Alkaline pH shifts Ca$^{2+}$ sparks to Ca$^{2+}$ waves in smooth muscle cells of pressurized cerebral arteries

THOMAS J. HEPPNER, ADRIAN D. BONEV, L. FERNANDO SANTANA, AND MARK T. NELSON

Department of Pharmacology, University of Vermont College of Medicine, Burlington, Vermont 05405-0068; and Department of Physiology and Biophysics, University of Washington, Seattle, Washington 98195

Received 16 July 2002; accepted in final form 15 August 2002

Am J Physiol Heart Circ Physiol 283: H2169–H2176, 2002; 10.1152/ajpheart.00603.2002.—The effects of external pH (7.0–8.0) on intracellular Ca$^{2+}$ signals (Ca$^{2+}$ sparks and Ca$^{2+}$ waves) were examined in smooth muscle cells from intact pressurized arteries from rats. Elevating the external pH from 7.4 to 7.5 increased the frequency of local, Ca$^{2+}$ transients, or “Ca$^{2+}$ sparks,” and, at pH 7.6, significantly increased the frequency of Ca$^{2+}$ waves. Alkaline pH-induced Ca$^{2+}$ waves were inhibited by blocking Ca$^{2+}$ release from ryanodine receptors but were not prevented by inhibitors of voltage-dependent Ca$^{2+}$ channels, phospholipase C, or inositol 1,4,5-trisphosphate receptors. Activating ryanodine receptors with caffeine (5 mM) at pH 7.4 also induced repetitive Ca$^{2+}$ waves. Alkalization from pH 7.4 to pH 7.8–8.0 induced a rapid and large vasoconstriction. Approximately 82% of the alkaline pH-induced vasoconstriction was reversed by inhibitors of voltage-dependent Ca$^{2+}$ channels. The remaining constriction was reversed by inhibition of ryanodine receptors. These findings indicate that alkaline pH-induced Ca$^{2+}$ waves originate from ryanodine receptors and make a minor, direct contribution to alkaline pH-induced vasoconstriction.

ryanodine receptors; voltage-dependent calcium channels; arterial diameter

IN SMOOTH MUSCLE, as in other cell types, Ca$^{2+}$ signaling is central to nearly all cellular processes, including gene expression, neurotransmitter release, memory, and muscle contraction. In arterial smooth muscle, Ca$^{2+}$ may have diverse effects depending on the spatiotemporal pattern of the Ca$^{2+}$ signal. For example, increased Ca$^{2+}$ influx through voltage-dependent Ca$^{2+}$ channels (VDCCs) elevates intracellular Ca$^{2+}$ concentrations ([Ca$^{2+}]_i$) globally (i.e., throughout the cell) and causes contraction by elevating myosin light chain kinase activity. In contrast, Ca$^{2+}$ sparks, which represent transient, local release of Ca$^{2+}$ through ryanodine-sensitive Ca$^{2+}$ channels or ryanodine receptors (RYRs) located in the sarcoplasmic reticulum membrane, act to oppose arterial smooth muscle contraction. RYR-mediated Ca$^{2+}$ sparks activate closely juxtaposed large conductance, Ca$^{2+}$-sensitive K$^+$ channels to promote membrane potential hyperpolarization and thus constitute an element in the negative feedback regulation of vascular tone (7, 18–20, 35, 40).

Another modality of Ca$^{2+}$ signaling observed in arterial smooth muscle cells are asynchronous Ca$^{2+}$ waves, which are usually defined as a change in intracellular Ca$^{2+}$ concentration that travels the length of the cell. Ca$^{2+}$ waves occur spontaneously in a small percentage of arterial smooth muscle (16, 18, 33), and their frequency and amplitude can be increased markedly by the addition of vasoconstrictor agonists such as norepinephrine, UTP, or phenylephrine (16, 18, 32, 33, 42). Whereas recent results suggest that Ca$^{2+}$ waves induced by vasoconstrictors are caused by the release of Ca$^{2+}$ from the sarcoplasmic reticulum through inositol 1,4,5-trisphosphate [Ins(1,4,5)P$_3$] receptors (17), the physiological role of Ca$^{2+}$ waves is unclear. Indeed, recent reports on this topic are contradictory: several studies have suggested that Ca$^{2+}$ waves contribute Ca$^{2+}$ for vasoconstriction (2, 15, 22, 34, 42, 47), whereas other studies suggest that phenylephrine-induced Ca$^{2+}$ waves do not contribute to arterial diameter regulation (33, 37).

