Simplification of the quasiperiodic route to chaos in agonist-induced vasomotion by iterative circle maps

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De Brouwer, S., D. H. Edwards, and T. M. Griffith. Simplification of the quasiperiodic route to chaos in agonist-induced vasomotion by iterative circle maps. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H1315–H1326, 1998.—We have shown that the patterns of vasomotion induced by histamine in isolated rabbit ear resistance arteries can be described in terms of iterative circle maps that model the dynamics of coupled nonlinear oscillators. Cyclopiazonic acid (CPA), an inhibitor of the sarcoplasmic reticulum Ca\textsuperscript{2+}-adenosinetriphosphatase pump, consistently transformed chaotic behavior into characteristic periodic oscillations known as mixed-mode responses, which consist of mixtures of large- and small-amplitude excursions and represent frequency-locked states. Quasiperiodicity, which reflects the interaction of oscillators with incommensurate frequencies, was also observed, although in a smaller number of experiments. The patterns of mixed-mode complexes found at different CPA concentrations allowed the derivation of firing numbers, i.e., number of large oscillations/sum of number of small and large oscillations, and the sequences in which they emerged conformed to Farey arithmetic. Two-dimensional return maps derived by Poincaré section of phase space representations of the dynamics were used to compute the mean number of rotations per iteration on the circle, i.e., the winding number. Plots of winding number against firing number revealed a devil’s staircase-type structure. Experiments with verapamil, a voltage-operated L-type Ca\textsuperscript{2+}-channel antagonist, confirmed that influx of extracellular Ca\textsuperscript{2+} was essential to sustain chaos, quasiperiodicity, and mixed-mode responses. Nonlinear coupling between cytosolic and membrane events in rabbit ear arteries thus results in a self-organized dynamics that collapses to that predicted by the theory of simple circle maps.

sarcoplasmic reticulum; cyclopiazonic acid; verapamil; mixed-mode dynamics; devil’s staircase; Poincaré section

PREVIOUS STUDIES HAVE SHOWN that the vasomotion induced by histamine in buffer-perfused rabbit ear resistance arteries is an endothelium-independent phenomenon that may be considered chaotic (11–13). This classification is based on observations of generic patterns of behavior that represent “universal” routes for the transition from regular to irregular dynamics and include a period-doubling cascade and intermittency (11). Estimates of a nonlinear statistical index, the correlation dimension, further indicate that vasomotion in rabbit ear arteries is of relatively low intrinsic complexity, being equivalent to the behavior of a nonlinear system with just four dynamic state variables (11). Analyses of the effects of specific pharmacological probes on experimental signals have identified two distinct components: 1) a “slow” cytosolic oscillation (period = minutes) generated by Ca\textsuperscript{2+} cycling between the cytosol and the sarcoplasmic reticulum (SR) and 2) a “fast” membrane oscillation (period = seconds) generated by voltage-dependent Ca\textsuperscript{2+} influx through L-type channels that is opposed by the coordinated activity of multiple membrane ion transport systems that promote hyperpolarization (8, 11, 12, 14).

Nonlinear interactions between these oscillators may permit the emergence of highly characteristic oscillatory behavior known as quasiperiodicity and mixed-mode dynamics, in addition to chaos (11, 13, 14). In the present study, we have used pharmacological interventions to gain insights into the intracellular coupling mechanisms ultimately responsible for these different patterns of response and to define the relationship between them. Although mixed-mode behavior may occasionally be observed in the presence of normal Ca\textsuperscript{2+} uptake by the SR, it appears consistently after pharmacological inhibition of the SR Ca\textsuperscript{2+}-adenosinetriphosphatase (ATPase) pump with cyclopiazonic acid (CPA) or thapsigargin (11, 13, 14). In the present study, graded concentrations of CPA were used to induce mixed-mode behavior, and because the action of this agent is readily reversible on washout in rabbit ear arteries, specific protocols could be reversed or repeated (14). The role of Ca\textsuperscript{2+} influx in the maintenance of the patterns of response observed in the presence of CPA was investigated with the Ca\textsuperscript{2+} channel antagonist verapamil.

In their simplest form, mixed-mode complexes may be classified according to the notation M\textsuperscript{n}, where M large amplitude excursions are associated with n small peaks. These represent the frequency-locked states of resonant oscillators that synchronize when the ratio of their “natural” frequencies is a rational fraction, i.e., p/q, where p and q are integers (26, 27). The dynamics of such behavior is therefore periodic and when represented in phase space can be visualized as a closed orbit on the surface of a three-dimensional torus. By contrast, when the dynamics is quasiperiodic, the frequency ratio of the coupled oscillators is irrational and the trajectories of their combined motion ultimately cover the surface of a torus completely because no orbit ever repeats twice. More complex chaotic orbits may arise when motion on the torus becomes unstable and its surface wrinkles and fragments, a scenario known as the quasiperiodic route to chaos (19, 27). The patterns of motion on the surface of a torus can be visualized in two dimensions by taking a planar section perpendicular to its axis (a Poincaré section) to construct a return map. When the behavior is periodic, the trajectories of the system intersect this plane at a finite number of points, whereas the intersections of quasi-periodic orbits lie on a closed curve that corresponds to the surface of the torus. Iterative one-dimensional maps constructed from Poincaré sections allow further
simplification of the dynamics, the least complicated representation being the sine circle map, which models the progression of trajectories around a circle corresponding to the surface of a smooth torus in cross section.

