Critical Role of Endothelial Nitric Oxide Synthase and Cyclooxygenase in Response of Rabbit Basilar Artery to Serotonin

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ABSTRACT—The modes of action of serotonin (5-HT) on the tone of the rabbit basilar artery were investigated in vitro with the aim of determining the exact role of the endothelium. After sacrificing the animal under pentobarbital anesthesia, 3-mm segments of the artery were removed and mounted in a 5-ml myograph for isometric tension recording. Vessels precontracted by histamine were relaxed by acetylcholine. Mean maximum relaxation at 10⁻⁴ M was reduced from 79% to 22% (P<0.001) by 10⁻⁵ M N⁶-nitro-L-arginine (L-NA), and from 73% to 63% (NS) by 3.10⁻⁶ M indomethacin. Intact non-precontracted vessels were contracted by 5-HT (10⁻⁹ M to 10⁻⁵ M): 10⁻⁵ M L-NA significantly increased the contractile force (approximately twofold), whereas 3.10⁻⁶ M indomethacin significantly decreased it (to approximately 35%). In histamine-precontracted vessels, 5-HT induced at low concentrations (3.10⁻⁹ M to 3.10⁻⁸ M) a reduction in tone and induced an increase in tone at higher concentrations. At 10⁻⁵ M, L-NA abolished the relaxant phase of the response, whereas 3.10⁻⁶ M indomethacin potentiated it. In uridine triphosphate-precontracted segments, there was not a net reduction in tone under 5-HT at 3.10⁻⁹ to 3.10⁻⁸ M, but further contraction appeared at higher concentrations. The presence of 10⁻⁵ M L-NA significantly increased the contraction to 5-HT, but 3.10⁻⁶ M indomethacin did not significantly reduce it. Endothelial lesion reduced by about 50% the contractile response of L-NA-treated arteries to 5-HT; and conversely, endothelial lesion increased approximately twofold the contraction of indomethacin-treated arteries to 5-HT. We conclude that 5-HT causes the release from the endothelium of two vasoactive factors, one of which is probably the vasodilator nitric oxide, but the size of the relaxation may depend on the prevailing level of nitric oxide synthase activation. The second factor is a cyclooxygenase-dependent contractile agent. However, the contraction to 5-HT was not modified by the presence of the thromboxane synthase inhibitor CGS 13080 (10⁻⁴ M), suggesting that thromboxane A₂ is not the main contractile agent released.

Keywords: Eicosanoid, Nitro-arginine, Indomethacin, Cerebral artery, Thromboxane synthase

Much evidence exists implicating serotonin (5-hydroxytryptamine, 5-HT) in pathological cerebrovascular phenomena. For example, it can be released from perivascular mast cells during inflammation, from platelets after subarachnoid and intracerebral hemorrhage or in other conditions favoring aggregation (e.g., hemodynamic or atherosclerotic platelet activation), from serotonergic neurons (axons) in close apposition to parenchymal vessels, from sympathetic fibers into which it may have been taken up. In previous work we have shown changes in mechanical reactivity to 5-HT of rabbit cerebral arteries after subarachnoid hemorrhage: at even brief delays after the hemorrhage (10 min, 24 h), there was significantly increased contraction (1), associated with increased sensitivity to extracellular calcium (2). In examining the modifications induced in rabbit middle cerebral artery reactivity, measured in vitro, by previous injection in vivo of a leukocyte activator, we found that aggregating platelets constricted the vessel far more on the injected side than the non-injected side (3). Such platelets are known to release large quantities of 5-HT and thromboxane A₂ (TXA₂), so that it is likely that 5-HT-induced constriction is critically important in such pathological situations.

