1 ml/hr. The time protocol is given in Figure 2C.

Both time protocols were selected based on the preliminary experiments about dose- and time-dependent effects of IMC or Rofecoxib on the increase of evoked rCBF in somatosensory area of the cortex after hind paw stimulation (current, 1.5 mA; duration, 5 sec; frequency, 5 Hz; Figs. 1, A and B and 2, A and B). It was observed that the inhibitory effect of IMC on the evoked rCBF was almost constant in doses 5–7 mg/kg body wt. (Fig. 1A) and was stable in the time interval 30–110 min after the beginning of drug infusion (Fig. 1B). In the case of Rofecoxib, its inhibitory effect on the evoked rCBF was constant in doses 2–5 mg/kg body wt. (Fig. 2A) and was stable in the time-interval 15–110 min after the beginning of drug infusion (Fig. 2B).

We examined the evoked rCBF responses to the graded stimulus frequencies before and after application of COX inhibitors.

Data Analysis. Arterial blood pressure was monitored during the experiments, and the MABP was calculated as the average at three time points (i.e., before, during, and immediately after each stimulation period). Arterial blood samples were serially collected before and immediately after each step of stimulation period and were analyzed for blood gas values.

The LDF signal and arterial blood pressure were recorded continuously on MacLab data acquisition software (AD Instruments, Australia), and the outputs from 20 successive measurements were accumulated. Data were digitized at 40 Hz and were saved on a disk for offline analysis. The rise time and the termination time of the evoked rCBF were defined as the times at the intersection of the extrapolated lines, which were drawn on the response curve from 90% to 10% of the peak, with the baseline (Fig. 3). The rise time is a hemodynamic latency, and it is the time at which the evoked rCBF curve leaves the baseline level after the onset of stimulation. The peak time is the time at which the

