Determinants of LV diastolic function during atrial fibrillation: beat-to-beat analysis in acute dog experiments


Cardiovascular Imaging Center and Section of Electrophysiology, Department of Cardiovascular Medicine, The Cleveland Clinic Foundation, Cleveland, Ohio 44195

Submitted 27 June 2003; accepted in final form 28 August 2003

The purpose of this study was therefore to better elucidate the physiological determinants of beat-to-beat changes in the LV diastolic function during AF and to quantify their relationship with the corresponding RR intervals. We used a combination of Doppler echocardiography and hemodynamic analysis to test the hypothesis that the determinants of LV diastolic function during AF depend largely on the beat-to-beat changes in preload that reflect the variability of individual RR intervals.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests and other correspondence: T. Tabata, Dept. of Digestive and Cardiovascular Medicine, The Univ. of Tokushima, 2-50-1 Kuramoto, Tokushima 770-8503, Japan (E-mail: tommy@clin.med.tokushima-u.ac.jp).

http://www.ajpheart.org 0363-6135/04 $5.00 Copyright © 2004 the American Physiological Society
METHODS

The experimental protocol was approved by the Animal Research Committee of the Cleveland Clinic Foundation. All experimental procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Surgical preparation. Twelve healthy mongrel dogs weighing between 25 and 35 kg were used in the present study. The animals were placed in the supine position and were initially premedicated with 20 mg/kg thiopental sodium intravenously and intubated with a cuffed endotracheal tube. The animals were ventilated with room air supplemented with oxygen as needed to maintain normal arterial blood gases with the use of a SAV respirator (Narkomed 2, North American Dräger; Telford, PA). Anesthesia was maintained with an inhalation mixture of oxygen and isoflurane (1.0–2.0%) throughout the experiment. Metabolic environments for the animals were main-

Hemodynamic assessment was performed in each animal. The peak systolic LV pressure (LVP) was measured from the LV pressure curve and LV end-diastolic volume (EDV) was obtained from the LV volume curve (Fig. 1, top). The maximum (dP/dt max) and minimum (dP/dt min) value of the first derivative of LV pressure curve and the time constant of isovolumic LV pressure decay (τ) was obtained by digitizing the waveform off-line. The τ was determined by fitting the pressure-time data (from the point of dP/dt max to LV pressure 5 mmHg amplified, filtered, digitized, and continuously displayed on a moni-
toring system (GE Marquette Medical Systems, Prucka-Cardiolab EP System).

Electrical stimulation. A programmable eight-channel stimulator (Master-8, AMPI) was used to generate the desired sequence of rectangular impulses for atrial pacing or nerve stimulation. The amplitude of the impulses was determined by current isolators (model A360, WPI) that also permitted alteration of the polarity of the impulse to reduce the effects of polarization at the electrode-tissue interface. After the surgical procedures were completed, stabilization period of 30 min at normal sinus rhythm was allowed. AF was then induced by a two-step maneuver as previously reported (17, 21). AF was triggered by brief burst (5–10 s) of right atrial pacing (5–10 mA, 1-ms pulses at 20 Hz) and maintained by sinus node fat pad stimu-

LVP

(mmHg)

dP/dt

(mmHg/s)

LV volume

(ml)

RVe

RR

RR

Mitral inflow

(cm/s)

Fig. 1. Top and middle, simultaneous recording of the LV pressure (LVP) and volume curve obtained by conductance catheter and right ventricular ECG (RVe). The first derivative of LV pressure curve (dP/dt) was analyzed off-line. RR1 and RR2 are preceding and prepreceding cardiac cycle lengths, respectively, for a given cardiac beat (*). Bottom, measurements of peak mitral inflow early diastolic velocity (E) and its velocity time integral (vE) and LV filling time (LV-FT).
higher than next LV end-diastolic pressures) to the equation \( P(t) = (P_0 - P_b) e^{-t/r} + P_b \), where \( P_b \) is the pressure decay asymptote, \( P_0 \) is the pressure at \( \frac{dP}{dt} \) at time \( t \) referenced to time of \( \frac{dP}{dt} \) occurrence (19). An interval of >25 ms was considered necessary to calculate \( t \). The RR intervals were measured from the right ventricular electrogram for >100 consecutive cardiac cycles. The RR, RR, and RR, intervals for a given cardiac beat (marked with an asterisk) were measured for each cardiac cycle along with all of the parameters during AF (Fig. 1, middle).

