Pharmacological Block of the Electrogenic Sodium Pump Disrupts Rhythmic Bursting Induced by Strychnine and Bicuculline in the Neonatal Rat Spinal Cord

LAURA BALLERINI, ENRICO BRACCI, AND ANDREA NISTRI
Biophysics Sector, International School for Advanced Studies (S.I.S.S.A.), 34014 Trieste, Italy

INTRODUCTION

Rhythmic bursts of activity can be experimentally observed in vertebrate spinal neurons, both in vivo and in vitro, and are presumably related to the generation of rhythmic motor patterns such as locomotion or scratching (for review see Rossignol and Dubuc 1994). Much effort has been spent in clarifying the cellular and network properties responsible for these rhythmic patterns with the use of a variety of experimental preparations. In the fictive swimming of the lamprey or tadpole spinal cord, reciprocal inhibition (mediated by glycine and/or γ-aminobutyric acid (GABA)) between antagonist pools of neurons activated by excitatory transmitters represents an essential mechanism for rhythmogenesis (Dale 1995; Grillner et al. 1991). In the rat spinal cord the intrinsic pacemaker properties of N-methyl-D-aspartate (NMDA)-sensitive neurons have been proposed to play a role in rhythmogenesis (Hochman et al. 1994).

Recent experiments on the neonatal rat spinal cord in vitro have indicated that pharmacological block of glycinergic and GABAergic inhibition elicits spontaneous network-driven rhythmic bursts (dependent on activation of non-NMDA glutamate receptors) that, unlike those of fictive locomotion, appear synchronously in all lumbar motoneurons (Bracci et al. 1996a). These spontaneous bursts are longer and slower than those observed during fictive locomotion induced by NMDA or serotonin (Cazalets et al. 1992), although they become as fast as those of fictive locomotion after application of the same drugs (Bracci et al. 1996a,b). In the presence of strychnine and bicuculline, rhythmic activity is likely to be generated by mechanisms different from reciprocal inhibition (because the main inhibitory processes are blocked) and from NMDA-dependent pacemaker neurons (because it can persist in the presence of NMDA receptor antagonists) (Bracci et al. 1996a). In view of the fact that suppression of chloride-mediated inhibition provides a simplified model of spinal circuits, it seems interesting to study the cellular mechanisms that allow the network to express rhythmic bursting.

With the use of pharmacological tools to target membrane sites of network neurons in an isolated spinal cord preparation, the present work probes conductance mechanisms that might allow generation of rhythmic bursting in the disinhibited spinal cord. To this end we have examined the potential role of 1) the Ca2+-sensitive K+ conductance(s) that are gradually activated during a burst in the lamprey spinal cord (El Manira et al. 1994); 2) the slow inward rectifier conductance, which is important in phasing thalamocortical oscillations (McCormick and Pape 1990); 3) the electrogenic Na+ pump, which produces a long-lasting hyperpolarization of
hippocampal neurons during recovery from glutamate-induced depolarization (Thompson and Prince 1986) and the electrogenic activity of which is required for spacing bursts induced by NMDA on midbrain neurons (Johnson et al. 1992). The present data provide for the first time evidence that in the absence of synaptic inhibition the activity of the electrogenic Na\(^+\) pump plays an important role in spinal rhythmogenesis.