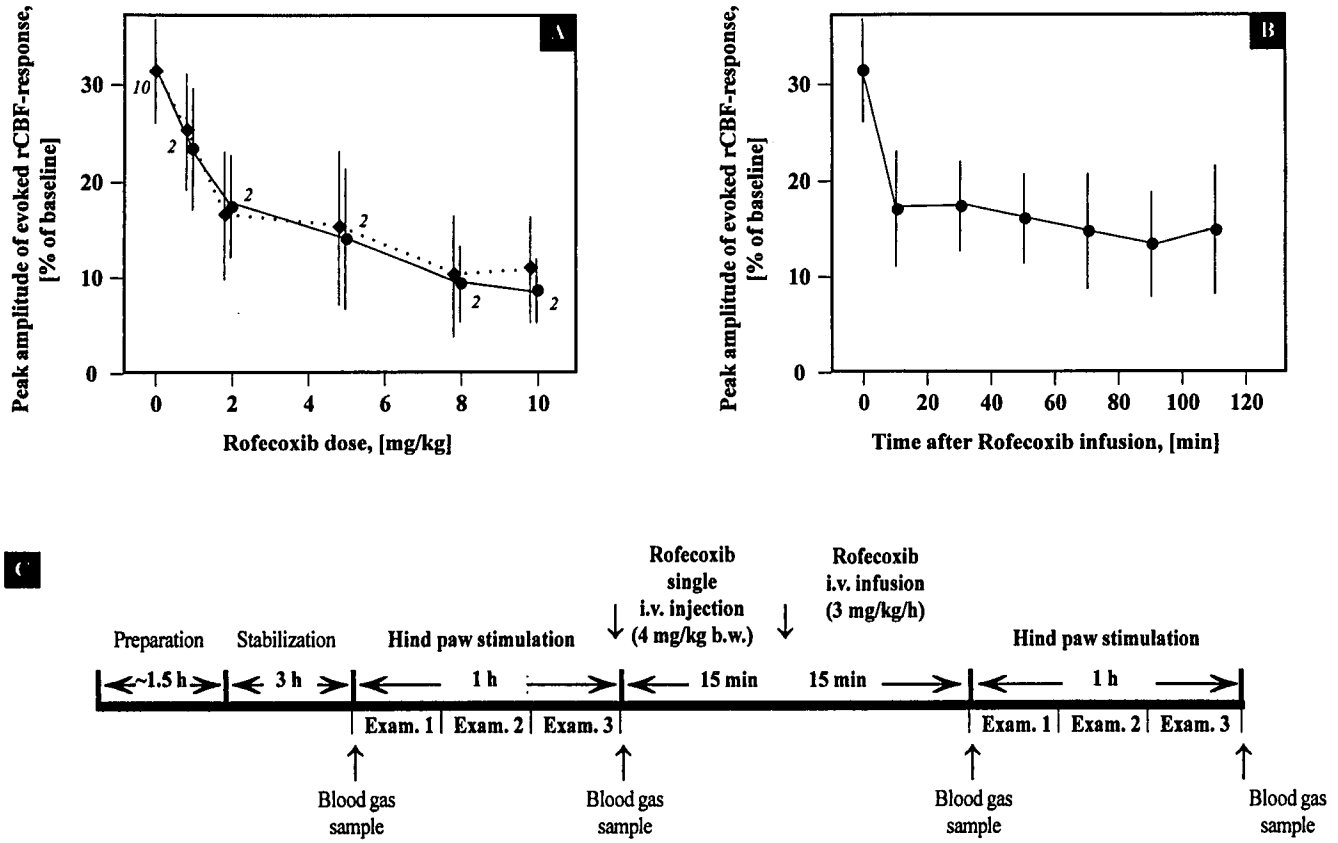

Figure 2. Experimental scheme of Rofecoxib application. (A) Dose-dependent effects of a single i.v. injection of Rofecoxib on the peak amplitude of evoked rCBF response in somatosensory cortex. Dotted line, 15 min after Rofecoxib application; solid line, 30 min after Rofecoxib application. Stimulating parameters: 1.5 mA, 5-sec duration, 5 Hz frequency. The number of rats in each group is marked on the points of dose-dependent curves. The peak amplitude was calculated as the percentage of baseline. Baseline level was considered to be 100%. (B) Time-dependent effect of continuous i.v. infusion of Rofecoxib on the peak amplitude of evoked rCBF response in somatosensory cortex. The dose and scheme of Rofecoxib application are the same as in C. Stimulating parameters: 1.5 mA, 5-sec duration, 5 Hz frequency. The peak amplitude was calculated as the percentage of baseline. Baseline level was considered to be 100%. (C) Experimental protocol. Experiment was carried out about 3 hr after the preparation of the animal. The evoked rCBF were examined before and after Rofecoxib application (4 mg/kg single i.v. injection; 15 min after that, 3 mg/kg/hr i.v. infusion). At each examination, 20 successive pulses of 0.2, 1, and 5 Hz frequency (5-sec duration, 1.5 mA), were applied at 60-sec intervals. The order of stimulus frequencies was selected randomly.

response curve of evoked rCBF reaches the maximum height. The termination time is the time at which the evoked rCBF curve returns to the baseline level after maximum response (21). The LDF signal was normalized towards the baseline level as percentage changes from the baseline. The response magnitude was calculated as an integral of the response curve from the rise time to the termination time.

Values were statistically analyzed by analysis of variance (ANOVA) using Student's *t* test, and are presented as mean ± SD.

Results

Effect of IMC and Rofecoxib on Physiological Variables. Physiological variables measured during the stimulation paradigms are listed in Table I (for IMC) and Table II (for Rofecoxib). They were within the normal ranges before IMC or Rofecoxib application. IMC (6.25 mg/kg body wt./hr, i.v.) led to a significant decrease of MABP ($P < 0.01$) and heart rate ($P < 0.05$) and did not affect PaO_2, $PaCO_2$, and pH (Table I). Rofecoxib did not