**Echocardiographic assessments.** Epicardial echocardiography was performed in 9 of 12 animals using commercially available equipment (Sequoia C512, Siemens Medical Solutions; Mountain View, CA) with a 3.5 MHz phased array transducer. From the four-chamber view, the pulsed Doppler mitral inflow velocity profile was obtained by placing the sample volume at the tips of the mitral leaflets. The peak pulsed Doppler early diastolic mitral inflow velocity (E) and its velocity time integral (E) were measured from the recording (Fig. 1, bottom). The E was measured off-line by digitizing the darkest portion of the tracings. The LV filling time (FT) was measured as the time from the onset to the end of mitral inflow E wave. We attempted to measure each parameter for >100 consecutive cardiac cycles; however, the number of data points involving mitral inflow E wave velocity was usually less due to intermittent suboptimal signal quality. Statistical analysis. The \( \frac{dP}{dt}_{max} \), \( \frac{dP}{dt}_{min} \), \( \tau \), and peak systolic LV at a given cardiac beat were correlated with the RR, interval as well as with RR,RR, and RR,RR, respectively. The relationship between the Doppler-derived parameters, including peak E wave velocity, E, LV-FT, and LV-EDV obtained by a conductance catheter at a given cardiac beat were correlated with their RR intervals. The difference in correlation coefficients in the relationship between \( \frac{dP}{dt}_{max} \) and RR, or RR,RR, as well as those between LV-FT and mitral inflow E wave velocity or E, was evaluated by Fisher’s Z transformation, followed by Student’s paired t-test. The LV-EDV was further correlated with the subsequent peak systolic LV. Finally, the \( \frac{dP}{dt}_{min} \) and \( \tau \) were correlated with the peak systolic LV in the same cardiac beat. A \( P \) value <0.05 was considered statistically significant.

**RESULTS**

**RR intervals during AF.** RR intervals during triggered AF ranged from 183 to 418 ms in all animals and significantly shortened compared with those during baseline sinus rhythm (316 ± 60 vs. 448 ± 83 ms; \( P < 0.0001 \)).

**Relationship between \( \frac{dP}{dt}_{max} \) and RR intervals.** As previously reported (21), there was a significant positive linear relationship between the preceding RR and subsequent \( \frac{dP}{dt}_{max} \) (Fig. 2A). A similar linear relationship was found when the parameter was the RR,RR, (Fig. 2B). The averaged data from all dogs confirmed that the correlation coefficient was significantly greater for the latter relationship during AF (r = 0.79 vs. \( r = 0.59 ; P < 0.01 \)). This finding indicates that both the mechanical restitution (through RR) and postextrasystolic potentiation (through RR,RR,) play important mechanistic role as determinants of beat-to-beat variability of contractility.

**Relationship between \( \frac{dP}{dt}_{min} \), peak systolic LV, and RR intervals.** In accordance with our working hypothesis, we sought to determine whether similar simple linear relationships existed between the RR variability and the diastolic parameters. However, unlike the strong linear correlation documented in Fig. 2, a simple linear regression analysis did not reveal a significant relationship. Thus we only found positive curvilinear trends between \( \frac{dP}{dt}_{min} \) and RR, intervals (or RR,RR,), as well as negative curvilinear trends for \( \tau \) (Fig. 3, A, D, B, and E). Interestingly, there were also positive curvilinear trends between peak systolic LV and the RR intervals (Fig. 3, C and F). Although the above analysis did not reveal simple linear relationships, it nevertheless confirmed the deleterious role of short RR intervals during AF. Moreover, it suggested the possible functional role of peak systolic LVP as an important factor determining LV diastolic function. In particular, because small values for both RR and RR,RR, were consistently associated with small peak systolic LVP (Fig. 3, C and F), we were prompted to elucidate its impact on the diastolic characteristics.