Constructions of circle maps have provided useful representations of a wide range of experimental systems including oscillating Josephson junctions (4, 29), fluctuations in crystal electrical conductivity (17), and cardiac arrhythmias (9). In the present study, we show that phase space portraits of rabbit ear artery vasomotion and the iterative maps derived from them by Poincaré section similarly provide evidence for the existence of motion on the surface of a torus. When the coupling between two oscillators in a quasiperiodic system becomes sufficiently strong, their frequencies may become locked into a rational ratio. Plots of such frequency-locked states as a function of the ratio of the natural frequencies of the oscillators generate a staircase function. The derivation of such a staircase from Poincaré sections of experimental signals of rabbit ear artery vasomotion confirms that frequency-locked states and an associated quasiperiodic transition to chaos closely conform to the scenario described by discrete, iterative circle maps.

**METHODS**

Experiments were performed on isolated ear preparations from male New Zealand White rabbits killed by injection of pentobarbital sodium (120 mg/kg iv) as previously described (11–14). First-generation vessels (1–1.5 cm long and ~150 µm in diameter) branching from the central artery were identified and perfused with oxygenated (95% O2-5% CO2) Krebs-Henseleit buffer (composition in mM: 120 NaCl, 5 KCl, 2.5 CaCl2, 1.3 NaH2PO4, 25 NaHCO3, 11 glucose, and 10 sucrose, pH 7.2–7.4) at 35°C in situ via a cannula secured in the central ear artery, which was itself ligated distally so as to divert all flow into the artery under study. The distal end of each vessel was cut to allow free outflow of perfusate, and side branches were ligated where possible. Average flow was maintained at 0.5 ml/min in all experiments, and superimposed fluctuations in flow and perfusion pressure were monitored continuously by a transonic flow probe and a pressure transducer connected via a side arm to the perfusion circuit. Most experiments were conducted in the presence of 50 µM N6-nitro-L-arginine methyl ester (L-NAME) to inhibit nitric oxide synthase and eliminate secondary effects dependent on nitric oxide production. Histamine hydrochloride, L-NAME, verapamil hydrochloride, and CPA were obtained from Sigma, and solutions were freshly prepared on the day of each experiment.

Theoretical considerations. The simplest circle map (the sine map) consists of the iteration

\[
\theta_{n+1} = f(\theta_n) = \theta_n + \Omega - (K/2\pi) \sin(2\pi\theta_n)
\]

where \(\theta_n\) is the angle (normalized to 2π) that corresponds to the nth iterate around the circle, K is an index of the strength of the coupling between the two oscillators generating the dynamics and thus the nonlinearity present in the system, and \(\Omega\) is the ratio of the frequencies of these oscillators in the absence of coupling (K = 0). This map may be considered as the prototype for more complex behavior generated by a general class of two-dimensional circle maps with which it possesses close dynamic similarity. In such maps, there are two iterated variables that couple a radial coordinate, \(r_n\), with an angular coordinate, \(\theta_n\), such that \(\theta_{n+1} = f(\theta_n, r_n)\) and \(r_{n+1} = g(\theta_n, r_n)\) where g and f are arbitrary functions but f is periodic such that \(f(0 + 1) = f(0) + 1\) (19, 27). In the sine circle map, \(r_n\) is constant and equal to 1.

The average number of rotations per iterate on a circle map, which is a measure of the actual periodicity of the system, may be calculated as the winding number

\[
W(K,\Omega) = \lim_{n \to \infty} \frac{\lfloor f(\theta_0) - \theta_0 \rfloor n}{n}
\]

In the case of the sine circle map it follows directly from Eq. 1 that \(W = \Omega\) only in the absence of coupling (i.e., K = 0). It follows that the nonlinearity present in the system modifies its “intrinsic” periodicity. In coupled systems, the variable \(\theta_n\) may converge under iteration to a series that is periodic (\(\theta_{n+p} = \theta_n + p\) with rational winding number \(W = p/q\), quasiperiodic (when the winding number is irrational), or chaotic (where the map behaves irregularly). In the case of periodic and quasiperiodic behavior, the relationship between the position of present and future states of the sine circle map changes monotonically and this smooth progression around the perimeter of a torus is reflected in the map (Fig. 1A). Chaos becomes possible only when iterates on the map no longer behave in a monotonic fashion, i.e., the map becomes noninvertible. For the sine circle map, it is readily shown that this occurs when the map develops a local maximum and a local minimum at \(\theta = 1 - (1/2\pi)\cos^{-1}(\Omega, K)\) or \(1/2\pi\cos^{-1}(\Omega, K)\), respectively (Fig. 1A). These extrema exist only for \(K > 1\), so that the line \(K = 1\) marks the possibility of chaos (Fig. 1B).