The mechanisms of such aggravated constrictory responses to 5-HT are largely unexplored, although it can

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be suspected that functional changes in the endothelium probably play a significant role. It has been known for a long time that endothelium-intact coronary arteries may relax in response to serotonin, generally by the activation of endothelial 5-HT₁-like receptors (4, 5). A large variety of non-cerebral vessels, both arteries and veins, have been shown to possess serotonergic receptors mediating relaxation of smooth muscle. Early work on feline cerebral arteries in vitro showed that the precontracted middle cerebral artery was dilated by 5-HT (after treatment with phenoxylbenzamine to destroy the receptors mediating contraction) (6); and in situ, the diameter of cat pial arteries /arterioles was increased (7, 8). In the latter case, the response to 5-HT varied in relation to the vessel diameter, the smaller arterioles/arteries dilating and the larger ones constricting. However, another study on cat pial arteries in situ did not confirm the existence of such a relationship or such dilatory responses (9). A recent study on human intracortical arterioles showed a biphasic response to the 5-HT₁₈ agonist sumatriptan, the small relaxation at low concentration being abolished by nitric oxide synthase inhibition (10). In another study, human cerebral arteries were found by immunohistochemistry to bear 5-HT₁B receptors on both the smooth muscle and the endothelium, but responses of precontracted segments (to 5-HT; agonists) were not tested (11). 5-HT was also shown to induce relaxation of porcine cerebral veins (inhibition of rhythmic contractions) by a mechanism independent of nitric oxide synthase (12).

In addition to this limited and controversial evidence that 5-HT can dilate intact cerebral arteries under specific conditions, there have been several reports indicating that endothelium removal/destruction may potentiate constrictory responses. In an in vitro study on feline basilar artery, we observed augmented constriction to 5-HT of perfused vessels when the endothelium was removed (13). Similar results have since been published concerning rabbit and dog basilar arteries (14–18), rabbit middle cerebral arteries (19), and goat middle cerebral arteries (20). Faraci and Heistad (21) used a nitric oxide synthase (NOS) inhibitor in vivo and found increased constriction of rabbit large cerebral arteries by injected 5-HT, but the origin of the nitric oxide (NO) was not demonstrated.

Conversely, other studies have suggested that 5-HT-induced constriction may involve one or more endothelial factors. In mouse pial arterioles studied in situ, evidence was adduced supporting the hypothesis that 5-HT may release an endothelial constrictor agent that was blockable by a cyclooxygenase (COX) inhibitor (22). A similar finding of constrictory COX-dependent activity was made in pial arterioles of stroke-prone spontaneously hypertensive rats, but not in those of control (Wistar-Kyoto) rats (23), but the endothelial origin of the constrictor was not actually demonstrated. Another in vitro study of rat cerebral (basilar) artery reactivity to 5-HT showed that the contractions involved a COX-dependent metabolite and provided evidence that TXA₂ was the agent responsible (24). Finally, a more recent study on small human cerebral (pial) arteries showed that 5-HT-induced contraction was to a large extent mediated by endothelin, via ET-A receptors (25).

In the face of these conflicting data on cerebrovascular responses to 5-HT, our primary aim was to test the hypothesis that the endothelium contributes to contractile and relaxant responses to 5-HT in the rabbit basilar artery model, by addressing the following questions: 1) What response can be obtained with 5-HT in the non-precontracted and the preconstricted segment? 2) Is the endothelium involved in these responses? 3) Do the responses involve metabolites of NOS and COX, and, in the latter case, is thromboxane involved in contraction? Such questions may be of considerable relevance to human cerebrovascular studies in view of the widely overlapping 5-HT receptor types present in both species.

MATERIALS AND METHODS

General procedures

Fauve de Bourgogne rabbits weighing between 2.5 and 3 kg were used in the study. All rabbits were anesthetized with acepromazine (17 mg/kg) and sodium pentobarbital (20 mg/kg) administered intravenously. After decapitation, the brain was removed and placed in a N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid buffer solution, as previously described (26). Three 3-mm long segments of each basilar artery were carefully removed under a surgical microscope. The artery segments were then placed in 5-ml organ baths containing a solution of the following composition: 126 mM NaCl, 5 mM KCl, 1.2 mM NaH₂PO₄, 1.3 mM MgCl₂, 20 mM NaHCO₃, 2.5 mM CaCl₂, 5.5 mM glucose, gassed with 4% CO₂ / 20% O₂ / 76% N₂ with a pH of 7.3 – 7.4.