**METHODS**

Experiments were carried out on spinal cord preparations isolated from neonatal rats (4–9 day old) under urethane anesthesia (6 ml ip of 10\% wt/vol solution) as described previously (Ballerini et al. 1995; Fisher and Nistri 1993). Spinal cords (from the midthoracic region to the cauda equina) were continuously superfused at 7.5 ml/min with oxygenated Krebs solution of the following composition (in mM): 113 NaCl, 4.5 KCl, 1 MgCl\(_2\), 2 CaCl\(_2\), 1 NaH\(_2\)PO\(_4\), 25 NaHCO\(_3\), and 11 glucose, pH 7.4. Dorsal and ventral root stimulations were performed via a miniature suction electrode with the use of stimuli 0.1 ms in duration, 1.5–2 times threshold intensity (2–5 V) for the shortest-latency synaptic response. For root recordings, lumbar ventral roots (predominantly L\(_5\), although L\(_2\) or L\(_3\) were also used) were connected to miniature suction electrodes filled with Krebs solution. For intracellular recordings, lumbar (L\(_4\) or L\(_5\)) motoneurons (functionally identified on the basis of their antidromic spike) were impaled with 3 M KCl-filled microelectrodes (30–60 M\(\Omega\)) under current-clamp conditions (either in bridge or discontinuous current-clamp mode at \(\sim 2\) Hz). Motoneuron resting membrane potential was \(-74 \pm 7\) (SD) mV (\(n = 15\)). Responses were amplified with an Axoclamp 2A unit, stored on video tape for further analysis, digitized at 1–10 kHz with pCLAMP program (Axon Instruments; version 6.2), and displayed on a chart recorder. Ventral root responses were recorded with either DC-coupled (usually with 30-kHz low-pass filter) or AC-coupled (100 Hz–10 kHz pass band) amplification. An additional 20-Hz low-pass filter was sometimes used. Details concerning the definition of bursts and their measurement (duration and frequency) are as previously reported (Bracci et al. 1996a); cycle period was measured from the start of a burst to the beginning of the next one. Data are expressed as means \(\pm\) SD with statistical significance evaluated with analysis of variance. The following drugs (applied via the bathing solution) were used: bicuculline methiodide (Sigma); strychnine nitrate (Sigma); apamin (Alomone Labs); carbachol (Sigma); ouabain (Sigma); and strophanthidin (Sigma).

**RESULTS**

Data were collected from 39 spinal cord preparations recorded either intracellularly from single motoneurons or extracellularly from one or two homologous ventral roots in the presence of strychnine (1 \(\mu\)M) and bicuculline (20 \(\mu\)M). Under these conditions network-driven rhythmic bursts characterized by intraburst oscillations developed spontaneously in motoneurons of the lumbar segments within \(\sim 10\) min (Bracci et al. 1996a). DC-coupled ventral root recordings were able to monitor potential changes in the motoneuron pool, which closely resembled those recorded intracellularly (compare Fig. 1, A and B, and Fig. 2, A and B). AC-coupled ventral root recordings were also suitable for detecting burst and intraburst oscillation frequency and duration (see Fig. 6). On average, in the presence of strychnine and bicuculline, burst frequency was 28 \(\pm\) 5 s and burst duration was 7.2 \(\pm\) 1.8 s. The relatively slow periodicity and long duration of bursts raised the question of the mechanism(s) responsible for their onset, maintenance, and termination. To examine this issue, it seemed useful to apply pharmacological blockers selective against membrane conductances or transport processes operating at network level.

**Effects of apamin**

Because in the lamprey spinal cord apamin, a selective blocker of the slow Ca\(^{2+}\)-activated K\(^+\) conductance (Sah 1996), prolongs cycle period and duration of bursts induced by NMDA (El Manira et al. 1994), it seemed of interest to test the action of this substance (0.4 \(\mu\)M) on bursting in the disinhibited spinal cord. This treatment was performed in six preparations, examples of which are illustrated with either intracellular (Fig. 1A) or extracellular recording from left and right L\(_5\) ventral roots (Fig. 1B): unlike the case of the

![Fig. 1. Spontaneous rhythmic bursting evoked by strychnine and bicuculline in the isolated spinal cord of the neonatal rat is affected by bath-applied apamin (0.4 \(\mu\)M). A: intracellular recordings from a lumbar motoneuron displaying regular bursting (a single bursting episode, asterisk, is expanded at right to reveal intraburst oscillatory structure) in the presence of strychnine and bicuculline (top) and after 20 min of application of apamin. Note decrease in cycle period and burst duration. Value alongside traces: membrane potentials at rest. B: comparable data recorded from left (l) and right (r) L\(_5\) ventral roots (different preparation from A) at slow (left) or fast (right) timebase. Asterisks: expanded burst episodes. Note similarity of extracellular records to intracellularly recorded responses and analogous action of apamin, which reduces cycle period and burst duration synchronously in both lumbar roots.](image-url)
Rhythmic bursting in thalamic relay neurons depends on the activation of an inward rectifier selectively blocked by Cs⁺ (McCormick and Pape 1990). This monovalent cation (4 mM) was then tested on rhythmic bursting of the rat spinal cord as illustrated in Fig. 2A with recordings from one L₅ ventral root. Although burst frequency was unaffected by this agent, burst duration was significantly increased, as shown also with the faster timebase responses in Fig. 2A, right. Individual oscillations became gradually longer within the burst episode, whereas they had similar duration before the application of Cs⁺. The action of Cs⁺ was reversed after