A plot of \(W(K,\Omega)\) as a function of K takes the form of a devil’s staircase, so called because for certain values of K and \(\Omega\) it has infinitely many steps (e.g., the sine circle map at \(K = 1\); Fig. 1C). There is then a self-similar fractal structure governed by Farey arithmetic, which provides a compact method for representing the sequences that are generated by the mixing of two basic states (2, 18, 25). Rational fractions of the form \(p/q\) may be organized into self-similar levels of increasing denominator q by Farey numbers according to the rule that the mediant between \(p/q_1\) and \(p/q_2\) is \((p_1 + p_2)/(q_1 + q_2)\) with the unit interval designated as \(0/1, 1/1\). For \(0 < K < 1\) in the sine circle map, the winding number locks in at every rational \(p/q\) present in a nonzero interval of \(\Omega\), although the staircase remains incomplete because there are quasiperiodic intervals between the frequency-locked steps. The regimes in \(K\)-space where \(W\) assumes rational values are called Arnol’d tongues, between which the winding number is irrational (Fig. 1B). The widths of the frequency-locked resonances grow progressively with K until, at \(K = 1\) (“criticality”), they expand to fill a critical line and exclude all quasiperiodic orbits. At \(K = 1\), the devil’s staircase is thus said to be complete, because the probability of finding a rational winding number equals one (Fig. 1C). Above \(K = 1\), an infinite number of smaller steps are squeezed out when two steps on the staircase overlap and the trajectories of the system are then able to jump between the overlapping resonances in an irregular fashion. This is the mechanism that allows the appearance of chaos, although for \(K > 1\) there may still be apparently ordered but superstable behavior associated with the previous mode-locked states. In two-dimensional circle maps, there is no unique value of K defining a horizontal critical line analogous to that for \(K = 1\) in the sine circle map, but rather a “critical curve” on the \(K\)-plane where the measure of the frequency-locked intervals is complete and above which they begin to overlap. In contrast to the behavior of the sine map beyond criticality, the surface of the torus underlying a two-dimensional circle map may no
longer be smooth but may become wrinkled or fragmented. Correspondingly, the intersections appearing on a Poincaré section may not be continuously distributed (18, 27).

In the case of vasomotion in rabbit ear arteries, the natural frequencies of the contributing cytosolic and membrane oscillators cannot be determined experimentally, and both may vary during interventions (see RESULTS) so that their ratio is not fixed. Some insight into the relationship between the winding number and the natural frequencies of the contributing oscillators and the strength of the coupling between them can be obtained by considering instability near a frequency-locked plateau in the sine circle map. The increment in phase \(\theta\) between two iterations can be obtained by considering instability near a frequency for small, and the map may be represented as the continuous differential approximation

\[
\frac{d\theta}{dn} = \Omega - K/2\pi \sin 2\pi \theta
\]

which can be integrated to give

\[
\theta = (1/\pi) \tan^{-1} \left[ (K/2\pi) - (w/\Omega) \tan (w+n) \right]
\]

where

\[
w = W(K,\Omega) = \left[ \Omega^2 - (K/2\pi)^2 \right]^{1/2}
\]

This illustrates, albeit for a specific region of the staircase, how the periodicity of the map (the winding number) reflects both the ratio of the natural frequencies of the two interacting oscillators and their coupling strength.

Derivation of circle maps from experimental data. Standard techniques from dynamic systems theory were used to analyze the interaction of the two contributing oscillators after preprocessing of experimental data by singular value decomposition. This transformation is a noise-reduction technique that “disentangles” phase space trajectories without altering winding numbers because it is a one-to-one linear mapping that preserves the topology of phase space (1). Measurements of flow or pressure at time \(t\), \(z(t)\), were embedded in an \(m\)-dimensional space by means of a time delay, \(\tau\), whose optimal value was found by inspection to be of the order of \(\approx 4\) s. This procedure generated a state matrix \(\mathbf{Z}(t), \mathbf{Z}(t+\tau), \ldots, \mathbf{Z}[t+(m-1)\tau]\) where \(\mathbf{Z}(t) = [z(t), z(t+\tau), \ldots, z(t+m\tau)]\) for signal lengths varying between \(n = 500-2,000\) points. Because the nearly periodic trajectories used to derive winding numbers lie on the surface of a torus, which on small scales of measurement has a topological dimension of \(2\) (8), an embedding dimension of \(m = 4\) was considered sufficiently large.

The eigenvalues of the first four principal eigenvectors generated by decomposition of the state matrix varied from preparation to preparation, but in each case the values obtained were insensitive to the length of the signal used. Their relative magnitudes were generally of the order 1, 0.8 ± 0.15, 0.8 ± 0.15, and 0.04 ± 0.01, thus confirming that the important dynamic information present in the original signal was preserved in the first three principal eigenvectors (1). The attractor was therefore reconstructed by plotting these eigenvectors against each other. Poincaré sections were then taken across suitably chosen planes, and the sequences in which trajectories intersected these planes were used to construct one-dimensional discrete iterative return maps analogous to those of Fig. 1A. The winding number for these maps was computed according to Eq. 2, with \(\theta_n\) the angle of the \(n\)th point on the Poincaré section measured with respect to an origin within the loop formed by the section, selected as the center of mass of the points on the plane. According to Eq. 2, computations of the winding number for a small set of points will depend on both \(\theta_0\) and \(n\). A robust estimate, i.e., independent of \(\theta_0\) was therefore obtained from a least-squares linear approximation of a plot of \(\theta(n)\) against \(n\) with
Fractal dimension of the devil’s staircase. An index of the global structure of the staircase can be obtained by calculating the dimension of the intervals between the steps as follows. For a given pair of intervals $p_i/q_i$ and $p_j/q_j$, the length of the interval between them is denoted $S_{ij}$. If the daughter state $(p_1 + p_2)/(q_1 + q_2)$ is found and the gaps between this state and its parents are denoted as $S_1$ and $S_2$, respectively, the fractal dimension of the staircase $D$ may be obtained from