The segments, mounted on L-shaped prongs, were allowed to equilibrate for 90 min at a temperature of 37°C, before the isometric tension measurements were begun. During the equilibration period, the incubating solution was changed every 15 min, and the vessels were frequently stretched until a stable resting tension of approximately 600 mg was obtained. Pilot experiments had ascertained that stable and reproducible contractions were obtained under these conditions. For tests of contraction, cumulatively increasing concentrations of 5-HT were added to the bath to obtain concentration-response curves. For tests of relaxation, the arteries were precontracted by histamine (10⁻⁵ – 3 × 10⁻⁵ M) to obtain a stable contraction, and then increasing concentrations of agonist (acetylcholine or 5-HT) were added. In another group of experiments, we
used as the precontracturant uridine-5'-triphosphate (UTP, $3 \times 10^{-5}$) because histamine itself has known effects on NOS and COX metabolite release (26–28). After each test, a minimum of four washes were made followed by a 30-min rest before the following test.

**Endothelium lesioning**

The endothelium was lesioned in certain experiments in arteries already mounted on the L-shaped prongs. A fine polished stainless steel tube was introduced into the artery lumen between the steel prongs. A stream of gas of the same composition as the mixture used to gas the incubating solution was allowed to pass through the artery segments for 2 min. After a wash and a minimum rest period of 30 min, further tests were undertaken on the lesioned artery.

The histological state of the endothelium was checked in some non-lesioned arteries and in the arteries which had been subject to endothelium-lesioning experiments by a modification of the en face silver nitrate method of Caplan et al. (29).

**Protocols**

In all experiments each segment served as its own control; i.e., control reactivity was first tested, followed by the experimental manoeuvre, and a second test of the reactivity. The effects of the nitric oxide synthase inhibitor $N^\omega$-nitro-L-arginine (L-NA, $10^{-5}$ M) or the cyclooxygenase inhibitor indomethacin ($3 \times 10^{-6}$ M) were investigated by introducing them into the baths 10 min before beginning the two tests. In specific experiments, endothelial lesion was performed between tests 1 and 2: in this case, the whole experiment was performed in the presence of either $10^{-5}$ M L-NA or $3 \times 10^{-6}$ M indomethacin. The inhibition of the relaxation to acetylcholine (ACh) (Fig. 1) attests to the efficiency of the L-NA concentration. The indomethacin concentration is in excess of that used previously to inhibit histaminergic agonist-induced production of COX metabolites in rabbit cerebral arteries (27). Experiments on the inhibitor of TXA$_2$ synthase, CGS 13080, were performed similarly by administering it (at $10^{-4}$ M) 30 min before test 2. In order to check on the possible effects of the solvent of the stock solution (concentration = 1000× bath concentration, in DMSO), the solvent was administered at the same concentration in the control test. Possible spontaneous differences in the reactivity to 5-HT in consecutive tests were checked in parallel runs in which DMSO only was given in both tests.

**Drugs**

Acetylcholine chloride was obtained from Merck (Darmstadt, Germany); serotonin creatinine sulfate, $N^\omega$-nitro-L-arginine, indomethacin and histamine dihydrochloride were obtained from Sigma (St. Louis, MO, USA). CGS 13080 was a gift from Ciba Geigy (Basel, Switzerland).

**Expression of results and statistics**

Calculations of relaxation were made by dividing the magnitude of the decrease in tension by the initial, stable, induced increase in tone. In the experiments using UTP as the precontracturant, in which there was no net relaxation, the results were expressed as change in tension (in mg) with respect to the initial, stable, induced tone.