**Effects of cesium application**

Rhythmic bursting in thalamic relay neurons depends on the activation of an inward rectifier selectively blocked by Cs⁺ (McCormick and Pape 1990). This monovalent cation (4 mM) was then tested on rhythmic bursting of the rat spinal cord as illustrated in Fig. 2A with recordings from one L₅ ventral root. Although burst frequency was unaffected by this agent, burst duration was significantly increased, as shown also with the faster timebase responses in Fig. 2A, right. Individual oscillations became gradually longer within the burst episode, whereas they had similar duration before the application of Cs⁺. The action of Cs⁺ was reversed after

**Fig. 2.** Effects of extracellularly applied Cs⁺ (4 mM) on strychnine- and bicuculline-induced bursting. A: extracellular recording from L₅ ventral root before (top), after 20 min of application of Cs⁺ (middle), and after 20 min of washout (bottom). Responses at right: expansions of events marked by asterisks. Note large prolongation of individual bursts with enhanced oscillatory activity. B: intracellular recording from lumbar motoneuron (different preparation from A) showing individual bursts before (left) and after application of Cs⁺ (right). Note similarity to effects recorded extracellularly and associated with 9-mV hyperpolarization from −70 mV initial resting potential.

In the lamprey spinal cord, in all preparations tested apamin largely decreased cycle period and burst duration (on average by 46 ± 10% and 47 ± 14%, respectively; P < 0.001), as also illustrated by the histograms in Fig. 3A. Expanded timebase tracings in Fig. 1, A, right, and B, right, show that intraburst oscillations persisted even if their number was usually diminished because of reduction in burst duration (Fig. 1, A and B). In the presence of apamin, bursts and intraburst oscillations still appeared synchronously in left and right L₅ ventral roots (Fig. 1B). The effects of apamin (which were not reversible for >2 h of washout) were not associated with any change in motoneuron resting membrane potential, as indicated by intracellular experiments (n = 3). Other pharmacological agents able to block slow Ca²⁺-activated K⁺ conductances are muscarinic agonists (Schwindt et al. 1988; Storm 1990). Carbachol (10 μM) was tested on the rhythmic pattern of the disinhibited spinal cord. Pooled data (see histograms in Fig. 3B) indicate that this agent significantly (P < 0.001) reduced cycle period (by 46 ± 16%) and burst duration (by 55 ± 19%; n = 5) in a manner analogous to that observed with apamin. It is unclear whether any intrinsic cholinergic drive might be modulating rhythmic activity: further experiments in which anticholinesterase inhibitors are used may provide a clue to this question. The present results thus suggest that in the rat spinal cord block of slow Ca²⁺-activated K⁺ conductances accelerated bursting without changing its intrinsic characteristics. It therefore appeared necessary to perform further tests aimed at identifying mechanisms essential for burst generation.

**Fig. 3.** Histograms of effects of apamin (A), carbachol (B), and Cs⁺ (C) on cycle period and burst duration. Data are expressed as % of control bursts. Double asterisks: statistically significant effect (P < 0.001). Number of experiments is indicated in the text.
20 min of washout (see Fig. 2A, bottom). Similar effects were also detected with intracellular recording (Fig. 2B; different preparation from A), which indicated that Cs\(^+\) increased burst duration by 80%. In this cell Cs\(^+\) application caused a resting membrane potential hyperpolarization of 9 mV. Neither repolarizing (via intracellular current injection) the cell to its initial resting potential (−70 mV) nor depolarization up to −55 mV membrane potential changed the action of Cs\(^+\) on burst duration (not shown). Similar results were obtained from eight preparations with either intracellular (n = 3) or ventral root recordings (n = 5). On average, burst duration was increased to 170 ± 47% of control (P < 0.001), whereas burst cycle period remained unaffected, as illustrated by the histograms in Fig. 3C. In intracellular recordings, Cs\(^+\) application produced an average hyperpolarization of 7 ± 4 mV. Although these findings outline a contribution of a Cs\(^+\)-sensitive inward current to the control of burst length, it seems that the predominant mechanism responsible for burst generation remained elusive. One possibility worth exploring was the role of the electrogenic Na\(^+\)/K\(^+\) pump, because this process is essential for the intrinsic rhythmicity of nigral neurons (Johnson et al. 1992). A recent modeling study (Li et al. 1996) has also indicated that such a pump activity would be important to support simulated bursting. It was therefore decided to test three different methods to block such a pump activity, namely application of ouabain or strophanthidin or omission of extracellular K\(^+\) (Glynn 1993; Horisberger et al. 1991) on spinal bursting.