**Relationship between Doppler echocardiography parameters and RR intervals, LV-EDV, and peak systolic LVP.** First, as demonstrated in Fig. 4A from a representative experiment, there was a significant positive linear relationship between the RR, intervals and Doppler-derived LV-FT in the same cardiac beat. This tendency was observed in all animals (mean \( r = 0.55 \), range 0.39–0.81). Second, LV-FT showed a significant positive linear relationship with peak mitral inflow E wave velocity (data for all animals are shown in Table 1, mean \( r = 0.52 \), range 0.42–0.64) and even stronger correlation with E (representative example in Fig. 4B; all data in Table 1, mean \( r = 0.87 \), range 0.74–0.95). Third, as could be deduced from the correlation between the RR intervals and the filling indexes, there was a significant positive linear relationship between RR interval and LV-EDV obtained from LV volume curve (representative example in Fig. 4C; mean \( r = 0.53 \), range 0.34–0.74). Finally, there was a strong positive linear relationship between LV-EDV and subsequent peak systolic LV (representative example in Fig. 4D; all data in Table 1, mean \( r = 0.82 \), range 0.79–0.90).

These findings demonstrated that longer RR intervals were associated with improved filling indexes (filling time FT and volume E) and LV-EDV that led to augmentation of the peak...
systolic pressure in the studied beat during AF. The question remained whether and to what extent did the increased systolic pressure affect the diastolic parameters.

Relationship between $dP/dt$ min and $\tau$, and the peak systolic LVP. As shown in Fig. 5, A–L, each of the 12 animals showed a strongly significant positive linear relationship between $dP/dt$ min and peak systolic LVP (Table 1, mean $r = 0.95$, range $0.87 \sim 0.98$) and a strongly negative curvilinear relationship between $\tau$ and peak systolic LVP in the same cardiac beat during AF (Fig. 6; Table 1, mean $r = -0.85$, range $-0.46 \sim -0.96$). A significant negative curvilinear relationship was found between the two parameters $\tau$ and $dP/dt$ min in each case (Fig. 7; Table 1, mean $r = -0.86$, range $-0.56 \sim -0.98$).

These findings revealed the complex sequel of relations between the diastolic parameters and the individual beat-to-beat variability during AF, and confirmed our working hypothesis that beats with longer coupling intervals RR 1 , as well as those with additional postextrasystolic booster (i.e., large RR 1 /RR 2 ), end up with an elevated LVP that in turn plays an important role for the improved diastolic characteristics during AF.

Fig. 3. Representative relationship between the minimum value of $dP/dt$ ($dP/dt_{\text{min}}$) time constant of isovolumic LV pressure decay ($\tau$), peak systolic LVP and RR 1 (A–C), or RR 1/RR 2 (D–F) in one animal. There were positive curvilinear trends between $dP/dt_{\text{min}}$ and RR 1 or RR 1/RR 2 and negative curvilinear trends between $\tau$ and RR 1 or RR 1/RR 2 . There were also positive curvilinear trends between peak systolic LVP and RR 1 or RR 1/RR 2 similarly to the relationship described for $dP/dt_{\text{min}}$.

Fig. 4. Representative relationship between preceding RR 1 interval and Doppler echocardiography parameters LV-FT and $E_vti$, LV end-diastolic volume (EDV) and peak systolic LVP in one animal. There was a significant positive linear relationship between the RR intervals and LV-FT in the same cardiac beat (A). LV-FT showed a significant positive linear relationship with mitral inflow $E_vti$ (B). There was also a significant positive linear relationship between the RR interval and LV-EDV (C). Finally, there was a strong positive linear relationship between LV-EDV and subsequent peak systolic LVP (D).
This study demonstrated that the determinants of LV diastolic function during AF depend largely on the beat-to-beat changes in preload that reflect RR variability in accordance with the following mechanism. The duration of the preceding RR interval directly affects the LV-FT (Fig. 4A). Furthermore, due to the close correlation between the LV-FT and the LV filling volume (evaluated by Evti; Fig. 4B), prolongation of RR interval results in an increase of the LV-EDV (Fig. 4C).