In general, the results of the second test were compared directly with those obtained previously in the same segment in the absence of the inhibitor (control test). In some cases,
values obtained from different segments were compared by an unpaired t-test or by analysis of variance (ANOVA).

Values are expressed as means ± S.E.M.; P<0.05 was considered a significant probability.

RESULTS

Responses to ACh

In histamine-precontracted segments, ACh induced a concentration-dependent relaxation with a maximum of 75 – 80% at 10^{-4} M. L-NA at 10^{-5} M severely limited the relaxation to about 20% (Fig. 1A). No significant inhibitory effect of indomethacin (3 × 10^{-6} M) was observed (Fig. 1B). The effects of ACh on histamine-contracted segments was systematically used to check the initial status of the endothelium (30).

Responses to 5-HT

**Contraction in intact arteries:** 5-HT alone induced concentration-dependent contractions that were significantly potentiated by the presence of L-NA at 10^{-5} M (Fig. 2A). In a similar series of experiments, the presence of indomethacin at 3 × 10^{-6} M substantially reduced the contractile response to 5-HT (Fig. 2B). In both cases, the pD₂ values of the 5-HT-induced contractions were not significantly different in the presence and the absence of the inhibitor (Table 1, lines 1 and 2).

**Relaxation in intact arteries:** a) Histamine precontraction. In control conditions, histamine-precontracted BA segments showed a biphasic reactivity: at low 5-HT concentrations (3 × 10^{-9} M, 10^{-8} M, and in some cases 3 × 10^{-9} M), a small relaxation was observed (Fig. 3: A and B, controls); at higher concentrations, further contraction was imposed on the existing precontraction (Fig. 3: A and B, controls). The maximum relaxation obtained (combining both control groups) was 10.1 ± 2.4% (n = 16, P<0.001 compared to zero) at 10^{-8} M 5-HT. When L-NA at 10^{-5} M was also present, the relaxations were converted to contractions (Fig. 3A). Conversely, when indomethacin at 3 × 10^{-6} M was present, the relaxant phase was potentiated and the contractile phase tended to be diminished (Fig. 3B).

b) UTP precontraction. The results in Fig. 4, A and B show that first, there was no net relaxant phase (see filled circles), i.e., that the level of contraction obtained with

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**Table 1.** Apparent pD₂ values of contractile responses to 5-HT in unprecontracted basilar arteries

| Experimental maneuver | Control | After maneuver | Statistics (paired t-test) |
|-----------------------|---------|----------------|---------------------------|
| 1. Administration of 10^{-5} M L-NA | 6.94 ± 0.50 (n = 11) | 6.55 ± 0.53 (n = 11) | NS / 0.37 |
| 2. Administration of 3 × 10^{-6} M indomethacin | 6.57 ± 0.42 (n = 9)* | 6.31 ± 0.33 (n = 9)* | NS / 0.50 |
| 3. Endothelium lesion (presence of 10^{-5} M L-NA) | 6.84 ± 0.51 (n = 7)* | 6.21 ± 0.69 (n = 7)* | P<0.05 / 0.59 |
| 4. Endothelium lesion (presence of 3 × 10^{-6} M indomethacin) | 6.25 ± 0.46 (n = 7) | 6.08 ± 0.31 (n = 7) | NS / 0.50 |

*Group size restricted to values obtainable in both the control and the second test. In some cases, the weakness of the responses precluded an estimation of pD₂.
increasing 5-HT concentrations never fell below that obtained initially with the UTP-induced precontraction. There was, however, a point of inflexion at $10^{-8}$ M 5-HT with a slight relative fall in the level of contraction at $3 \times 10^{-8}$ M 5-HT. Second, with the inhibitor present (open circles), the curve was shifted upwards (L-NA, Fig. 4A) or downwards (INDO, Fig. 4B). Paired t-tests between the pairs of values in Fig. 4A and Fig. 4B did not reveal significant differences. Combining the two sets of control data (hence $n = 11$ for controls) and comparing the three groups by ANOVA (control vs L-NA vs indomethacin), significant differences were found for L-NA vs control at $3 \times 10^{-8}$ M, $10^{-7}$ M, $3 \times 10^{-6}$ M and $10^{-5}$ M 5-HT (Tukey test).