**Pharmacological inhibition of the electrogenic Na\(^+\)/K\(^+\) pump**

Figure 4 illustrates the effects of ouabain (10 μM) on an intracellularly recorded motoneuron. In this example, in the presence of strychnine and bicuculline, the resting membrane potential was −70 mV and spontaneous bursts took place at regular intervals (Fig. 4, top). Single antidromic stimulation (during quiescent periods) evoked an action potential with an amplitude of 75 mV (Fig. 4, right). Such a stimulation did not influence subsequent bursts. After a latency of ~5 min from the start of the ouabain application, the motoneuron membrane potential slowly depolarized and, after 15 min in the presence of ouabain, it reached −64 mV, when bursts appeared irregularly and had highly variable duration, because events lasting tens of seconds coexisted with short discharges (see Fig. 4, middle). At this stage a full antidromic spike could still be evoked, although it was of reduced amplitude (63 mV) owing to depolarization (see Fig. 4, right middle). After 20 min of application (see Fig. 4, bottom), the motoneuron membrane potential declined to −50 mV and the antidromic spike amplitude was 38 mV. This phase was characterized by long quiescent periods that were interrupted by spontaneous bursts of irregular duration so infrequently as to make impractical any quantitative analysis of residual events (not shown). To check the viability of the spinal network under these conditions, dorsal root stimuli (of intensity sufficient to activate polysynaptic pathways) were delivered during quiescent periods and were found to evoke long-lasting bursts similar to the ones appearing spontaneously, as illustrated in Fig. 4, bottom. Further exposure to ouabain slowly led to suppression of electrical excitability. Similar results were obtained from eight spinal cord preparations recorded either intracellularly (n = 4) or extracellularly from left and right L4 ventral roots (n = 4), in which, in the presence of ouabain, irregular bursts appeared synchronously (not shown). These effects could not be reversed on ouabain washout.

Analogous data were obtained with bath application of strophanthidin (4 μM), another selective blocker of the electrogenic sodium pump, as illustrated by simultaneous recordings from left and right L4 ventral roots in Fig. 5. After a latency of 6–10 min from the beginning of strophanthidin application, regular rhythmic bursts (induced by strychnine and bicuculline; see Fig. 5, top pair of traces for control condition) were gradually converted into chaotic activity (shown at 30 min in Fig. 5, middle pair of traces), characterized by bursts of variable duration and unpredictable onset, similar to those observed in the presence of ouabain. Like the case of regular bursts observed in the presence of strychnine and bicuculline only (Fig. 5, top pair of traces), even this chaotic activity (Fig. 5, middle pair of traces) appeared synchronously in left and right ventral roots. Further exposure (40 min) to strophanthidin brought about nearly complete suppression of bursting even if dorsal root stimulation...
elicited bursts of variable duration (not shown). Recovery from strophandin was observed, albeit extremely slowly: regular rhythmicity returned only after 2–3 h of washout (Fig. 5, bottom pair of traces). Similar results were obtained in four preparations in which ventral root recording was preferred to intracellular recording to perform continuous long-lasting monitoring of burst suppression and recovery. When normal Krebs solution was replaced by K+−free solution (n = 4 or n = 3 preparations for extra- or intracellular recording, respectively), changes in the spontaneous bursting pattern (evoked by strychnine and bicuculline) started after ~20 min and developed fully after 40–60 min, as exemplified in Fig. 6A for an AC-coupled recording from L5 right ventral root. Large control bursts (Fig. 6A, top) were converted into short episodes of variable amplitude and interposed with small and brief spontaneous events without any ordered pattern (Fig. 6A, middle). Return to control solution (with strychnine and bicuculline) gradually reestablished bursting patterns after 40 min of washout (Fig. 6A, bottom). Figure 6B shows similar changes developing in an intracellularly recorded motoneuron (resting potential −72 mV) in which the regular bursting pattern was replaced by irregular activity of shorter duration and with spontaneous fluctuations in baseline potential (−81 mV at rest). Recovery required very long washout periods (>40 min), which compromised intracellular recording stability.