In the present study, we demonstrate that extracellular alkalization constitutes a novel mechanism for the induction of Ca$^{2+}$ waves in intact, pressurized cerebral arteries. We find that extracellular pH in the range of 7.0–7.4 has a minimal impact on Ca$^{2+}$ spark or Ca$^{2+}$ wave frequency. At pH 7.5, however, Ca$^{2+}$ spark frequency is significantly increased, and, above pH 7.5, Ca$^{2+}$ wave frequency is significantly increased. At pH 7.8 or higher, repetitive Ca$^{2+}$ waves occurred in virtually every smooth muscle cell. The alkaline pH-induced Ca$^{2+}$ waves occurred in the presence of inhibitors of VDCCs or Ins(1,4,5)P$_3$ receptors but are
blocked by inhibition of RyRs. These alkaline pH-induced Ca\(^{2+}\) waves appear to play a minor role in arterial vasoconstriction whenVDCCs are inhibited.

**METHODS**

**Tissue preparation.** Adult female rats (−250 g) were euthanized with pentobarbital sodium followed by thoracotomy as approved by the Office of Animal Care Management at the University of Vermont. Posterior cerebral arteries and cerebellar arteries were removed from the brain and placed in cold HEPES-buffered saline. After the connective tissue was removed, segments of the artery (2–4 mm in length; 50–120 \(\mu\)m) were placed in a chamber specially designed to measure Ca\(^{2+}\) responses in pressurized arteries. Arteries were tied to glass cannulas containing the same solution as the superfusing bath and applied for at least 10 min before Ca\(^{2+}\) responses were recorded unless indicated otherwise.

**Ca\(^{2+}\) imaging and analysis.** Smooth muscle cells in the arterial wall were scanned with a laser scanning confocal microscope (OZ; Noran Instruments) controlled by an O2 workstation (Silicon Graphics) by using an Intervision software package. A Nikon Diaphot microscope with a ×60 water immersion lens (1.2 numerical aperture; Nikon) was used to visualize the artery. Cerebral arteries were incubated in an immersion lens (1.2 numerical aperature; Nikon) was used to visualize the artery. Cerebral arteries were incubated in an

**Drugs and solutions.** Ca\(^{2+}\) sparks and waves were measured in a HEPES solution of the following composition (in mM): 134 NaCl, 6 KCl, 1 MgCl\(_2\), 2 CaCl\(_2\), 10 HEPES, and 10 glucose (pH 7.4). NaOH was used to adjust the pH of HEPES solutions. Arterial diameter experiments used PSS of the following composition (in mM): 119 NaCl, 4.7 KCl, 23.8 NaHCO\(_3\), 1.2 KH\(_2\)PO\(_4\), 1.6 CaCl\(_2\), 1.2 MgCl\(_2\), 0.023 EDTA, and 11.0 glucose (pH 7.4). PSS was continuously bubbled with 95% O\(_2\)-5% CO\(_2\). To adjust the pH of the PSS to a more alkaline pH, bubbling was stopped, and the container with PSS was opened to allow CO\(_2\) to escape. As CO\(_2\) slowly dissipated from the PSS, the pH gradually increased. When the desired alkaline pH value was reached, the container with PSS was tightly capped, preventing any further changes in pH. The alkalized PSS was then superfused over the cannulated artery. Caffeine, nisoldipine, diltiazem, and U-73122 were obtained from Sigma. 2-Aminothydroxyphenylborate (2-APB) was obtained from Tocris, ryanozide was from LC Laboratories, and xestospongion C and verapamil were obtained from Calbiochem.

**Statistics.** Results are expressed as means ± SE where applicable. Unless otherwise noted, \(n\) is the number of cells. Significance between groups was evaluated with a one-way ANOVA followed by the appropriate multiple comparison test for significance (Dunn’s pairwise multiple comparison procedure or Kruskal-Wallis analysis of variance on ranks).