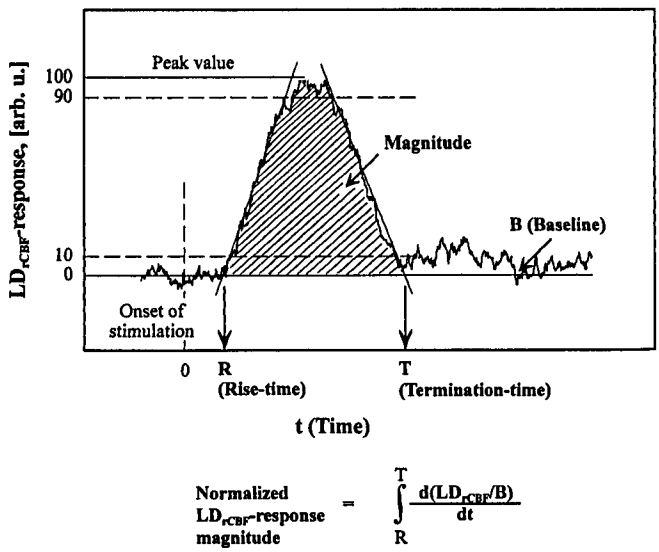

Figure 3. Diagram illustrating normalization of the LDF signal to the baseline level and calculation of the time-parameters of evoked rCBF response.

Table I. Physiological Variables Before and After IMC Application[a]

Parameter	Before IMC	After IMC	*P*
MABP	93.40 ± 10.88	78.70 ± 6.52	$P < 0.001$
Heart rate	408 ± 13	396 ± 12	$P < 0.05$
pH	7.44 ± 0.04	7.42 ± 0.04	ns
$PaCO_2$	34.04 ± 2.93	33.18 ± 1.73	ns
PaO_2	122.08 ± 13.94	119.27 ± 16.72	ns

[a] The number of animals is 11. Mean ± SD.

Table II. Physiological Variables Before and After Rofecoxib Application[a]

Parameter	Before Rofecoxib	After Rofecoxib	*P*
MABP	110.23 ± 12.47	102.90 ± 8.86	ns
Heart rate	414 ± 10	407 ± 13	ns
pH	7.44 ± 0.05	7.44 ± 0.04	ns
$PaCO_2$	36.24 ± 3.30	35.78 ± 2.41	ns
PaO_2	118.01 ± 12.55	120.22 ± 14.34	ns

[a] The number of animals is eight. Mean ± SD.

significantly affect all tested physiological parameters (Table II). The hind paw stimulation did not cause any change in MABP during stimulation before and after drug application, which is consistent with the previous studies in our laboratory (21, 22).

Effect of IMC and Rofecoxib on the Response Magnitude of the Evoked rCBF. Neither COX inhibitors significantly affected the baseline level of CBF. The mean baseline levels of CBF before and after IMC application were 19.54 ± 3.44 and 18.20 ± 3.80 arbitrary units (arb.u.), respectively. Before and after Rofecoxib application, the mean baseline levels of CBF were 16.52 ± 3.50 and 14.85 ± 3.02 arb.u., respectively.

Variations in the evoked rCBF as a function of stimulus frequency are shown in Figure 4. In both cases, before and after drug application, the response magnitudes of the evoked rCBF increased with increasing stimulus frequency up to 5 Hz.

Intravenous application of IMC led to a significant reduction of the response magnitudes of the evoked rCBF in all ranges of frequencies. The response magnitudes of the evoked rCBF at 0.2, 1, and 5 Hz stimulation, after IMC application, were 61.3% ± 18.3%, 72.5% ± 18.6%, and 66.1% ± 17.0%, respectively, of that before IMC application.

Intravenous application of Rofecoxib led to a significant reduction of the response magnitudes of the evoked rCBF at 1 and 5 Hz stimulation; they were 66.7% ± 11.3% and 58.5% ± 16.5%, respectively, of that before Rofecoxib application. Rofecoxib did not significantly affect the response magnitude of the evoked rCBF at 0.2 Hz stimulation ($P > 0.05$).

