NO Synthase Blockade Induces Chaotic Cerebral Vasomotion via Activation of Thromboxane Receptors

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Background and Purpose—Instability of the vascular tone (vasomotion) develops in several cerebrovascular diseases associated with endothelial dysfunction. The aim of the present study was to characterize cerebral vasomotion induced by diminished NO production with quantitative evaluation and chaos analysis. We tested the hypothesis that activation of thromboxane receptors mediates chaotic vasomotion after NO synthase (NOS) inhibition.

Methods—Measurements of vascular tension were carried out in isolated rat middle cerebral arteries. The extent of vasomotion was characterized by tension instability, whereas vasomotion complexity was assessed by chaos analysis.

Results—Blocking the basal NO release by Nω-nitro-L-arginine (L-NA) induced vasomotion, which was further enhanced and became irregular after UTP administration. The NO donor sodium nitroprusside was able to reverse this effect, and stable steady-state conditions reappeared. The guanylyl cyclase inhibitor 1H-(1,2,4)oxadiazolo[4,3-a]quinoxaline-1-one (ODQ) or coapplication of ODQ and L-NA had an effect identical to that of L-NA alone. Vasoconstriction by K+ failed to induce vasomotion in intact vessels or in the presence of L-NA or ODQ. The thromboxane receptor antagonist ICI 192605 dose-dependently attenuated the vasomotion induced by L-NA and UTP, and the thromboxane-receptor agonist U-46619 induced significant vasomotion in intact vessels.

Conclusions—The lack of NO in cerebral vessels provokes vulnerability to chaotic vasomotion, which can be triggered by the administration of UTP, whereas excess NO reverses it to stable conditions. The vasomotion after blockade of the NO-cGMP pathway is mediated by activation of thromboxane receptors. (Stroke. 2001;32:2609-2614.)

Key Words: cerebral circulation • nitric oxide • thromboxanes • vasomotor system • rats

Vasomotion, representing spontaneous rhythmic changes of the vessel diameter, has been described in several vascular beds; its physiological or pathophysiological importance is still not understood. It can be characterized as an adaptation strategy of the vasculature, because change in the pattern or amplitude of vasomotion is capable of setting the vascular resistance and conductance to a desired level.1,2 Alteration of the vascular tension from a stable level to a sine-wave vasomotion was shown to increase vascular conductance.3 However, irregular high-amplitude vasomotion represents collapsed regulation of the vascular tone, which results in inadequate blood supply of the tissues. The appearance of large-scale chaotic patterns in vasomotion reflects the inability of the vascular regulation to respond adequately to various stimuli.

Vasomotion has been described both in the microcirculation and in large vessels of the human brain2,4,5 as well as in experimental animals (cat,6 rat,7,8 and rabbit).9 Examining the characteristics of vasomotion may be a promising tool in diagnostic procedures, because changes in vasomotion are suspected to be the early signs of tissue hypoxia.1,4,5,9 Vasomotion has been reported to appear under various pathophysiological conditions, such as hypertension,10 hypoperfusion,9 and brain ischemia,11 and may predict worsening of these conditions.

Subarachnoid hemorrhage and traumatic brain injury are often associated with instability of the cerebrovascular tone, which may initiate diffuse angioplastic reactions.12 The loss of endothelial NO formation,13–15 increased thromboxane levels,16 and the action of different vasoconstrictor metabolites, especially nucleotides,17,18 are all capable of producing long-lasting vasospasm experimentally. The change of these parameters can be observed in patients with subarachnoid hemorrhage or clinical vasospasm; however, therapeutic approaches are of limited success, partly because the mechanism of the development of vasospasm is still not well characterized. Experimental and clinical observations together strongly suggest that vasospasm is a dynamic pathophysiological mechanism: the severity of the disease and the angiographic signs are poorly correlated and can change abruptly.

In light of the chaos theory and with the help of powerful computing tools, such as chaos analysis, it has become
possible to characterize the unpredictable behavior of dynamic diseases; in some cases, clinical applications are under trial.\textsuperscript{19} To our knowledge, application of chaos analysis in the development of cerebrovascular vasomotion has not been undertaken yet, although it provides an independent addition to the more conventional measurements. Changes of the vascular tension or diameter can be analyzed in this manner, and the output can easily be understood without the intricate knowledge of the underlying mathematics. If a stable equilibrium in vascular tone regulation becomes chaotic, it indicates that the system is unreliable, and its behavior to other stimuli is unpredictable.