These results demonstrate the similarity of the effects observed after application of ouabain, strophanthidin, or K+−free solution.

**DISCUSSION**

The principal finding of the present investigation is the novel demonstration that regular bursts generated by an excitatory network were converted into chaotic paroxysmal activity after pharmacological block of the electrogenic Na+ pump by ouabain, strophanthidin, or K+−free solution. Pharmacological block of inward rectifier or of Ca2+-dependent K+ conductances resulted in modifications of burst frequency and/or burst duration, while in both cases regular rhythmicity persisted.

**Characteristics of spinal bursts**

In the neonatal rat spinal cord, pharmacological block of inhibition converts the alternating flexor-extensor and left-right activity of fictive locomotion (induced by serotonin and/or NMDA) into patterns characterized by bilateral synchronous rhythmic activity (Cowley and Schmidt 1995). Coapplication of strychnine and bicuculline to the same preparation in control solution elicits highly synchronous spontaneous bursting generated by a premotoneuronal network via α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-sensitive glutamate receptors (Bracci et al. 1996a). These data thus demonstrate that rhythmicity does not necessarily depend on inhibitory (glycinergic and GABAergic) interactions. What are the mechanisms responsible for such a regular bursting? To answer this question, we bath applied pharmacological agents while recording from motoneurons, which can be considered as the output elements of the rhythm-generating interneuronal network.

**Role of apamin-sensitive conductances**

In the lamprey spinal cord membrane potential oscillations are due to a dynamic interplay between depolarizing and hyperpolarizing conductances, one of which, namely the apamin-sensitive Ca2+-dependent K+ one, plays a major role in burst termination (El Manira et al. 1994), although its function in controlling fictive swimming remains controversial (Meer and Buchanan 1992). Rat lumbar motoneurons also possess a sustained Ca2+-dependent K+ current (Takahashi 1990a). In the present study apamin significantly decreased cycle period and burst duration, suggesting that apamin-sensitive conductances were not the main burst-terminating factor. Carbachol, which is supposed to block the slow Ca2+-dependent K+ current (Sah 1996; Schwindt et al. 1988), induced analogous effects. The effects of apamin or carbachol might result from a mere boost in network excitability due to reduction in spike frequency adaptation, which enhances the repetitive firing behavior within the network itself. In fact, increasing network excitability via application of other agents such as serotonin or NMDA has similar accelerating effects (Bracci et al. 1996a,b).

**Role of cesium-sensitive conductances**

Neonatal rat motoneurons possess a slowly developing inward current evoked by hyperpolarization (inward rectifier) that is reversibly abolished by external application of Cs+ (Takahashi 1990b). This current contributes to maintaining motoneuron resting membrane potential relatively depolarized (Takahashi 1990b; note also hyperpolarization induced by Cs+ in the present study). An analogous current has been found in cat thalamic relay neurons, where it is responsible for intrinsic membrane potential oscillations.
FIG. 6. Effects of K\(^+\)-free solution on strychnine- and bicuculline-induced bursting. A: extracellular recording (AC coupled; low-pass filtered) of control bursting (top), after 40 min in K\(^+\)-free solution (middle), and after 40 min return to control strychnine and bicuculline medium. Note reversible disruption of bursting activity. B: intracellular records of spontaneous bursting before (top) and after 25 min of application of K\(^+\)-free solution. Note membrane hyperpolarization with disruption of bursting activity with large-amplitude events interposed with irregular baseline fluctuations. (McCormick and Pape 1990). In the rat spinal cord, blockade of inward rectifier current by Cs\(^+\) significantly increased burst duration and the number of intraburst oscillations. Conversely, burst frequency was unaffected by Cs\(^+\). These data suggest that inward rectifier currents were not essential for rhythmic burst generation and for intraburst oscillations. The observation that Cs\(^+\) affected burst duration but not burst frequency suggests that distinct mechanisms are responsible for burst triggering and for burst maintenance, the latter being at least in part controlled by slow inward rectifiers.