$$\sum \left( \frac{S_i}{S_j} \right)^D = 1 \quad (6)$$

if the construction is continued until a sufficiently large number of gaps of sizes $S_i$ are found (18, 27). This formulation of fractal dimension may be regarded as a generalization of the Hausdorff box counting algorithm (19). Numerical estimates of the fractal dimension of the staircases generated by the sine circle map and more general two-dimensional circle maps provide evidence for universal scaling behavior with $D = 0.87$ at the onset of criticality (4–6, 15). Estimates of $D$ for the sine circle map based on only two gaps in the staircase have been found to be accurate to within a few percent (15).

Statistics. The principal frequencies of the mixed-mode oscillations observed at different concentrations of CPA and verapamil were determined by fast Fourier transform and, in the case of verapamil, were compared by the Student’s $t$-test, $P < 0.05$ being considered as significant. It was not always possible to identify definitively the frequency corresponding to the fast membrane oscillator because of overlapping harmonics generated by the slow component.

RESULTS

Mixed-mode behavior was observed in two arteries after administration of histamine or the combination of histamine plus L-NAME (see, e.g., Figs. 2 and 5A). In the example of Fig. 2, administration of 50 µM L-NAME transformed periodic mixed-mode complexes induced by histamine into a quasiperiodic signal in association with a rise in perfusion pressure. Phase space portraits constructed from these time series confirmed that the trajectories of the dynamics could be visualized as motion on the surface of a torus, with a Poincaré section approximating a closed loop in the quasiperiodic case and thus providing evidence for the existence of two interacting oscillators with an incommensurate frequency ratio (Fig. 2). In a further 34 arteries 1 or 2.5 µM histamine induced obviously chaotic oscillations in the presence of 50 µM L-NAME. CPA consistently (i.e., in >95% of all preparations) transformed such responses into behavior possessing the typical characteristics of mixed-mode dynamics before ultimately abolishing rhythmic activity at high concentrations and causing a concentration-dependent fall in mean perfusion pressure (Figs. 3–6). Chaotic behavior tended to persist at low CPA concentrations (see, e.g., Figs. 3A and 6B) but was sometimes transformed (~70% of all preparations) into quasiperiodicity before the appearance of mixed-mode complexes (see, e.g., Fig. 4A). Chaotic patterns could nevertheless occasionally emerge from simpler patterns of quasiperiodic or mixed-mode dynamics on administration of CPA (see, e.g., Fig. 4B) or increases in the concentration of CPA present in the perfusate (see, e.g., Fig. 6B).

In most instances, mixed-mode complexes were periodic or nearly periodic and exhibited stable $M^n$ patterns (Figs. 2–6), whose frequency decreased progressively with CPA concentration before vasomotion was abolished (see, e.g., Fig. 3A). The number of small oscillations, $n$, present in each complex was inversely related to the concentration of CPA administered, so that the simplest patterns, i.e., $1^1$ or $1^2$, became apparent just before the disappearance of all oscillatory activity (Figs. 3 and 6). Fast Fourier transforms were used to estimate the “effective” (rather than natural) frequencies of the contributing slow and fast subsystems during typical periodic mixed-mode behavior in which both components could clearly be distinguished ($n = 44$; Fig. 3B). Both oscillators exhibited an approximately linear decline in frequency as a function of the log of CPA concentration, and a linear least-squares fit to the experimental data showed that this was approximately sixfold more pronounced in the case of the fast subsystem, thus reflecting convergence of the two oscillators toward a single frequency (i.e., an exactly synchronized $1^0$ state) before the abolition of rhythmic activity.
mixed-mode patterns (exhibited a linear falloff that was similar to that emerged over a range of CPA concentrations, also concentration of CPA was varied (Figs. 5
by Farey arithmetic could also be identified as the forming to the sequences of rational numbers related the form 1
Theoretically, there may be daughter concatenations of components of typical mixed-mode signals as a function of log [CPA]
A

Fig. 3. A representative traces from an artery perfused with 1 µM Hist and 50 µM L-NAME in which chaotic fluctuations in flow (top) and pressure (bottom) were transformed into mixed-mode behavior on administration of cyclopiazonic acid (CPA) with an associated fall in perfusion pressure. Chaos and mixed-mode states are identified as χ and as 12 and 10, respectively, and are evident in both signals. CPA progressively slowed oscillatory behavior (and in this example increased its amplitude) before vasomotion was finally abolished. Frequencies indicated were calculated from period between large-amplitude components of mixed-mode complexes (note 8-min time gap between peaks in traces for 9 µM CPA). [CPA], CPA concentration. B: plots of frequencies of principal slow (χ) and fast (ψ) components of typical mixed-mode signals as a function of log [CPA] (dotted lines). Frequency of 10-type mixed-mode complexes is plotted separately because slow and fast oscillators then behave synchronously (●, solid line). In each case, a linear least-squares approximation was applied to the data points.