Blockade of the NO pathway is reported to induce vasomotion in the large cerebral arteries as well as in the microcirculation of the rat brain,\textsuperscript{2,6,20–24} but limited further data are available regarding the mechanisms of this action. Activation of thromboxane receptors after the inhibition of NO synthesis in rat middle cerebral arteries (MCAs), which reportedly induces vasomotion in some vessels,\textsuperscript{25} has been demonstrated recently. The aim of the present study was to characterize vasomotion in the rat cerebral vasculature with classic quantitative evaluation and chaos analysis of the pattern of vasomotion and to test the hypothesis that the activation of thromboxane receptors may mediate vasomotion after NO synthase (NOS) inhibition.

Materials and Methods

Adult male Wistar rats (n=35) were exsanguinated rapidly under deep ether anesthesia. Ring segments of the MCAs were prepared for measurement of isometric force as described previously.\textsuperscript{26} Special care was taken to preserve the endothelium during preparation. The segments were transferred to 5-mL organ baths filled with a modified Krebs solution of the following composition (mmol/L): NaCl 119, KCl 4.6, NaH\textsubscript{2}PO\textsubscript{4} 1.2, CaCl\textsubscript{2} \cdot 2H\textsubscript{2}O 1.5, MgCl\textsubscript{2} \cdot 6H\textsubscript{2}O 1.2, NaHCO\textsubscript{3} 15, and glucose 10, and the bath solution was bubbled continuously with a humidified gas mixture (90\% O\textsubscript{2}/10\% CO\textsubscript{2}). The MCA segments were mounted on 2-L-shaped tungsten wires (50-\textmu m diameter): one wire was fixed to the bath, and the other was fixed to a force transducer. The vessels were allowed a 90-minute equilibration period, during which the resting tension was adjusted to 1.5 to 2 mN, and the bath solution was warmed to 37°C with repeated washing every 25 to 30 minutes. Thereafter, each segment was exposed to 124 mmol/L K\textsuperscript+ Krebs solution to elicit a reference contraction. After a 60-minute resting period, the functional integrity of the endothelium was tested by application of bradykinin (0.01 to 10 \mu mol/L) after precontraction induced by 150 \mu mol/L UTP. Segments that did not exhibit at least 20\% relaxation of the precontraction were considered to have damaged endothelium and were excluded from the study. Spontaneous vasomotion was recorded continuously for 5 minutes during steady-state conditions and 15 minutes after the application of the NOS inhibitor N\textsuperscript{\textast}nitro-L-arginine (L-NA, 100 \mu mol/L), the guanylyl-cyclase inhibitor 1H-(1,2,4)oxadiazolo[4,3-a]quinoxaline-1-one (ODQ, 10 \mu mol/L), the thromboxane-receptor agonist U-46619 (1 \mu mol/L), or the respective vehicles. Twenty minutes after the application of L-NA, vasomotion was recorded in the presence of UTP (150 \mu mol/L) or 40 \mu mol/L K\textsuperscript+ Krebs solution. The combined effects of L-NA (100 \mu mol/L) and UTP (150 \mu mol/L) on vasomotion were also found in the presence of the thromboxane receptor antagonist 4-(Z)-6-(2-0-chlorophenyl-4-0-hydroxyphenyl-1,3-dioxan-cis-5-yl)hexenoic acid (ICI 192605, 10 or 50 \mu mol/L) or its vehicle. After recording vasomotion in the presence of L-NA and UTP together, vessels received 1 mmol/L sodium nitroprusside (SNP). All solutions were prepared the day of the experiment and were kept on ice until administration. ODQ and ICI 192605 were dissolved in dimethyl sulfoxide; all other drugs were dissolved in saline.

After completing the experiments, 250-second segments of the recordings were digitized and further evaluated by using computer protocols. All values in the text and figures are presented as mean\pm SEM; the number of segments studied is expressed as n. Changes of the vascular tension are expressed as percentage of the maximal reference contraction elicited by 124 mmol/L K\textsuperscript+ Krebs solution. Vasomotion was quantified by calculation of the average distance of all data points from their mean. Statistical analyses were carried out by using ANOVA followed by the Newman-Keuls test for post hoc comparisons or the Student unpaired \textit{t} test, whichever was appropriate. A value of \textit{P}<0.05 was considered significant.