Effects of endothelial lesion on contraction to 5-HT:
In the continual presence of $10^{-5}$ M L-NA, the second contraction to 5-HT was reduced in amplitude, compared to the control, by the passage of the gas stream through the artery (Fig. 5A). The pD$_2$ value of the curve was significantly reduced from 6.84 to 6.21 (Table 1, line 3). On the contrary, in the continual presence of $3 \times 10^{-6}$ M indomethacin, the second contraction to 5-HT was larger than
the control contraction obtained before the endothelial lesion (Fig. 5B), but the pD₂ values were not significantly different (Table 1, line 4). The functional efficacy of the lesion was confirmed in the latter set of experiments by measuring responses to 10⁻⁵ M ACh on histamine-precontracted segments: the mean relaxation was 71.0 ± 8.8% before lesion and 25.6 ± 6.4% after lesion (n = 7, P < 0.01, paired t-test). This test could not be used in the L-NA group because of the strong inhibition of the response by L-NA. In some of these experiments, the destruction of the endothelium was confirmed by the absence of endothelial borders seen with the silver staining method, compared with unlesioned segments.

Effects of CGS13080 on contractions: The effects of the thromboxane synthase inhibitor CGS 13080 at 10⁻⁴ M were determined by administering it to the baths 30 min before the second test. Four segments received only the solvent in both tests (DMSO, 1/1000 in the bath) (Fig. 6A), and eight received CGS 13080 before test 2 (Fig. 6B). L-NA at 10⁻⁵ M was present throughout the experiment in order to inhibit an NO-dependent relaxant component, thus favoring the detection of differences in COX-dependent responses. The responses in the two tests with DMSO were identical in Fig. 6A, indicating good stability of the responses to 5-HT. No significant differences could be detected between the tests made in the presence and in the absence of CGS 13080 (Fig. 6B), suggesting there was no significant stimulation of thromboxane synthase activity by 5-HT.

DISCUSSION

In the present study, we found that both cyclooxygenase and nitric oxide synthase participate importantly in the overall response of rabbit basilar arteries to 5-HT. Inhibition of a NOS-dependent component increased the 5-HT-induced contraction, and inhibition of a COX-dependent component reduced it. Both these components were shown
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to be largely endothelium-dependent. Regarding responses to ACh (Fig. 1), the great reduction in relaxations obtained by adding 10^-5 M L-NA shows that relaxation was mediated via NO and largely blocked at this concentration of inhibitor. This is in good agreement with results obtained by Parsons et al. (31) on the rabbit middle cerebral artery. We did not test for the possible contribution of potassium channels in the smooth muscle since there is evidence that their contribution is weak or null in this vessel (32, 33).

The possible relevance of these results to human cerebrovascular pharmacology is suggested by the fact that both human cerebral arteries/arterioles and rabbit cerebral arteries contract to 5-HT1 agonists such as sumatriptan and appear to react essentially via 5-HT1B/D receptors (10, 11, 34). No part whatsoever of the rabbit cerebral artery contraction can be inhibited by ketenserin (5-HT2A-receptor antagonist) (ref. 34 and R. Sercombe, unpublished results), which differentiates these vessels from rat and cat cerebral arteries. Human temporal artery contraction also depends largely on 5-HT1B receptors, with a minor role for 5-HT2A receptors (35).