**RESULTS**

**Effects of external pH from 7.0 to 7.5 on Ca\(^{2+}\) sparks.** In pressurized cerebral arteries (60 mmHg; 37°C), all cells examined exhibited Ca\(^{2+}\) sparks at external pH values of 7.0, 7.4, and 7.5. Increasing the pH between 7.0 and 7.4 had no significant effect on Ca\(^{2+}\) spark frequency; however, increasing the pH to 7.5 induced a significant increase in Ca\(^{2+}\) spark frequency (Fig. 1, A and B). At higher pH values, longer-lasting Ca\(^{2+}\) events became so numerous that an accurate measurement of Ca\(^{2+}\) spark frequency was not possible.

**Alkaline pH increases Ca\(^{2+}\) waves.** In pressurized cerebral arteries (60 mmHg, 37°C) at pH 7.4, a small percentage (7% of cells; \(n = 98\)) exhibited Ca\(^{2+}\) waves. At pH values of 7.8 or higher, virtually 100% of the cells (\(n = 207\)) exhibited Ca\(^{2+}\) waves (Fig. 2A). The frequency of waves in a given cell also increased markedly with alkaline pH (Fig. 2B). At pH 8.0, the mean Ca\(^{2+}\) wave frequency was 11.7 ± 0.6 waves-cell\(^{-1}\)-min\(^{-1}\), but ranged as high as 44 waves-cell\(^{-1}\)-min\(^{-1}\). These repetitive waves, which were asynchronous, originated near one end of the cell and then traveled to the opposite end of the cell (Fig. 2A) with an average velocity of 57.7 ± 0.9 \(\mu\)m/s (\(n = 7\)) at pH 8.0.

Interestingly, Ca\(^{2+}\) waves were not limited to the larger (60–100 \(\mu\)m) resistance arteries used throughout this study but were also found in arterioles. Figure 3 illustrates pH 8.0-induced Ca\(^{2+}\) waves propagating in smooth muscle cells in a pressurized cerebral arte-
riole (20–30 μm diameter). As with the larger arteries, 100% (n = 42) of the smooth muscle cells examined exhibited Ca\(^{2+}\) waves at pH 8.0 and had a similar frequency (Fig. 4).

Ca\(^{2+}\) influx through VDCCs is not required for alkaline pH-induced Ca\(^{2+}\) waves. To determine the origin of the Ca\(^{2+}\) underlying Ca\(^{2+}\) waves, the effects of VDCC inhibitors on Ca\(^{2+}\) waves was examined. Application of the VDCC inhibitors verapamil (10 μM) (48), diltiazem (10 μM) (25), or nisoldipine (100 nM) (36) for up to 30 min did not reduce the frequency of ongoing Ca\(^{2+}\) waves (Figs. 4 and 5A). To examine the role of VDCCs in the initiation of Ca\(^{2+}\) waves, arteries were incubated with diltiazem (50 μM) for 10 min at pH 7.4 before the pH was increased to 7.8–8.0. However, preincubation with a VDCC inhibitor failed to prevent the induction of Ca\(^{2+}\) waves at pH 7.8–8.0 (Fig. 4). Alkaline pH-induced Ca\(^{2+}\) waves also persisted for at least 20 min (the longest time period cells were examined in Ca\(^{2+}\)-free solution) in nominal absence of external Ca\(^{2+}\) (Fig. 4). These findings indicate that over the time of the experiments, external Ca\(^{2+}\) and Ca\(^{2+}\) entry through VDCCs or through other pathways do not play a significant role in the initiation or maintenance of alkaline pH-induced Ca\(^{2+}\) waves.

It is conceivable that alkaline pH affects the release of factor(s) that would alter Ca\(^{2+}\) wave probability in the smooth muscle cells. To test the dependence of alkaline pH-induced Ca\(^{2+}\) waves on the endothelium, we removed the endothelium by pushing 3–5 ml of air through the vessel or by a combination of forcing air through the lumen and scraping the lumen with a hair. A lack of effect of the endothelial-dependent vasodilator acetylcholine was taken as evidence of endothelial denudation. Removal of the endothelium did not significantly affect Ca\(^{2+}\) wave activation induced by pH 8.0 (Fig. 4).

AJP-Heart Circ Physiol • VOL 283 • DECEMBER 2002 • www.ajpheart.org
Ins(1,4,5)P₃-mediated Ca²⁺ release is not involved in alkaline pH-induced Ca²⁺ waves. Previous studies have shown that Ca²⁺ waves induced in smooth muscle by vasoconstrictors are dependent on Ca²⁺ release through Ins(1,4,5)P₃ receptors, reflecting agonist-induced activation of PLC and elevation of Ins(1,4,5)P₃.