Effect of IMC and Rofecoxib on the Time Parameters of Normalized Evoked rCBF. Stimulation at frequencies ranging from 0.2 to 5 Hz using a fixed duration of 5 sec yielded frequency-dependent response curves of rCBF (Fig. 5). As it is impossible to detect the velocity of the evoked rCBF increase by LDF, in the present study, we calculated the time parameters of evoked rCBF curves before and after application of COX inhibitors (Tables III and IV). The rise time of 0.5 sec was nearly constant between 0.2 and 5 Hz stimulation before and after IMC or Rofecoxib application. In both cases, after drug application, the evoked rCBF at 0.2–5 Hz stimulations peaked earlier as compared with that before their application ($P < 0.05$). The termination time at 0.2 Hz stimulation decreased significantly after IMC application (Table III), but was not affected at 1 and 5 Hz stimulation, as well as after Rofecoxib infusion (Table IV).

Discussion

The present study suggests that the non-selective COX-1 and COX-2 inhibitor IMC and highly selective COX-2 inhibitor Rofecoxib significantly reduce (about 50%–60%) evoked increases in rCBF induced by frequency-dependent somatosensory stimulation in rats *in vivo*. This observation is in agreement with previously published data of Dahlgren *et al.* (10) demonstrating that IMC

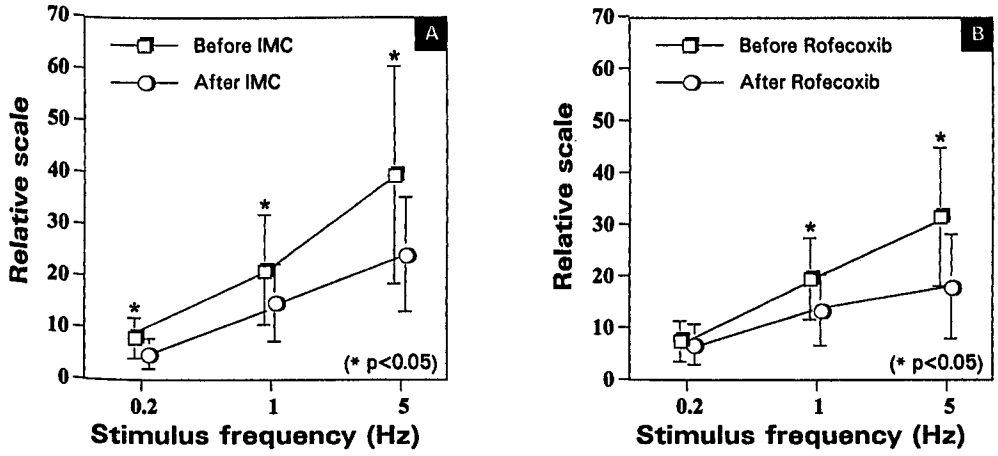

Figure 4. Changes in the response magnitudes of evoked rCBF at varying frequencies of hind paw stimulation before and after IMC (A) or Rofecoxib (B) application. Note that IMC significantly reduces the response magnitudes of the evoked rCBF in all range of frequencies ($P < 0.05$, Student's *t* test). Error bars indicate SD, $n = 11$. Rofecoxib reduces significantly the response magnitudes of the evoked rCBF at 1 and 5 Hz stimulation ($P < 0.05$, Student's *t* test), but does not affect significantly the evoked rCBF at 0.2 Hz stimulation. Error bars indicate SD, $n = 8$.