Plots of the frequency of such 10 complexes, which emerged over a range of CPA concentrations, also exhibited a linear falloff that was similar to that observed for the slow component of more complex mixed-mode patterns (n = 19).

Concatenations of periodic mixed-mode states conforming to the sequences of rational numbers related by Farey arithmetic could also be identified as the concentration of CPA was varied (Figs. 5B and 6B). Theoretically, there may be daughter concatenations of the form 1p/q between states of the form 1q and 2q, but these were not always identified experimentally. Moreover, in some instances the dynamics was clearly mixed mode in form but the overall behavior was aperiodic because the number of small oscillations present was variable (see, e.g., Fig. 5A). Transitions between states satisfying Farey arithmetic were sometimes unstable, with the system drifting reversibly between different frequency-locked modes after a few cycles (e.g., 12 and 11; Fig. 5C). In some arteries it was not possible to classify the observed complexes according to the standard method of notation, and they are most appropriately described as chaotic while nevertheless retaining features reminiscent of mixed-mode dynamics (see, e.g., Fig. 6B).

In five experiments, the concentration of CPA perfusing the arteries was first increased in a graded fashion to the point at which vasomotion was abolished and then decreased to restore oscillatory activity (see, e.g., Fig. 6A). The precise patterns of mixed-mode dynamics observed were generally found to differ during these reciprocal experimental protocols at equivalent concentrations of CPA. This is likely to reflect hysteresis rather than transient behavior, because at least 20 min of response were always recorded at each CPA concentration to ensure that a steady state had been attained. In four arteries, CPA was administered in a graded fashion until vasomotion was abolished and was then washed out with Holman’s buffer containing the same concentration of histamine and L-NAME for 30–40 min. When the experimental protocol with CPA was repeated, identical patterns of mixed-mode dynamics were usually not seen at equivalent concentrations of CPA in such consecutive experiments (see, e.g., Fig. 6B). The unpredictable nature of these observations can be interpreted as being characteristic of a chaotic system.

Two-dimensional projections of the attractor of a nearly periodic 12 mixed-mode state induced by administration of CPA are shown in Fig. 7, B and C, and a Poincaré section through the associated attractor is shown in Fig. 7D. Careful inspection of the experimental trace shows that the peaks of the signal vary slightly from complex to complex. The spatial distribution of the points on the corresponding Poincaré section confirms that the dynamics is not strictly periodic, because the data points cluster into regions of the plane in such a way as to suggest the existence of an underlying torus whose surface is not smooth, but fragmented. This behavior is to be expected generically in two-dimensional circle maps close to the transition to chaos (4). In the case of fluctuations in flow that were clearly chaotic (Fig. 8), phase space portraits show that the trajectories of the dynamics were much less well localized to specific regions of phase space than those of the mixed-mode dynamics depicted in Fig. 7, and the corresponding Poincaré section also has a more complex structure. One-dimensional circle maps derived from the Poincaré sections of these signals are shown in Figs. 7E and 8E and in the irregular case exhibit a well-defined minimum and maximum. This noninvertibility confirms that the underlying behavior is chaotic.

Plots of winding number against firing number, which in the present study is defined as the fraction M/(M + n) for a nearly periodic mixed-mode oscillation of the form Mₙ and (M₁ + M₂)/(M₁ + M₂ + n₁ + n₂) for a concatenation of the form M₁⁰M₂, reveal a devil’s
Fig. 4. Trace showing complex effects of CPA in arteries perfused with 2.5 µM Hist and 50 µM L-NAME. A: administration of 2 µM CPA initially transformed chaotic behavior (χ) into quasiperiodic dynamics (Q) before emergence of stable 12 mixed-mode complexes. B: preparation in which graded increases in CPA transformed a quasiperiodic signal into chaos before inducing mixed-mode behavior. Vasomotion ceased at higher [CPA] in both preparations, with perfusion pressure declining towards preconstriction levels (not shown).

Fig. 5. Traces illustrating a spectrum of mixed-mode patterns in arteries perfused with 1 µM Hist and 50 µM L-NAME. A: aperiodic dynamics. B: high-order concatenations of frequency-locked states in presence of CPA. C: frequency-locked behavior showing unstable drift between 12 and 11 patterns in presence of 2 µM CPA. These complexes were stable both at higher and lower [CPA].

staircase-type structure, although the staircase was incomplete (Fig. 9). In theory, the staircase could be constructed indefinitely for circle maps at criticality if there was sufficiently high experimental resolution, because between any two periodic states there will be an infinite number of other periodic states and concatenations. Then the firing numbers would form an infinite self-similar staircase devil’s staircase as illustrated in Fig. 1 for the sine circle map. However, evidence for the existence of mode-locked states that correspond to narrow Arnol’d tongues and thus narrow steps on the staircase was found only in a few preparations [e.g., (1)3 10 (Fig. 5B) and (1)2(1)2 (Fig. 6B)]. An estimate of the fractal dimension D of the staircase was obtained from the intervals between the 13 and 11 states and the mediant 12 state as described in METHODS, the value obtained being 0.63.