Alkaline pH could also conceivably activate PLC and elevate Ins(1,4,5)P₃ levels (1, 2). Inhibition of PLC (U-73122, 2 μM), after the induction of Ca²⁺ waves with external pH 7.8–8.0, however, was ineffective in blocking pH 8.0-induced Ca²⁺ waves (Fig. 4), suggesting that this pathway is not involved in the genesis and
maintenance of Ca\(^{2+}\) waves. To directly test the dependence of Ca\(^{2+}\) waves on Ins(1,4,5)P\(_3\) receptors, two inhibitors of Ins(1,4,5)P\(_3\) receptors, xestospongin C (13) and 2-APB (31), were employed. Preincubation of arteries with xestospongin C (20 \(\mu\)M) for 20 min (37\(^\circ\)C) in external pH 7.4 followed by wave induction by external pH 8.0 in the continued presence of xestospongin C was ineffective in abolishing Ca\(^{2+}\) waves (Fig. 4). 2-APB (100 \(\mu\)M) also failed to abolish Ca\(^{2+}\) waves at pH 8.0 (Figs. 4 and 5B). These results suggest that Ca\(^{2+}\) release through Ins(1,4,5)P\(_3\) receptors does not contribute to Ca\(^{2+}\) waves induced by alkaline pH.

**Ca\(^{2+}\) waves originate from RyRs.** Because Ca\(^{2+}\) influx or Ca\(^{2+}\) release through Ins(1,4,5)P\(_3\) receptors does not appear to be the immediate source of Ca\(^{2+}\) for Ca\(^{2+}\) waves, we examined the role of Ca\(^{2+}\) release through RyRs. In contrast to the results obtained with xestospongin C, 2-APB, and U-73122, treatment with the selective RyR inhibitor ryanodine (10 \(\mu\)M) abolished alkaline-induced Ca\(^{2+}\) waves within 10–15 min of application (Fig. 4 and Fig. 6A). Similarly, preincubation (20 min) of the artery with ryanodine (10 \(\mu\)M) completely prevented the induction of Ca\(^{2+}\) waves by the subsequent elevation of extracellular pH to 7.8 or higher (Fig. 4). These findings indicate that alkaline pH-induced Ca\(^{2+}\) waves originate from RyRs.

**Fig. 3.** Alkaline pH-induced Ca\(^{2+}\) waves in cerebral arterioles. Repetitive Ca\(^{2+}\) waves were found in virtually all smooth muscle cells from arterioles when external pH was increased to 8.0. Every third image is displayed (\(~100 ms\) between images). First two rows are consecutive. Third row is from the same file recorded 12 s after the second row to show Ca\(^{2+}\) waves in additional cells. Color scale bar represents the percentage of the maximum fluorescence. Images were acquired every 33.33 ms.

**Fig. 4.** Ryanodine inhibited Ca\(^{2+}\) waves but not inhibitors of voltage-dependent Ca\(^{2+}\) channels (VDCCs), inositol 1,4,5-trisphosphate [Ins(1,4,5)P\(_3\)] receptors, PLC, Ca\(^{2+}\) free solution, or the removal of the endothelium. Waves were induced by external pH 8.0 except for caffeine, which was in pH 7.4. *Significantly different from pH 8.0 control (\(P < 0.05\)). Numbers above bars refer to numbers of cells.

**Fig. 5.** Inhibitors of VDCCs or InsP\(_3\) receptors do not significantly alter pH 8.0-induced Ca\(^{2+}\) waves. A: pH 8.0-induced Ca\(^{2+}\) waves recorded from the same posterior cerebral artery before (left) and 10 min after the addition of nisoldipine (100 nM) (right). B: pH 8.0-induced Ca\(^{2+}\) waves recorded from the same posterior cerebral artery before (left) and 15 min after the addition of 2-aminophenylborate (2-APB, 100 \(\mu\)M) (right).
Because Ca\(^{2+}\) waves originate from RyRs, we hypothesized that caffeine, which elevates RyR activity by increasing the apparent Ca\(^{2+}\) sensitivity of the RyR (39), should be able to activate Ca\(^{2+}\) waves. We have previously shown that steady application of caffeine, in micromolar concentrations, increases Ca\(^{2+}\) spark and associated transient large conductance, Ca\(^{2+}\)-sensitive K\(^{+}\) current frequency in cerebral artery smooth muscle (14, 18, 26, 40). Furthermore, bolus application of higher concentrations of caffeine (10 mM) induces a ryanodine-sensitive, global Ca\(^{2+}\) transient (34). Here, we demonstrate that the steady application of an intermediate caffeine concentration (5 mM) is capable of inducing repetitive Ca\(^{2+}\) waves at pH 7.4 (Figs. 4 and 6B). This finding indicates that activation of RyRs can induce and sustain repetitive Ca\(^{2+}\) waves.