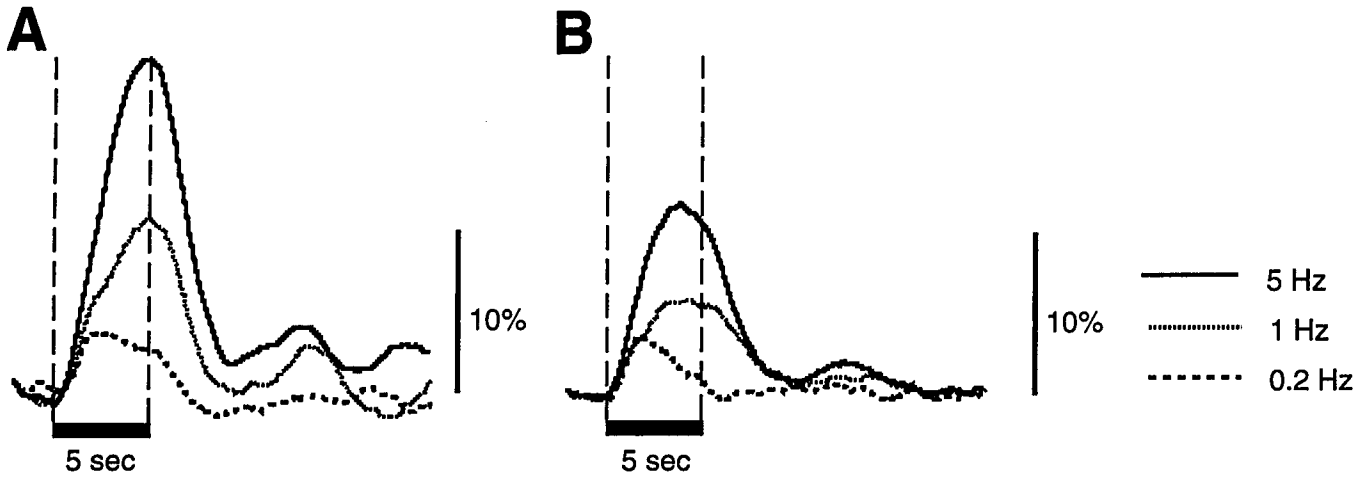

Figure 5. Normalized evoked rCBF curves at varying frequencies of hind paw stimulation before and after application of IMC. The response curves were normalized to the baseline level and were averaged by the number of animals used. Note that the normalized evoked rCBF before application of COX inhibitor peaked later as compared with that after its application, in all range of frequencies ($P < 0.05$, Student's t test), although the rise time of 0.5 sec was nearly constant, regardless of the stimulus frequency. y-axis, LDF signal in the percentage of baseline; x-axis, time in seconds.

Table III. Time Parameters of the rCBF Response Curves Before and After IMC Application[a]

Stimulus frequency	Condition	Rise time (sec)	Peak time (sec)	Termination time (sec)
0.2 Hz	Before IMC	0.55 ± 0.23	2.56 ± 0.54	6.32 ± 0.58
	After IMC	0.54 ± 0.30	1.96 ± 0.43[b]	5.03 ± 0.78[b]
1 Hz	Before IMC	0.54 ± 0.22	4.21 ± 0.84	8.02 ± 1.47
	After IMC	0.51 ± 0.30	3.87 ± 0.76[b]	8.32 ± 1.21
5 Hz	Before IMC	0.56 ± 0.18	4.72 ± 0.62	8.92 ± 1.58
	After IMC	0.55 ± 0.25	3.77 ± 0.44[b]	9.21 ± 1.52

[a] The number of animals is 11. Mean ± SD.
[b] There is a significant difference in parameters before and after IMC application ($P < 0.05$).

Table IV. Time Parameters of the rCBF Response Curves Before and After Rofecoxib Application[a]

Stimulus frequency	Condition	Rise time (sec)	Peak time (sec)	Termination time (sec)
0.2 Hz	Before Rofecoxib	0.55 ± 0.25	3.44 ± 0.32	5.97 ± 0.56
	After Rofecoxib	0.55 ± 0.30	2.57 ± 0.41[b]	5.52 ± 0.81
1 Hz	Before Rofecoxib	0.53 ± 0.27	4.82 ± 0.64	8.48 ± 1.05
	After Rofecoxib	0.55 ± 0.21	4.05 ± 0.62[b]	7.72 ± 1.10
5 Hz	Before Rofecoxib	0.54 ± 0.22	4.79 ± 0.55	9.15 ± 1.28
	After Rofecoxib	0.52 ± 0.32	3.87 ± 0.53[b]	8.78 ± 1.02

[a] The number of animals is eight. Mean ± SD.
[b] There is a significant difference in parameters before and after Rofecoxib application ($P < 0.05$).