In six preparations, the contribution of Ca2+ influx to the genesis of mixed-mode dynamics was investigated with verapamil. High concentrations abolished rhythmic activity completely and reduced perfusion pressure, but low concentrations tended to transform mixed-mode complexes into quasiperiodic or nearly periodic sinusoidal responses, although chaos occasionally became evident as a transient phenomenon (Figs. 10 and 11). In three of six preparations, low concentrations of verapamil (0.03 µM) induced a “paradoxical” constric-
administration of higher concentrations (see, e.g., Fig. 10). Although verapamil influenced the form and amplitude of the overall dynamic behavior, in marked contrast to CPA, it did not influence the frequency of either contributing oscillator until vasomotion abolished. The peak in the power spectrum corresponding to the slow oscillator was $0.014 \pm 0.001$ Hz in the presence of CPA alone and $0.014 \pm 0.001$ Hz in the additional presence of 0.3 µM verapamil. In the case of the fast oscillator, these frequencies were $0.034 \pm 0.004$ Hz in the presence of CPA alone and $0.032 \pm 0.006$ Hz in the additional presence of 0.3 µM verapamil. In neither case were these values significantly different from each other.

**DISCUSSION**

The present study has provided insights into the dynamic patterns generated by nonlinear interactions between the cytosolic and membrane oscillators that contribute to vasomotion in rabbit ear arteries. These include chaos, quasiperiodicity, and sequences of mixed-mode complexes that conform to Farey arithmetic and can be organized into a devil’s staircase-type structure. The findings thus provide evidence for a quasiperiodic route to chaos via overlapping frequency-locked resonances that can be represented by iterative maps on the circle.

Physiological perspectives. In all experiments, histamine was used to induce rhythmic activity and the resulting fluctuations in pressure and flow were usually chaotic, although simpler quasiperiodic and mixed-mode patterns were occasionally apparent. The contribution of Ca$^{2+}$ sequestration by internal stores to these distinct dynamic patterns was investigated by manipulating the activity of the vascular smooth muscle SR Ca$^{2+}$-ATPase with CPA. Intermediate concentrations of this inhibitor almost always transformed chaotic responses into mixed-mode patterns whose frequency and complexity were inversely related to the concentration of CPA administered, and in ~70% of preparations quasiperiodic dynamics could also be identified. Experiments with verapamil, which modulates L-type Ca$^{2+}$ channel activity, confirmed that oscillatory behavior was sustained by Ca$^{2+}$ influx from the extracellular space, because concentrations $\leq 1$ µM generally suppressed vasomotion completely (12). Low concentrations of verapamil often converted mixed-mode responses to simpler nearly sinusoidal or quasiperiodic behavior, although chaos also sometimes became evident as a transient phenomenon. These observations
confirm that the interaction of the participating oscillators is nonlinear and suggest that coupling between them is effected via Ca\(^{2+}\) movements in the subplasmalemmal space that are influenced by the buffering capacity of the SR and influx of Ca\(^{2+}\) via voltage-operated channels. High concentrations of CPA suppressed the activity of both oscillators completely and caused marked reduc-

Fig. 7. Derivation of a circle map from a nearly periodic flow signal. A: trace exhibiting characteristics of a \(^1\) mixed-mode oscillation (drug concentrations: CPA, 1 µM; Hist, 1 µM; l-NAME, 50 µM). B and C: 2 projections of a 3-dimensional phase portrait of dynamics rotated through \(\pm 90^\circ\). D: Poincaré section showing intersection of positively directed trajectories with plane normal to the paper indicated by line in B and C. This suggests the cross section of a torus, although the spatial localization of the points indicates that the surface of the torus has fragmented, as would be typical near the onset of chaos in a 2-dimensional circle map. E: circle map constructed from Poincaré section, showing clustering in 3 regions of plane. F: angular rotation as a function of iteration number. Slope of this line gives winding number.

Fig. 8. Derivation of a circle map from a chaotic flow signal. A: time series (drug concentrations: CPA, 2.5 µM; Hist, 1 µM; l-NAME, 50 µM). B and C: phase space portraits show that trajectories of irregular experimental signal are less well localized than in Fig. 7, although global structure of underlying attractor is similar. D and E: Poincaré section is complex, and circle map derived from this section exhibits a minimum and a maximum. This implies noninvertibility and confirms that the underlying dynamics is chaotic. F: it was still possible to derive a winding number from Poincaré section, although chaotic nature of flow signal precludes derivation of a firing number.
tions in perfusion pressure. This may be explained by reduced sequestration of Ca\(^{2+}\) entering the subplasmalemmal space, with consequent impairment of the superficial buffer barrier function of the peripheral SR (3, 7, 14, 16, 28). The resulting elevation in near-membrane Ca\(^{2+}\) concentration will promote membrane hyperpolarization by stimulating Ca\(^{2+}\)-activated K\(^+\) channels and enhancing Na\(^+\)-K\(^+\)-ATPase activity secondary to Na\(^+\)-influx via Na\(^+\)/Ca\(^{2+}\) exchange (8, 14, 21, 22, 24). Dynamic coupling between stores and the membrane may be facilitated by anatomic colocalization of the Na\(^+\)/Ca\(^{2+}\) exchanger and the Na\(^+\)-K\(^+\)-ATPase to specific regions of the plasmalemma closely adjacent to the peripheral SR (14, 20, 28). Spatial factors involved in the regulation of Ca\(^{2+}\) movements could also contribute to the paradoxical constrictor response evoked by low concentrations of verapamil in the presence of CPA. Local reductions in high near-membrane Ca\(^{2+}\) concentration may alter the balance between competing mechanisms of contraction and dilatation involving ion transport systems that differ in their proximity to peripheral SR buffering sites.