Consequently, the increased LV-EDV determines larger peak dP/dtmin, minimum value of the first derivative of left ventricular pressure curve; E, peak early diastolic mitral inflow velocity; Evti, early diastolic mitral inflow velocity time integral; EDV, end-diastolic volume; FT, filling time; LVP, peak systolic left ventricular pressure; NA, not available, τ, time constant of isovolumic left ventricular pressure decay. *P < 0.0001 vs. regression coefficient between FT and E.

**Table 1.** Correlation coefficients between Doppler echocardiography and LV pressure-volume derived indexes in each animal

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>FT vs. E</th>
<th>FT vs. Evti</th>
<th>EDV vs. LVP</th>
<th>dP/dtmin vs. LVP</th>
<th>τ vs. LVP</th>
<th>dP/dtmin vs. τ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.93</td>
<td>−0.88</td>
<td>−0.91</td>
</tr>
<tr>
<td>2</td>
<td>0.44</td>
<td>0.83</td>
<td>0.79</td>
<td>0.96</td>
<td>−0.96</td>
<td>−0.98</td>
</tr>
<tr>
<td>3</td>
<td>0.42</td>
<td>0.74</td>
<td>0.84</td>
<td>0.95</td>
<td>−0.92</td>
<td>−0.93</td>
</tr>
<tr>
<td>4</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.95</td>
<td>−0.92</td>
<td>−0.92</td>
</tr>
<tr>
<td>5</td>
<td>0.53</td>
<td>0.95</td>
<td>0.87</td>
<td>0.98</td>
<td>−0.95</td>
<td>−0.92</td>
</tr>
<tr>
<td>6</td>
<td>0.54</td>
<td>0.93</td>
<td>0.79</td>
<td>0.95</td>
<td>−0.92</td>
<td>−0.94</td>
</tr>
<tr>
<td>7</td>
<td>0.64</td>
<td>0.91</td>
<td>0.90</td>
<td>0.97</td>
<td>−0.90</td>
<td>−0.91</td>
</tr>
<tr>
<td>8</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.93</td>
<td>−0.57</td>
<td>−0.63</td>
</tr>
<tr>
<td>9</td>
<td>0.52</td>
<td>0.89</td>
<td>0.82</td>
<td>0.87</td>
<td>−0.46</td>
<td>−0.56</td>
</tr>
<tr>
<td>10</td>
<td>0.55</td>
<td>0.80</td>
<td>0.83</td>
<td>0.93</td>
<td>−0.80</td>
<td>−0.71</td>
</tr>
<tr>
<td>11</td>
<td>0.54</td>
<td>0.89</td>
<td>0.69</td>
<td>0.97</td>
<td>−0.94</td>
<td>−0.97</td>
</tr>
<tr>
<td>12</td>
<td>0.49</td>
<td>0.89</td>
<td>0.87</td>
<td>0.98</td>
<td>−0.92</td>
<td>−0.95</td>
</tr>
</tbody>
</table>

Means ± SD 0.52 ± 0.06 0.87 ± 0.07* 0.82 ± 0.06 0.95 ± 0.03 −0.85 ± 0.16 −0.86 ± 0.14

Fig. 5. Relationship between peak systolic LVP and dP/dtmin for animals 1–12 (A–L). There was a strong positive linear relationship between LVP and dP/dtmin.
systolic LVP (Fig. 4D). Finally, the LV diastolic function evaluated by dP/dt\text{min} and τ directly depends on the peak systolic LVP (Figs. 5 and 6) and therefore on the preceding RR variability. In brief, prolongation of the RR intervals during AF augments LV filling volume, increases the following peak systolic LVP and thereby results in improved subsequent LV relaxation.

**Force-interval relationship and Frank-Starling mechanism effect on LV systolic function during AF.** Although evaluation of LV performance during AF is inherently difficult because of its beat-to-beat variability, the role of the preceding RR intervals has been well established in determining LV systolic function (1, 9, 11, 12, 16, 18, 20, 21). Specifically, the LV systolic variables show positive and negative linear relationships with RR\textsubscript{1} and RR\textsubscript{2} intervals, respectively (9, 11, 12, 18). These phenomena were explained by interaction of the force-interval relationships: mechanical restitution governed by RR\textsubscript{1} and postextrasystolic potentiation determined by RR\textsubscript{2} (18), as well as by the Frank-Starling mechanism (1). Extrapolating from these mechanisms, Suzuki et al. (20) and we (21) have reported that LV systolic variables at RR\textsubscript{1}/RR\textsubscript{2} = 1 in the linear regression line could be a good indexes of the averaged LV systolic function during AF.