Effect of Ca\(^{2+}\) waves on arterial diameter. Alkaline pH constricts cerebral arteries (3, 4, 10, 12, 28, 43). To test the contribution of Ca\(^{2+}\) waves to alkaline pH-induced vasoconstriction, arterial diameter was measured before and after the addition of caffeine. External pH 8.0 solution constricted arteries to 73.6 ± 10.9% of a 60 mM K\(^{+}\)-induced vasoconstriction. Inhibition of VDCCs (50 \(\mu\)M diltiazem) relaxed the pH 8.0-induced vasoconstriction by 82.1 ± 10.9% of a 60 mM K\(^{+}\)/H\(_{11001}\) solution, constricted arteries to 73.6 ± 10.9% of a 60 mM K\(^{+}\)/H\(_{11001}\) solution. These findings suggest that alkaline pH-induced Ca\(^{2+}\) waves originate from RyRs.

DISCUSSION

This study identifies a mechanism whereby changes in extracellular pH are able to transform RyR-mediated Ca\(^{2+}\) signals from Ca\(^{2+}\) sparks to Ca\(^{2+}\) waves. Ca\(^{2+}\) sparks were present in all cells examined at pH 7.0, 7.4, and 7.5. Ca\(^{2+}\) spark activity was unchanged at external pH values of 7.0 and 7.4, but an increase in pH to 7.5 significantly increased Ca\(^{2+}\) spark frequency. At higher pH values, Ca\(^{2+}\) sparks could no longer be accurately identified but were replaced by longer-lasting, repetitive Ca\(^{2+}\) waves. Ca\(^{2+}\) wave frequency was pH dependent and reached a maximum at pH 7.8.

Source of Ca\(^{2+}\) release for alkaline pH-induced waves. Alkaline pH has been shown to increase Ca\(^{2+}\) influx into vascular smooth muscle cells through VDCCs (15, 23, 24, 44, 51). VDCC-mediated increases in intracellular Ca\(^{2+}\) should elevate the activity of Ca\(^{2+}\)-sensitive RyRs (39) and might be predicted to contribute to the induction of Ca\(^{2+}\) waves. However, pharmacological inhibition of VDCC activity did not prevent the induction of pH-induced waves nor did it inhibit pH-induced waves after activation. Therefore, the additional Ca\(^{2+}\) influx through VDCCs that occurs under alkaline conditions does not appear to be the main factor in Ca\(^{2+}\) wave induction or maintenance. Alkalization can also increase the open probability of Ins(1,4,5)P\(_{3}\) receptors (21, 52), possibly through the activation of PLC (1, 2). However, our results do not support a role for Ins(1,4,5)P\(_{3}\) receptor activation underlying pH-induced Ca\(^{2+}\) waves, because selective inhibitors of Ins(1,4,5)P\(_{3}\) receptors (xestospongin C and 2-APB) and PLC (U-73122) failed to prevent or abolish alkaline pH-induced Ca\(^{2+}\) waves. Instead, our findings suggest that alkaline pH-induced Ca\(^{2+}\) waves originate from RyRs.