Chaos analysis was carried out by the TSAS program written by Yoshihiro Yamamoto (which can be accessed at ftp://psas.p.u-tokyo.ac.jp). This program used the Grassberger-Procaccia\textsuperscript{27} algorithm (D2 method), which calculates the correlation dimension from a sample of digitized data. The signals were digitized at 6 Hz. The important parameters of D2 calculations were chosen according to suggestions of Griffith\textsuperscript{2}: embedding dimension=12, and \tau=13 data points. Phase-plane portraits were constructed with a delay of 12 data points.

Results

Qualitative Analysis of L-NA–Induced Vasomotion

Under steady-state conditions, all vessels studied had a stable baseline tone with insignificant vasomotion except for one segment, which was excluded from the study because it showed rhythmic activity from the beginning of the experiment. Application of 100 \mu mol/L L-NA resulted in vasostriction and induced vasomotion with frequencies \approx 7 cpm; the activity usually started after 10 minutes of drug application with constrictive bursts. Because vasomotion developed spontaneously in only one third of the L-NA–treated vessels, a quantitative statistical analysis could not be applied. To trigger vasomotion, we applied UTP, which generates propagating elevations of [Ca\textsuperscript{2+}] (Ca\textsuperscript{2+} waves) in the rat cerebrovascular smooth muscle.\textsuperscript{28} Indeed, after a 20-minute incubation with L-NA, an additional application of 150 \mu mol/L UTP increased vascular tone further, and high amplitude vasomotion appeared in all of the vessels studied (Figure 1). No difference could then be observed between vessels, which acted differently after L-NA alone. Vasomotion did not show any respective frequency, and the tension sometimes reached baseline dilation and maximal contraction within a short period of time. Application of 1 mmol/L SNP reduced vascular tone and eliminated vasomotion (Figure 1). To characterize the dynamic activity of the vessels, phase-plane portraits were performed with a delay of 2 seconds. Under steady-state conditions and in the presence of the NO donor SNP, the trajectories formed a fixed point, suggesting a stable equilibrium. However, the application of L-NA, and more prominently, L-NA+UTP, formed trajectories resembling strange attractors (Figure 1). Calculating chaotic dimension values revealed that cases with fixed point–like trajectories are nonchaotic, because the minimum number of variables needed to describe their action is <1. In cases in which the formation of NO was blocked, chaotic dimension values were higher, raising the possibility of low-dimensional chaos. Further investigations were carried out in MCAs treated with L-NA and UTP, because under this condition, vasomotion was always apparent.
Is NOS Blockade–Induced Vasomotion Dependent on the Action of cGMP?
The selective guanylyl cyclase inhibitor ODQ (10 μmol/L), the NOS blocker L-NA (100 μmol/L), or both compounds applied together induced high-amplitude vasomotion in the presence of 150 μmol/L UTP to a similar extent and with similar appearance (Figure 2). Application of UTP alone or together with the vehicle of ODQ did not induce sustained vasomotion, except for occasional short-lived dilatory bursts.

Is NOS Blockade–Induced Vasomotion Dependent on the Increased Vascular Tone?
After the incubation of the vessels with L-NA or ODQ, administration of 150 μmol/L UTP increased not only vasomotion but also the vascular tone. Therefore, we tested whether strong vasoconstriction alone or in combination with the blockade of NO or cGMP synthesis may induce vasomotion by itself. Theoretically maximal contraction induced by 124 μmol/L K’ Krebs solution was stable; no spontaneous constrictions or dilatations appeared (vasomotion 1.2±0.5%, n=9). The 40 mmol/L K’ Krebs solution administered 20 minutes after L-NA (100 μmol/L) or ODQ (10 μmol/L) induced contraction of the vessels to an extent similar to that found with 150 μmol/L UTP but failed to induce any kind of vasomotion (vasomotion 0.9±0.7% [n=5] and 1.1±0.6% [n=5], respectively).

Is NOS Blockade–Induced Vasomotion Dependent on the Activation of Thromboxane Receptors?
Incubating the MCAs with the selective thromboxane receptor antagonist ICI 192605 (10 μmol/L) slightly reduced the vasomotion appearing after the coadministration of L-NA (100 μmol/L) and UTP (150 μmol/L). In 3 of 12 cases examined, the irregular pattern was replaced by a highly regular sine-wave tension oscillation, indicating a simplification of the regulatory cycle (Figure 3B). Higher doses of ICI 192605 (50 μmol/L) totally eliminated vasomotion (Figure 3).