(in a 10 mg/kg dose, single injection) changes the rCBF response to nose stimulation with a reduction of the increase in cortical CBF by about 30%. Recently, Niwa *et al.* (19) reported that the selective COX-2 inhibitor NS-398 attenuates the increase in somatosensory cortex blood flow produced by vibrissal stimulation. Furthermore, the flow response to vibrissal stimulation is impaired in whisker barrel cortex of COX-2 null mice (19). On the other hand, the same authors observed that the selective COX-1 inhibitor SC-560 reduced the resting CBF but did not affect the increase in somatosensory cortex blood flow produced by neuronal stimulation (25).

There are many differences in methodology and experimental design of the studies mentioned above that do not allow a comparative analysis between them. It is necessary to have in mind also that there is only a single amino acid difference between COX-1 and COX-2 isoforms, and this may be critical for the level of selectivity of COX inhibitors, giving rise to the problem of a loss of selectivity at higher doses (18). In this group of compounds, Rofecoxib is one of the few highly selective COX-2 inhibitors that has no effect on COX-1 over the whole range of doses used and concentrations achieved in clinical practice. An ID_{50} for COX-1 could not be calculated because inhibition by Rofecoxib

was not seen with single doses up to 1000 mg (18, 26). We found that Rofecoxib does not affect physiological variables as well as the baseline flow. Thus, the inhibitory effect of Rofecoxib on the evoked rCBF increase described in the present study provides direct proof of the participation of COX-2 products in the regulation of function/flow coupling.

Based only on the inhibitory effect of IMC on the evoked rCBF response, it is impossible to conclude that COX-1 products also participate in the regulation of function/flow coupling. There are some contradictions about the inhibitory effect of IMC on the evoked rCBF. We observed that IMC reduces not only the rCBF response to graded neuronal stimulation, but that the drug significantly reduces heart rate and MABP. These experimental facts give rise to ideas about the influence on autoregulation of IMC and, therefore, about two IMC-sensitive pathways (COX dependent and COX independent) for regulation of cerebrovascular resistance during neuronal stimulation in somatosensory cortex.

IMC-Sensitive and COX-Independent Mechanisms for Regulation of the Function/Flow Coupling in Somatosensory Cortex. Based on previously published data (27–29) as well as on the observation that IMC does not affect CBF at rest condition, in spite of the significant decrease in MABP, we assume that IMC may not be affecting autoregulation in a dose of 6.25 mg/kg/hr (applied i.v.). Probably, the decrease in MABP after IMC application is the result of a decreased heart rate (Table I) (1, 30). Nevertheless, the effect of IMC on the regional blood pressure in the brain is not known, and it may be different from the effect on the MABP. In this case, it is possible that IMC puts a severe strain on autoregulation in a local area of the cortex and thus goes beyond the limit of vasodilating ability of the vessels after neuronal activation. This possibility may explain, at least partially, the inhibitory effect of IMC on the evoked rCBF response.

However, there are some experimental facts demonstrating that IMC does not have a crucial effect on the vasodilating limit of cerebral vessels, as well as that IMC acts predominantly through oxygen-dependent mechanism(s). Because a direct measurement of cerebrovascular resistance by LDF is impossible, we used the ratio MABP/CBF to estimate the changes in the resistance of blood vessels by IMC (31). If the ratio MABP/CBF is accepted as 100% before IMC application, after its application, the ratio decreases to 87.3% ± 19.3%, but the difference is not statistically significant ($P = 0.067$). Therefore, it may be assumed that IMC does not affect significantly the resistance of cerebral vessels. On the other hand, IMC (1–30 mg/kg body wt., i.v.) is a well-known vasoconstrictor (30, 32, 33). It has been observed that it decreases CO_2-induced vasodilation (1, 2, 12, 14, 34) and after subsequent application of prostacyclin, the CO_2-vasodilating reactivity of blood vessels is restored to control levels and above (34). It is described in many papers that IMC does not affect hypoxia-induced vasodilation (1, 2, 10, 14) or cerebrovascular resistance during pathologically neuronal activity (status epilepticus) associated with a reduced supply of oxygen (hypoxia and ischemia) (13). As IMC is a well-known COX inhibitor (1–3) and COX is an oxygen-requiring enzyme, it may be supposed that the effect of IMC on the evoked rCBF response is mediated by a prostanoid-dependent pathway. As Rofecoxib, which is selective for COX-2, had the same effect on the evoked rCBF as non-selective IMC, it suggests that the IMC can also work by COX-2 isoform.