Quasiperiodic transition to chaos via frequency-locked resonances. The complex patterns of vasomotion observed experimentally were reduced to one-dimensional iterative circle maps after construction of phase space portraits by time-delayed embedding, singular value decomposition, and Poincaré section. When the experimental signals were irregular, these circle maps were noninvertible, confirming that the dynamics could lie beyond criticality within a chaotic regime. The form of the Poincaré sections suggests, however, that rabbit ear artery vasomotion is of intrinsically higher complexity than the behavior of the sine circle map, because they were typical of higher-order circle maps that nevertheless exhibit identical universal scaling properties at criticality (4–6, 27). In the case of nearly periodic mixed-mode signals, Poincaré sections thus often revealed intersection points that clustered in specific regions of the sectioning plane in such a way as to suggest motion on the surface of a fragmented torus. In the context of chemical reactions, similar experimental observations have been documented by Argoul et al. (2), Masek and Swinney (18), and Richetti et al. (25) for the coexisting chaos, quasiperiodicity, and mixed-mode dynamics that can be observed in the Belousov-Zhabotinskii (B-Z) reaction under small variations in reaction conditions. Below criticality, quasiperiodicity and frequency-locked states are generic in circle maps, and transitions between them often became evident during the experimental protocols of the present study. The more frequent occurrence of mixed-mode dynamics may simply reflect the fact that frequency-locked regions are statistically more probable near criticality in circle maps, whereas the probability of quasiperiodicity increases as the coupling strength between the oscillators decreases to zero. Indeed, above criticality, chaos and frequency-locked regions are densely interwoven but quasiperiodic behavior is no longer permitted as the Arnol’d tongues overlap completely (see METHODS).

In two-dimensional circle maps, points that mark the possibility of chaos lie on a bell-shaped critical curve by 10.220.32.247 on July 3, 2017 http://ajpheart.physiology.org/ Downloaded from http://ajpheart.physiology.org/.

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**Fig. 10.** Effects of verapamil in a preparation in which CPA initially transformed a quasiperiodic signal (Q) into \(^{1\text{st}}\)-type complexes. A low concentration of verapamil (0.03 µM; A) stimulated appearance of chaos (\(\chi\)) and “paradoxically” elevated perfusion pressure, whereas higher concentrations (B) induced dilatation and ultimately abolished chaotic vasomotion without emergence of simpler mixed-mode patterns of response.
that relates the strength of the coupling between the contributing oscillators, $K$, to the ratio of their natural frequencies, $\Omega$, on the $K$-$\Omega$ plane (4). Although neither parameter could be measured directly in the present experiments, it is evident that changes in the relative frequency of the membrane and cytosolic oscillators are likely to be an important determinant of the patterns of vasomotion observed. Inspection of the experimental traces indicates that the effective frequency ratio of these oscillators lies between 1/10 and 1/20, and this may be taken as an approximate index of $\Omega$. In the case of the simplest 1$^\text{st}$ frequency-locked state induced by CPA, this ratio increases to unity because there is then exact synchrony. Progressive inhibition of the SR Ca$^{2+}$-ATPase thus resulted in an overall CPA concentration-dependent reduction in both the frequency and complexity of the dynamics, with the simplest patterns being apparent close to the point at which rhythmic activity ceased. As is to be expected, this was associated with an increase in the winding number (calculated from Poincaré sections of the experimental signals) from $\sim 0.2$ to $\sim 0.6$. The continuum approximation of the sine circle map near a mode-locked plateau (outlined in METHODS) illustrates qualitatively how the winding number will increase as the natural frequencies of the contributing oscillators converge and/or the coupling between them decreases.

Fast Fourier transforms showed that the frequency of the membrane oscillator decreased approximately sixfold more rapidly than that of the cytosolic oscillator after increases in CPA concentration, whereas verapamil did not affect the principal frequency of either oscillator until vasomotion was abolished. Furthermore, plots of the frequency of the synchronized 1$^\text{st}$ mixed-mode state as a function of CPA concentration closely matched the slower dominant frequency of more complex mixed-mode patterns. These observations suggest that the overall dynamics is “driven” by the cytosolic oscillator and the influence of the membrane oscillator is correspondingly weak. We have previously shown that a ryanodine-sensitive, Ca$^{2+}$-induced Ca$^{2+}$ release (CICR) mechanism underpins the activity of the intracellular subsystem (12, 14). The intrinsic nonlinearity of CICR readily explains the effects of CPA on the frequency of this subsystem, because free cytosolic Ca$^{2+}$ will trigger the emptying of stores only when they are sufficiently full (10). The diminished sequestration resulting from administration of CPA will consequently prolong the Ca$^{2+}$ cycling time for intracellular uptake and release.