The thromboxane agonist U-46619 at a dose of 1 μmol/L induced vasomotion in control segments with an irregular pattern similar to that of UTP and L-NA combined (Figure 4).

Dynamic analysis of the data showed that the phase-plane portraits of the L-NA + UTP–induced vasomotion are similar.

Figure 1. Qualitative analysis of NOS blockade–induced vasomotion in isolated rat MCAs. The top row of graphs shows original recordings; vascular tension was expressed as percentage of the reference contraction. The bottom row of graphs shows the phase-plane portraits of the respective time series with a delay of 2 seconds. \( T_1 \) and \( T_{11} \) are expressed as percentage of the reference contraction. Under steady-state conditions, vasomotion is insignificant, and the phase-plane trajectory forms a fixed point. After administration of L-NA (100 μmol/L), small-amplitude vasomotion appears with a trajectory forming a noisy cycle. Additional application of UTP (150 μmol/L) after NOS blockade induces high-amplitude irregular vasomotion with a strange trajectory. Administration of the NO donor SNP (1 mmol/L) completely eliminates vasomotion.

Figure 2. Vasomotion after administration of UTP (150 μmol/L) is significantly stronger in the presence of L-NA (100 μmol/L) or ODQ (10 μmol/L) than in vehicle-treated or untreated control segments. Coadministration of L-NA and ODQ does not enhance further the effect of either drug alone. Vasomotion was calculated as the average deviation of all data points from the mean and is expressed as the percentage of the reference contraction. Values are mean±SEM; n indicates the number of segments studied. **P<0.01 vs control.
Calculating the chaotic dimension values confirmed that both agents initiate vasomotion with a similar pattern (D2 values were 1.22 ± 0.04 for L-NA/UTP [n = 10] and 1.17 ± 0.03 for U-46609 [n = 6]; \( P = \text{NS} \)).

Discussion
Basal NO release suppresses cerebral vasomotion and maintains baseline vascular tone. When the formation of NO is inadequate, significant vasoconstriction develops with a consequent drop in blood flow. Under the condition of diminished NO synthesis, the pattern of vasomotion contains chaotic components, which refer to unpredictable behavior. This unpredictable pattern of vasomotion can be triggered by UTP, and stable conditions reappear only after supplementation of NO. Inhibition of cGMP synthesis has effects similar to those of U-46619–induced vasomotion (Figures 1 and 4). Calculating the chaotic dimension values confirmed that both agents initiate vasomotion with a similar pattern (D2 values were 1.22 ± 0.04 for L-NA + UTP [n=10] and 1.17 ± 0.03 for U-46609 [n=6]; \( P = \text{NS} \)).

Evidence that these receptors play a role in cerebrovascular vasomotion.

This is the first indication that UTP may enhance the vascular effects of NOS blockade. Under pathological situations, when UTP is released in large quantities by platelets and degrading tissue, this interaction may markedly influence vascular tone and reactivity. In the absence of NO, vasomotion appears in different vascular beds and has been previously described in isolated basilar arteries as well as the microcirculation of the parietal cortex, supplied by the MCAs (see review). Griffith, Parthimos, and colleagues have studied the fractal nature of vasomotion in isolated rabbit ear arteries and have found similar synergism between NOS blockade and histamine. In intact cerebral arteries, intraluminally applied UTP induces dilation through the action of NO and endothelium-derived hyperpolarizing factor. However, when UTP is applied to both intraluminal and extraluminal surfaces, this effect is masked by the
stronger vasoconstriction. In the case of impaired NO synthesis, the imbalance between these 2 opposite effects may enhance vasomotion.