We determined that the concentration of IMC in the brain tissue was about 2% to 3% of its i.v. injected concentration (see Appendix). It has been established that this concentration is enough to irreversibly inhibit COX and prostanoid synthesis in the brain of normal rats (1, 2).

The results raise two possibilities: a participation of COX products in the regulation of evoked rCBF increase in response to graded somatosensory stimulation, and/or some other effects of IMC associated with direct influence of neuronal activity in somatosensory cortex or influence of another biochemical mechanisms for regulation of function/flow coupling.

A direct effect of IMC on neuronal activity in the somatosensory area is not likely because it is reported that IMC (up to 30 mg/kg body wt., i.v.) does not affect oxygen consumption and regional cerebral glucose utilization under neuronal activation in the brain (1, 13, 14, 27).

It has been established that IMC blocks not only prostanoid-dependent, but also nitric oxide-dependent pathways for regulation of the relationship between rCBF and neuronal activity (29, 35, 36), and it is a result of direct and/or indirect inhibition of guanylate cyclase and suppression of cGMP production (35, 36). It has been assumed also that IMC may affect the crosstalk between cyclic nucleotide metabolizing systems and thus may suppress rCBF response to different stimuli (29, 37). It has been observed that other non-selective COX inhibitors (diclofenac and sulindac) have no effect on the hypercapnia-induced vasodilation in contrast to IMC (38, 39). All these results demonstrate that a part of rCBF increase, evoked by neuronal activity, may be IMC sensitive, but is not caused by COX inhibition. However, it should be noted that prostacyclin, applied after and during i.v. infusion of IMC, facilitates the release of nitric oxide and cGMP production and potentiates their action in the coronary vessels (40). It is most likely that IMC inhibits evoked rCBF increase mainly through a prostanoid-dependent mechanism.

COX-Dependent and IMC-Sensitive Mechanisms for Regulation of the Function/Flow Coupling in Somatosensory Cortex. If IMC and Rofecoxib participate through COX inhibition and suppression of prostanoid synthesis in the regulation of evoked rCBF during somatosensory stimulation, at least two possible trigger signals for COX activation by neuronal activity may be hypothesized: depolarization-induced enhancement in phospholipase A2 activity by K^+, and/or local CO_2 increase and

long-lasting pH changes in a discreetly activated region of the cortex, both leading to production of free arachidonate, subsequent COX activation, and prostanoid synthesis, as well as release of other vasodilators (Ca^{2+}, nitric oxide, adenosine, etc.) (1, 12, 30).

There are many contradictions in respect to prostanoids as regulators of cerebrovascular resistance. Some of these products are known as vasodilators (prostacyclin), some as vasoconstrictors (prostaglandin F_{2a} and thromboxanes), and for some of them the results are conflicting (prostaglandins E_1 and E_2) (1–3, 41). The ratio between their concentrations is an important factor in the regulation of evoked rCBF during neuronal stimulation in somatosensory cortex. Our results demonstrate that IMC and Rofecoxib, inhibitors of the total prostanoid synthesis, significantly reduce evoked rCBF in response to somatosensory stimulation, suggesting that the overall effect of prostanoids is to vasodilate cerebral vessels during neuronal stimulation *in vivo*. As the washout of prostanoids from the brain into the blood stream is rapid (1), these substances are obvious candidates for the regulation of the function/flow coupling.