Although the inhibition of NOS by L-NA and cyclooxygenase by indomethacin may not be 100% at the concentrations used, these concentrations are generally considered to inhibit these two enzymes specifically (36, 37), and lower concentrations of indomethacin inhibited relaxations of rabbit middle cerebral artery induced by histamine-1 receptor agonists and mediated by prostaglandins (27). It can be concluded therefore that 1) EDRF in the form of NO antagonizes the constrictor responses to 5-HT, and 2) a constrictor agent, presumably generated by 5-HT-induced stimulation of arachidonic acid metabolism through the COX pathway, contributes very significantly to the overall contraction. The experiments involving endothelium lesion, carried out in the presence of L-NA or indomethacin (Fig. 5), led to mutually opposite changes in the amplitude of the 5-HT responses, which demonstrates that the effects of lesion were not linked to functional damage of the smooth muscle. The responses to acetylcholine in the indomethacin-treated group revealed a high degree of efficiency of endothelial inactivation. We conclude that the endothelium is the major site of origin of the NOS and COX metabolites contributing to the 5-HT responses, which differentiates the present relaxant (NOS-dependent) effect of 5-HT from that found in porcine cerebral veins involving 5-HT7 smooth muscle receptors (38). After destruction of the endothelium of non-precontracted isolated rabbit basilar artery, several groups (14, 15, 17, 18) found an increased contraction to 5-HT. Likewise, endothelium removal/destruction in the cat basilar artery (13), the canine basilar artery (16) and the goat middle cerebral artery (20) increased contractions in vitro to 5-HT. All these results thus concur with an endothelium-mediated, NO-dependent depression of 5-HT-induced contractions in cerebral arteries. In our experiments, the increase in contraction of endothelium-lesioned segments or segments treated with L-NA is probably not due to removal of spontaneous NO release. In 6 resting segments of rabbit basilar artery, we tested the action of L-NA at 10^-5 M: the arteries contracted by 0 mg (n = 5) or 235 mg (n = 1), indicating an insignificant amount of spontaneous NO release. It is thus probable that 5-HT stimulated the release of NO from the endothelium.

The present results differ from those of previous investigations (13, 14) in that we were able to demonstrate a significant relaxant effect of 5-HT on histamine-precontracted vessels, this effect being abolished by L-NA and potentiated by indomethacin. Indeed, in the presence of the latter, the relaxation attained a maximum of almost 30%, under 10^-8 M 5-HT. This may be an under-estimation of the total dilatory component, since the contractile response to 5-HT was not completely abolished by indomethacin.

The existence of such 5-HT receptor-induced L-NA-sensitive relaxation was demonstrated in human and bovine intraparenchymal arterioles (about 180 μm) using the 5-HT1B/D agonist sumatriptan (10), and 5-HT1B receptors were detected immunohistochemically on both the smooth muscle and the endothelium of human pial arteries (50 – 500 μm) (11).

Why has such an endothelium-dependent relaxation of cerebral arteries not been reported before? We suggest there are two factors that favor this observation in our experiments. First, the precontracting agent, histamine, is known to cause the release of endothelial dilator substances (26 – 28), so that it cannot be excluded that there was a synergistic action of 5-HT and histamine on the release of or the effects of EDRF(s), favoring the observation of a phase of net relaxation observed over a short range of concentrations of 5-HT. This suggestion is supported by the observations on UTP-precontracted segments (Fig. 4). In this case, although an inflexion in the 5-HT contraction curve was found at the same concentrations as the relaxant phase in histamine-precontracted segments, there was not an overall reduction in tone. UTP may induce an endothelium-dependent relaxation in some vessels (38, 40) but in our hands this does not seem to be the case in rabbit cerebral arteries (ref. 41 and R. Sercombe, unpublished results).

Second, our experimental conditions were different from those in previous reports. Most in vitro studies of arteries use incubating solutions in equilibrium with 95% O2/5% CO2, involving a high concentration of molecular oxygen (PO2 about 500 mmHg). The associated higher oxidative potential must entail a more rapid and complete oxidation of the nitric oxide produced by 5-HT than in our experi-
ments (PO2 130 – 150 mmHg). Kelm et al. (42) showed that the half-life of NO would be reduced by about 40% at a PO2 of 500 mmHg compared to 150 mmHg. Given that the 5-HT-induced relaxation appeared rather moderate in the absence of indomethacin, a substantial increase in the lability of the NO could render such a response undetectable.