NO has been shown recently to have cGMP-independent effects. Previous studies in cerebral vessels have shown that the guanylyl cyclase inhibitor ODQ (10 μmol/L) and L-NA (100 μmol/L) constrict rat MCAs to a similar extent and that after the blockade of guanylyl cyclase, administration of L-NA fails to cause additional contraction. With a focus on vasomotion, ODQ had an effect similar to that of L-NA in the present study, and coadministration of the 2 drugs had an identical effect as well. In an in vivo study of Lindauer et al., ODQ has been found to induce smaller amplitude vasomotion than does NO blockade. This may be related to the dosage of ODQ in that study, because it induced only 46% reduction of the cGMP levels, whereas L-NA decreased it by 65%. Furthermore, because administration of a constant level of exogenous cGMP did not eliminate the flow oscillations after L-NA or ODQ, they concluded that the inhibitory effect of NO on microcirculatory vasomotion is not mediated by cGMP. This seems to be in contradiction to our conclusion, which may be ascribed to the different mediator mechanisms of NO in large pial vessels and at the level of microcirculation. However, we propose an alternative hypothesis that could explain both observations together: when soluble guanylyl cyclase is not stimulated by NO, oscillatory changes of cGMP levels develop. If this is the case, a constant level of exogenous cGMP cannot eliminate the vasomotion induced by NO blockade. Fluctuations of the cGMP levels may evoke vasomotion via thromboxane receptors, because the sensibility of these receptors is regulated by cGMP-dependent protein kinase.

Vascular tone modulates cerebral vasoreactivity; eg, decreased or increased blood pressure may induce cerebral vasomotion in vivo. Because both L-NA and UTP have vasoconstrictor effects, it had to be clarified whether the increase in vasomotion in the presence of L-NA and UTP was a consequence of the increased vascular tone. Vasoconstriction induced by K+ failed to induce vasomotion and also failed to show a UTP-like synergistic effect after NO or guanylyl cyclase inhibition. Therefore, the contractile effect of L-NA and UTP may not be responsible for the vasomotion that develops in the absence of NO.

Both L-NA and UTP are well-known vasoconstrictors, and it is widely accepted that NO blockade enhances vasomotion or flow motion. However, the synergism in the induction of vasomotion of these 2 compounds is surprising and may refer to a common underlying mechanism. Thromboxane A2 (TXA2), an effective vasoconstrictor prostanoïd, has recently been found to mediate the contractive response to both NO blockade and UTP. Because thromboxane has been found to mediate vasomotion in various vascular beds, it makes a good candidate for this hypothetical common oscillator. In the present study, the thromboxone receptor antagonist ICI 192605 dose-dependently suppressed, whereas the thromboxone agonist U-46619 induced, vasomotion. Furthermore, the pattern of the thromboxone receptor agonist–induced vasomotion is similar to that caused by NO inhibition. Taken together, these observations suggest that activation of thromboxone receptors contributes to the induction of vasomotion after NO blockade.

This hypothesis is also supported by our in vivo observations in rats. In a recent study, we found cerebrocortical blood flow fluctuations in 5 of 17 cases after blockade of NO synthesis. However, in the 12 cases in which the thromboxone receptor antagonist SQ 29548 was applied in a dose of 1.25 mg/kg before induction of NO blockade, this phenomenon never occurred. These results also indicate that the thromboxone receptor–mediated vasomotion of large cerebral vessels after NO blockade appears to influence the blood flow of the brain.

Pronounced release of TXA2 and UTP from aggregating platelets and the damaged tissue has been demonstrated in the different types of stroke and traumatic brain injury. These substances, together with the diminished NO synthesis, which is a common feature in these pathophysiological states, may induce the instability of the cerebrovascular tone and aggravate ischemic brain damage. Activation of thromboxone receptors appears to be a key event in this process, inasmuch as the action of UTP is mediated, at least in part, by TXA2. Furthermore, dysfunction of the NO-cGMP pathway leads to increased microglial TXA2 production and to disinhibition of thromboxone receptors. The complex interactions of these factors are summarized in Figure 5.

Calculated chaotic dimension values of the L-NA+UTP–induced vasomotion and the U-46619–induced vasomotion were almost identical. These data indicate the importance of thromboxone receptors in the mediation of cerebral vasomotion. Shifting the working point of vasomotor regulation from a stable equilibrium to a chaotic state is potentially reversible, as demonstrated in the present study by the application of a thromboxone receptor inhibitor or an NO donor. However, in a chaotic regulatory situation, the response of the vessels to different stimuli cannot be foreseen, inevitably leading to an irreversible unresponsive state of deregulation, as seen in clinical vasospasm. Investigation of the pattern of vasomotion can be a useful tool in predicting the behavior of cerebral vessels well before the clinical signs of vasospasm occur.

The main finding in the present study is that the lack of NO in cerebral vessels provokes vulnerability to chaotic vasomotion. The unpredictable behavior can be triggered by the administr-
tion of UTP, and excess NO reverses it to stable conditions. Cerebral vasomotion after blockade of the NO-cGMP pathway is mediated by the activation of thromboxane receptors.

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References


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