Plots of winding number against firing number (derived by visual inspection of the experimental signals) revealed a devil’s staircase-type structure analogous to that reported for mixed-mode dynamics in chemical systems (2, 25, 26), although the staircase exhibited just three well-defined steps corresponding to low-order states (1$^1$, 1$^2$, and 1$^3$) and was therefore incomplete. Figure 1B illustrates theoretically how the probability of observing a given mode-locked state should be highest for winding numbers that represent the simplest rational ratios (e.g., 0/1, 1/2, 1/1) because there is a hierarchical order with respect to the width of the Arnol’d tongues related to their Farey numbers. It is therefore to be expected that such states will be the easiest to detect experimentally, given the limited pharmacological resolution possible in a complex biological system. Moreover, measurements derived from a Poincaré section through the phase space portrait of experimental signals are highly unlikely to correspond exactly to the critical line or curve that defines the onset of chaos in a circle map, which occurs at a unique set of control parameters, i.e., $K = 1$ for the sine circle map and a specific value of $K$ for each value of $\Omega$ in two-dimensional maps. Away from criticality, the dynamics of the system will lie in a region of the $K$-$\Omega$ plane where the staircase is intrinsically incomplete.
Below criticality, frequency-locked steps occur within relatively narrow ranges and are interspersed with quasiperiodic dynamics. Beyond criticality, the patterns of overlap of superstable Arnol’d tongues become increasingly complicated. Near the critical line or curve only small tongues overlap, whereas at larger values of K wide tongues overlap and high-order states tend to be squeezed out and destroyed by low-order superstable states (see, e.g., Fig. 1B).

The finding that the estimated fractal dimension of the staircase (~0.63) was lower than the universal value that characterizes the complete devil’s staircase of circle maps at criticality (~0.87) also suggests that the dynamics does not correspond exactly to a critical line or curve. The dynamics could, for example, take a complex path across the K-Ω plane under variations of CPA concentration such that its intersection with the Arnol’d tongues distorts their relative widths and estimates of the true dimension of the staircase. Numerical investigations of the scaling behavior of mode-locked intervals in the sine circle map also demonstrate that intervals adjacent to different Arnol’d tongues are compressed at different rates with increasing nonlinearity (i.e., coupling constant K) so that universality in terms of the fractal dimension of the staircase is lost (18). Intervals to either side of wide low-order states are maximally squeezed because of strong resonance, so that calculations employing such intervals lead to a lower value of D than the intervals to either side of narrower tongues that are minimally squeezed. Maselko and Swinney (18) thus obtained values of D in the range 0.67–0.97 for the devil’s staircase in the B-Z reaction, according to which parent states and mediant terms may converge asymptotically to the basins of attraction of separate periodic or chaotic attractors.

Many superstable states can be observed beyond criticality during iteration of circle maps simply by selecting different values of the starting angle θ0, so that sensitivity to initial conditions and hysteresis are present even in the simplest sine circle map. In the present study, identical dynamic patterns were generally not observed at the same concentration of CPA when specific experimental protocols were either repeated or reversed in the same artery. More complex mathematical simulations of mixed-mode behavior with continuous-time systems of differential equations consisting of three interacting nonlinear variables also demonstrate sensitivity to initial conditions and hysteresis (23). Under changes in a single control parameter, such models show that mixed-mode states (e.g., 11, 12, or 13) lie on isolated branches of limit cycles that coexist with regions of chaos formed by complex mixing of parent states to form concatenations, so that the system may converge asymptotically to the basins of attraction of separate periodic or chaotic attractors when different starting points are selected (23). The present experimental observations that high concentrations of CPA sometimes promoted chaotic dynamics after lower concentrations had induced periodic behavior are therefore an expected, rather than surprising, finding in a nonlinear system.

In conclusion, nonlinear crosstalk between the cytosolic and membrane oscillators in rabbit ear arteries allows the emergence of frequency-locked states. Poincaré sections and the one-dimensional iterative maps derived from them thus permit the construction of an incomplete devil’s staircase. The findings provide strong evidence for motion on a torus and suggest that a quasiperiodic route to chaos via overlapping mode-locked resonances is dependent on the kinetics of the SR Ca2+ pump and Ca2+ influx through voltage-operated channels. The dynamics of rabbit ear artery vasomotion thus collapses to behavior that can be described by circle maps, although the Poincaré sections suggest that the surface of the underlying torus may be fragmented. Such findings are more characteristic of two-dimensional circle maps than the simpler one-dimensional sine prototype. The behavior of continuous-time systems subject to external periodic forcing such as the driven pendulum, Josephson junctions, and charge-density waves has analogously been shown to collapse to two-dimensional circle maps (4). Dynamic vasomotion in rabbit ear arteries may, however, be considered “self-organized,” because frequency-locked states appear spontaneously in the absence of an external driving force.

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