Is Frank-Starling mechanism involved in LV diastolic function during AF? On the other hand, there were fewer reports (5, 14–17) paying attention to the LV diastolic function during AF. We expected to find simple rules, similar to those governing the systolic performance that could be used to estimate LV diastolic function based on the force-interval relationship and/or Frank-Starling mechanism. However, the present study did not show simple linear relationship between LV diastolic parameters and RR\textsubscript{1} interval or RR\textsubscript{1}/RR\textsubscript{2} ratio. Nakamura et al. (16) have previously reported that τ did not show correlation with RR\textsubscript{1} interval or RR\textsubscript{1}/RR\textsubscript{2} ratio and that τ was fairly constant for a wide range of these parameters. These authors concluded that there should be independent mechanisms for regulating contractility and relaxation. To a certain degree, these results are consonant with our findings that no simple linear relationship existed between LV diastolic parameters and RR\textsubscript{1} or RR\textsubscript{1}/RR\textsubscript{2} ratio. However, in the present study, τ was never constant when RR intervals varied greatly, and long RR intervals consistently yielded small τ (Fig. 3B). Careful evaluation of LV performance during AF is inherently difficult because of its beat-to-beat variability, the role of the preceding RR intervals has been well established in determining LV systolic function (1, 9, 11, 12, 16, 18, 20, 21). Specifically, the LV systolic variables show positive and negative linear relationships with RR\textsubscript{1} and RR\textsubscript{2} intervals, respectively (9, 11, 12, 18). These phenomena were explained by interaction of the force-interval relationships: mechanical restitution governed by RR\textsubscript{1} and postextrasystolic potentiation determined by RR\textsubscript{2} (18), as well as by the Frank-Starling mechanism (1). Extrapolating from these mechanisms, Suzuki et al. (20) and we (21) have reported that LV systolic variables at RR\textsubscript{1}/RR\textsubscript{2} = 1 in the linear regression line could be a good indexes of the averaged LV systolic function during AF.

Is Frank-Starling mechanism involved in LV diastolic function during AF? On the other hand, there were fewer reports (5, 14–17) paying attention to the LV diastolic function during AF. We expected to find simple rules, similar to those governing the systolic performance that could be used to estimate LV diastolic function based on the force-interval relationship and/or Frank-Starling mechanism. However, the present study did not show simple linear relationship between LV diastolic parameters and RR\textsubscript{1} interval or RR\textsubscript{1}/RR\textsubscript{2} ratio. Nakamura et al. (16) have previously reported that τ did not show correlation with RR\textsubscript{1} interval or RR\textsubscript{1}/RR\textsubscript{2} ratio and that τ was fairly constant for a wide range of these parameters. These authors concluded that there should be independent mechanisms for regulating contractility and relaxation. To a certain degree, these results are consonant with our findings that no simple linear relationship existed between LV diastolic parameters and RR\textsubscript{1} or RR\textsubscript{1}/RR\textsubscript{2} ratio. However, in the present study, τ was never constant when RR intervals varied greatly, and long RR intervals consistently yielded small τ (Fig. 3B). Careful evaluation of LV performance during AF is inherently difficult because of its beat-to-beat variability, the role of the preceding RR intervals has been well established in determining LV systolic function (1, 9, 11, 12, 16, 18, 20, 21). Specifically, the LV systolic variables show positive and negative linear relationships with RR\textsubscript{1} and RR\textsubscript{2} intervals, respectively (9, 11, 12, 18). These phenomena were explained by interaction of the force-interval relationships: mechanical restitution governed by RR\textsubscript{1} and postextrasystolic potentiation determined by RR\textsubscript{2} (18), as well as by the Frank-Starling mechanism (1). Extrapolating from these mechanisms, Suzuki et al. (20) and we (21) have reported that LV systolic variables at RR\textsubscript{1}/RR\textsubscript{2} = 1 in the linear regression line could be a good indexes of the averaged LV systolic function during AF.
analysis revealed that both diastolic parameters, dP/dt\text{min} and τ, had strong linear and curvilinear relationships, respectively, with peak systolic LVP (Figs. 5 and 6), which directly correlated to LV-EDV (Fig. 4D) and was thus associated with the duration of the preceding RR interval (Fig. 4C). From these results, one should conclude that LV relaxation was more dependent on the Frank-Starling mechanism (via LV-EDV) than directly on the force-interval relationships (via RR1/RR2).