Recently, specific prostanoid receptors have been characterized on the smooth muscle of cerebral vasculature (42–44). Therefore, the mechanism of regulation of the evoked rCBF during somatosensory stimulation by prostanoids is probably receptor mediated. This does not exclude the possibility that COX inhibitors act also as receptor antagonists. It has been observed that IMC-sensitive CO_2 reactivity of cerebral arterioles is restored by vasodilator prostacyclin (34), which makes a block of prostanoid receptors by IMC doubtful. Nevertheless, this possibility needs verification.

Our results also demonstrate that IMC and Rofecoxib in the doses applied (6.25 and 3 mg/kg/hr, respectively) do not completely inhibit the rCBF increase in somatosensory area during neuronal stimulation (Fig. 4), suggesting that the neuronal stimulation also unlocks also the synthesis of other vasodilators (adenosine, acetylcholine, nitric oxide, etc.). The decrease in the evoked rCBF response magnitude and the shorter peak time after application of COX inhibitor suggest that probably the velocity of the evoked rCBF increase does not change markedly in all frequency ranges. The shortening of termination time of the evoked rCBF at 0.2 Hz stimulation, as well as the shortening of peak time in all range of frequencies after IMC or Rofecoxib application (Tables II and IV), suggests that COX inhibitors predominantly influence the second part of the evoked rCBF response curve (Fig. 5). Presumably, prostanoids take part in the regulation of rCBF, evoked by somatosensory stimulation, later than other vasodilating substances.

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Appendix

HPLC Analysis of IMC in the Brain Tissue. Three rats (390.0 ± 26.5, mean ± SD) were used to determine the concentration of IMC in the brain tissue of the end of experiment (1 hr 50 min). Another two rats were used as controls, and three rats were used to determine the concentration of IMC in the brain tissue 50 min after its i.v. application, according to the time-protocol shown in Figure 1.

The concentration of IMC in the brain tissue was analyzed by HPLC as described in Nowack *et al.* (45). A series 200 LC pump and model 1020 personal integrator from Perkin Elmer (Norwalk, CT), model 7125 injector from Rheodyne (Torrance, CA), and post-column reactor URA-100 (Kratos, Hofheim, Germany) with an LKB pump were used. The HPLC separation was performed on a Spheriosorb ODS 2 (5 μm)-Saule column and a mobile phase containing 70% methanol in 0.025 M KH_2PO_4, pH 4.0, and post-column derivatization by 0.1 N NaOH (0.12 ml/min, 75°C). The flow rate was 0.6 ml/min. The injected volume was 10 μl. Fluorimetric detection was used—λex = 310 nm, λem = 380 nm.

The concentrations of IMC in the brain tissue were similar 50 min and 1 hr 50 min after its application: 0.129 ± 0.004 μg/g tissue and 0.124 ± 0.005 μg/g tissue, respectively. These concentrations are about 2%–3% of its i.v. applied concentration, and they are enough to irreversibly inhibit COX and prostanoid synthesis in the brain of normal rats (1, 2). IMC was not detected in the brain of control animals.

HPLC Analysis of Rofecoxib in the Brain Tissue. Three rats (382.5 ± 18.0, mean ± SD) were used to determine the concentration of Rofecoxib in the brain tissue at the end of the experiment (1 hr 30 min). Another two rats were used as controls, and three rats were used to determine the concentration of Rofecoxib in the brain tissue 30 min after its i.v. application, according to the time-protocol shown in Figure 2.

The concentration of Rofecoxib in the brain tissue was analyzed by HPLC as described in Jamali and Sattari (46). A C18 analytical column packed with 5-μm reversed phase particles and a variable UV spectrophotometric detector set at 272 nm was used. The mobile phase consisted of 77% water, 23% acetonitrile, 0.1% acetic acid, and 0.03% triethylamine. The flow rate was 1 ml/min.

The concentration of Rofecoxib in the brain tissue 30 min after drug application was 0.164 ± 0.010 μg/g tissue. Rofecoxib was not detected in the brain of control animals.