Role of the electrogenic sodium pump

In hippocampal neurons prolonged glutamate application causes a large-amplitude depolarization followed by a profound hyperpolarization that persists for tens of seconds and is generated by the electrogenic Na\(^+\) pump, which reestablishes the normal Na\(^+\) and K\(^+\) gradients (Thompson and Prince 1986). Bursts observed in the disinhibited spinal cord are characterized by motoneuron depolarizations comparable (in amplitude and duration) with those induced by exogenous glutamate in hippocampal neurons. Intracellular recordings from motoneurons have, however, revealed that these bursts were not followed by hyperpolarization. One possibility is that the electrogenic Na\(^+\)Ca\(^{2+}\) pump (see Friedman et al. 1992) masked the motoneuron hyperpolarization, although lack of selective inhibitors of this process makes it difficult to study it directly. Because of the high sensitivity of this exchanger to membrane potential (Blaustein 1988), its contribution, if any, to the burst decay recorded from a motoneuron might be expected to be limited to the slowest and shallowest depolarizing component. We rather favor the view that the lack of hyperpolarization might be explained by the large volume-to-surface ratio of motoneurons, which allows these cells to generate strong electrical signals with relatively small changes in internal ionic concentrations. In spinal interneurons the volume-to-surface ratio is smaller, however (because of their reduced soma diameter) (Lev-Tov and O’Donovan 1995). Thus burst depolarization (presumably mediated by glutamate) (Bracci et al. 1996a) might cause a significant dissipation of interneuronal gradient, leading to sustained operation of the electrogenic Na\(^+\) pump, although this phenomenon would not be measurable with intracellular recordings from motoneurons. The hyperpolarizing current resulting from electrogenic Na\(^+\) extrusion might take interneurons below threshold, thus terminating network excitation for several seconds. Although this mechanism has not been demonstrated before in the spinal cord, an analogy may be drawn from the crucial pacemaking role of the electrogenic Na\(^+\) pump in the intrinsic bursting activity of midbrain dopaminergic neurons (Johnson et al. 1992). To test the applicability of this notion to bursting of the disinhibited spinal cord, we selectively blocked the electrogenic sodium pump during rhythmic bursting by bath application of ouabain, strophanthidin, or K\(^+\)-free solution (Glynn 1993; Horisberger et al. 1991). In all cases, highly regular rhythmicity was lost and the network generated irregular bursting synchronously in both sides of the cord, suggesting that side-to-side connections were functionally intact under these conditions. Ouabain or strophanthidin quickly converted rhythms into chaotic behavior characterized by irregular appearance of long-lasting bursts and short discharges. In both cases a single pulse to the segmental dorsal root (during quiescent periods) evoked a paroxysmal burst lasting >1 min, thus showing that the spinal pathways were apparently functional in the presence of these agents. Moreover, ventral root stimulation was able to evoke a motoneuronal antidromic action potential even after 20 min of drug application. Although ouabain effects were irreversible (Canessa et al. 1992), during strophanthidin washout bursting activity slowly returned to a regular rhythm. K\(^+\)-free experiments gave similar results,
although with much slower onset (>40 min), presumably due to the buffering ability of spinal cells that can long counteract depletion of extracellular K⁺ (Czéh et al. 1988). A comparably slower action of the K⁺-free solutions versus ouabain in blocking a delayed hyperpolarization of spinal neurons mediated by the Na⁺ pump activity has also been observed by Davidoff and Hackman (1980). In the present study, after 1 h of exposure to K⁺-free solution, bursts were converted into single discharges of variable amplitude, lacking any intraburst structure and appearing at irregular intervals. These findings strengthen the observations obtained with ouabain or strophanthidin because they make unlikely any nonspecific action common to addition of blockers as well as to removal of an extracellular ion. These observations are therefore consistent with the view that the Na⁺ pump played a crucial role in pacing bursts. Nevertheless, it is currently unclear whether inhibition of the Na⁺ pump had a direct or indirect action in burst disruption, because secondary effects due to accumulation of internal Na⁺ with alterations in transmembrane gradients for other ions such as Ca²⁺ could also play a role. In summary, then, the operation of the electrogenic Na⁺ pump appeared to be necessary to ensure slow rhythmicity in the absence of synaptic inhibition.

This work was supported by a project grant from Telethon-Italy (Grant n.823), by a network grant of the Human Capital and Mobility Programme from the European Union, and by research grants from Istituto Nazionale di Fisica della Materia and Consiglio Nazionale delle Ricerche to A. Nistri.

Address for reprint requests: L. Ballerini, Biophysics Laboratory, International School for Advanced Studies, via Beirut 2-4, 34014 Trieste, Italy.

Received 19 June 1996; accepted in final form 5 September 1996.

REFERENCES