Mechanistic relationship between peak systolic LVP and LV diastolic function during AF. During sinus rhythm, Weisfeldt et al. (25) has already reported that LV relaxation is determined primarily by peak systolic LVP. An increase in systolic LVP at longer filling period caused increase in dP/dt\text{min} reflecting faster initial LVP fall (4). Brausaert et al. (2) has reported that a more rapid relaxation should be corresponding to an acute reduction in LV systolic volume simply by virtue of restoring forces. It has been reported (14) that interventions that enhance the systolic shortening augment relaxation. In the present study, during AF, we found that dP/dt\text{min} did not correlate directly with preceding RR1 interval, but these parameters were significantly correlated with peak systolic LVP. This indicates that the longer RR intervals produced greater LV-EDV and, according to the Frank-Starling mechanism, greater subsequent peak systolic LVP thus promoting greater LV contractility and relaxation.

Potential benefit of slowing the ventricular rate during AF. We have previously reported the benefits of slower ventricular rate during AF on LV diastolic function (17, 24, 26, 27). In these studies, the improvement of diastolic parameters was demonstrated during selective atrioventricular nodal vagal stimulation by analysis of averaged values of diastolic parameters for >100 consecutive cardiac beats. In particular, prolongation of the average RR interval caused an improvement of the average dP/dt\text{min}. The results from the present study, based on beat-to-beat analysis, further expand these results and provide mechanistic explanation for the observed improvement of LV diastolic function by vagally induced slowing of the ventricular rate in AF.

Study limitations. Because of the nature of animal studies, certain limitations should be considered in our experiments. First, AF was acutely induced by burst stimulation of the right atrium and was perpetuated by periodical selective vagal stimulation to the sinus node in anesthetized open-chest dogs. The resultant ventricular responses were fast and random, but their sequence might be different in patients with chronic AF. Moreover, although the present data are strongly suggestive about the benefits of rate control, separate studies are needed during controlled slowing because filling volume and peak systolic LVP might have different dependence in the range of substantially longer RR intervals (13). Second, we used healthy
mongrel dogs in this study. However, the relationships between filling intervals, peak systolic LVP, and LV relaxation during AF might be disturbed in patients with organic heart disease. Third, it is generally accepted that τ is a more reliable diastolic index than dP/dt_{min} because it is not influenced by preload or afterload and is derived from multiple pressure measurements throughout the entire period of isovolumic relaxation (8). Furthermore, the LV relaxation determined by τ (in contrast to dP/dt_{min}) was reported to be independent of systolic pressure (7, 22). This seeming discrepancy with our results most likely reflects the fact that the above studies were performed during sinus rate but not AF. In the present study, changes of both τ and dP/dt_{min} corresponded well to the peak systolic LVP. Furthermore, there was a significant curvilinear relationship between the τ and dP/dt_{min}, suggesting that both parameters are reliable indices for beat-to-beat analysis during AF.

We conclude that for individual beats during AF, the LV relaxation is determined by the peak systolic LVP, which, in turn, is proportional to the preceding LV filling interval. Slowing of the ventricular rate during AF might benefit the diastolic function by utilizing a functional link between the prolonged LV filling interval and the subsequent higher peak systolic LVP, promoting greater LV relaxation.

ACKNOWLEDGMENTS

The authors thank Deborah A. Agler, Don G. Hills, and William J. Kowaleski for expert assistance during the echocardiographic studies and for the surgical preparations. We also acknowledge technical support from St. Jude Medical (St. Paul, MN) and Medtronic (Minneapolis, MN) in leads and pacing equipment.

GRANTS

This work was supported by American Heart Association Grant AHA-9808489A and National Heart, Lung, and Blood Institute Grant RO1-HL-60853-01A1 (to T. N. Mazgalev).

REFERENCES