pH and apparent Ca\(^{2+}\) sensitivity of RyRs. RyR activity, measured at the single channel level in bilayers, increases steeply with alkaline pH (30, 41). With the use of Ca\(^{2+}\) as the charge carrier in skeletal or cardiac tissues (Fig. 7). This suggests that pH-induced Ca\(^{2+}\) waves make a small, direct contribution to alkaline pH-induced vasoconstriction.
RyRs, the open probability of the RyR increases about twofold between pH 7.1 and pH 7.5 (41). Changes in external pH are directly translated into changes in internal pH values. In the mesenteric artery smooth muscle, internal pH has been shown to change with a ratio of 0.73 relative to the change in external pH (6). This change is quite rapid, reaching the half-peak intracellular response in strips of mesenteric artery in 38 s (6). From these observations and the results of the current study, we propose that an elevation in external pH acting through changes in intracellular pH, increases the apparent Ca$^{2+}$ sensitivity of RyRs, resulting in an increase in the probability of regenerative Ca$^{2+}$ waves. The observation that caffeine, which increases the apparent sensitivity of RyRs to Ca$^{2+}$ also induces Ca$^{2+}$ waves (Fig. 6B), is consistent with this model.

**Function of Ca$^{2+}$ waves.** The physiological role of Ca$^{2+}$ waves is unclear, with much attention in previous studies having been focused on their contribution to contraction. Several studies using α-adrenergic receptor activation to induce Ca$^{2+}$ waves suggest that waves contribute to contraction of vascular smooth muscle (15, 42), whereas other studies suggest that asynchronous Ca$^{2+}$ waves have minimal or no impact on vascular tone (33, 37). Our findings suggest that vasoconstriction induced by extracellular alkaline pH depends primarily on influx of Ca$^{2+}$ through VDCCs (see Fig. 7). From the effects of Ca$^{2+}$-wave inhibition by ryanodine, Ca$^{2+}$ waves account for a small percentage (~18%) of the pH 8.0-induced vasoconstriction. It should be noted that alkaline pH-induced Ca$^{2+}$ waves represent activation of RyRs, whereas Ins(1,4,5)P$_3$ receptors have a prominent role in agonist-induced Ca$^{2+}$ waves. RyRs and Ins(1,4,5)P$_3$ receptors communicate with different cellular targets and could explain differential effects of agonist and pH-induced Ca$^{2+}$ waves.

Different spatiotemporal patterning of Ca$^{2+}$ events may selectively activate specific cellular processes. For example, increases in "global Ca$^{2+}$" bring about vasoconstriction, whereas Ca$^{2+}$ sparks play a key role to reduce cell excitability through the activation of Ca$^{2+}$-sensitive K$^+$ channels and an attendant decrease in Ca$^{2+}$ entry through VDCCs (19, 34, 35, 38, 53). Ca$^{2+}$ waves evoked by caffeine have been shown to activate Ca$^{2+}$-dependent Cl$^-$ channels in rat portal vein (34). Ca$^{2+}$ mobilization may also contribute to the modulation of transcription factors such as nuclear factor of activated T cells and cAMP-responsive binding protein in smooth muscle (8, 45, 46, 49). In nonexcitable cells, Ca$^{2+}$ waves have been shown to increase the efficiency, as well as the selectivity, of Ca$^{2+}$-dependent transcription factor(s) (11, 29). Although the function of alkaline pH-induced Ca$^{2+}$ waves is unclear at this time, it is conceivable that they may regulate both Ca$^{2+}$-sensitive ion channels and transcription factors.

pH plays an important role in the regulation of the cerebral vasculature (3, 4, 10, 12, 28, 43). Arterial pH values >7.44 are observed under clinical conditions of alkalemia (9), and arterial pH can increase to 7.8 before death results (27). This study describes a unique pH-induced shift in Ca$^{2+}$ signaling in cerebral arterial smooth muscle cells; a shift that may contribute to alkaline pH-induced vasoconstriction. This direct contribution appears to be relatively minor, suggesting that alkaline pH-induced Ca$^{2+}$ waves may serve additional functions. Although the function of these waves is not entirely clear, the fact that Ca$^{2+}$ signaling shifts dramatically between sparks and waves within a narrow pH range that corresponds to clinical alkalemia is provocative.

The authors thank Drs. B. Etherton, M. Gomez, L. Gonzalez Bosc, D. Hill-Eubanks, G. Petkov, K. Thorneloe, M. Talyor, and G. Wellman for critical comments on this manuscript. This work was supported by National Institutes of Health Grants HL-44455, HL-63722 (to M. T. Nelson), and NS-39405 (to L. F. Santana, University of Puerto Rico, and M. T. Nelson), and the Totman Trust for Human Cerebrovascular Research.

**REFERENCES**


34. Mironneau J, Arnaudeau S, Macrez-Lepretre N, and Boit-