Seager et al. (43) found a depression by indomethacin of contractile responses to 5-HT in precontracted rabbit BA segments, which is compatible with our findings. The inhibition of contraction by indomethacin or by endothelial lesion is also completely in agreement with the demonstration by Rosenblum and Nelson (22) of endothelial-dependent contraction by 5-HT of mouse pial arterioles in vivo, an effect which was inhibited by acetylsalicylic acid and indomethacin. Another group (24) has provided evidence in rat basilar arteries for the participation of TXA2 in 5-HT-induced contractions: in the presence of the 5-HT2 receptor antagonist ketanserin, they found that thromboxane receptor antagonism reduced the contraction of 5-HT, and could abolish the first (specific) phase of contraction to the 5-HT1 receptor agonist 5-carboxamidotryptamine, as could thromboxane synthase inhibition. In contrast, analysis in vivo of feline pulmonary vasoconstrictor responses to 5-HT by another group (44) showed that there was no component due to thromboxane receptor activation (despite highly potent activity of a thromboxane mimetic).

In the present work we examined the hypothesis of thromboxane release by 5-HT by incubating the arteries with an inhibitor of thromboxane synthase (CGS 13080 at 10^-4 M), but we failed to find the slightest reduction of the contractile response (Fig. 6). This, at first sight, although COX inhibition reduced the contractile response, it seems that TXA2 contributed very little to the overall effect. This seems in agreement with results of De Moraes et al. (45) on human umbilical artery. It is possible that the major portion of the COX-dependent contraction was mediated by a prostaglandin (PG) such as PGH2, PGF2α or PGE2.

Two other eicosanoids might be considered possible complementary factors in basilar arteries, the isoprostane 8-isoPGF2α and 20-hydroxyeicosatetraenoic acid (20-HETE), if they can be released by 5-HT. Both can contract certain vessels and both have been shown, in some cases, to be dependent on COX activity and the endothelium for this action (46 – 49). 8-iso-PGF2α and 8-iso-PGE2 have been shown specifically to strongly constrict rat cerebral arterioles in vivo (50). Interestingly, the treated vessels remained slightly constricted even after 6 wash-outs, and in our experiments with 5-HT on 1-NA-treated arteries, we also had great difficulty in eliminating the contraction.

In human small pial arteries (381 ± 21 μm), Thorin et al. (25) found that 5-HT-induced contractions were largely mediated by endothelin (ET) release from the endothelium. However, their experiments completely preclude the involvement of COX metabolites since the whole experimentation was performed under 10^-5 M indomethacin. Nonetheless, one could envisage that, if 5-HT induces ET release from the rabbit basilar artery endothelium, and if ET acts via a COX metabolite (but not TXA2) as suggested by Munger et al. (51) for the renal circulation, then some of the contraction could be induced via ET.

Our results thus seem compatible with three different mechanisms of vasomotor actions by 5-HT in the rabbit basilar artery. Besides a direct contractile action on smooth muscle (residual effect after indomethacin blockage), 5-HT stimulates endothelial NOS, releasing NO or a labile nitroso compound that causes the smooth muscle to relax. This effect occurs notably at concentrations from 3 × 10^-9 M upwards (Fig. 3). It seems likely that, in vivo, the precise contribution of 5-HT-released NO will depend on the prevailing level of endothelial NOS activation. 5-HT also stimulates the production of a COX-dependent eicosanoid or a radical by the endothelium. The present experiments indicate that thromboxane A2 is not the major COX metabolite responsible for the indomethacin-sensitive fraction of the contraction to 5-HT in these arteries. It is well known that endothelial cells play a major role in determining vascular reactivity, and the present data on a cerebral artery underscore the complex nature of their participation in the reactivity to the essentially pathological stimuli mediated by 5-HT.

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