Endurance Training in the Spontaneously Hypertensive Rat
Conversion of Pathological into Physiological Cardiac Hypertrophy

Carolina D. Garciarena, Oscar A. Pinilla, Mariela B. Nolly, Ruben P. Laguens, Eduardo M. Escudero, Horacio E. Cingolani, Irene L. Ennis

Abstract—The effect of endurance training (swimming 90 min/d for 5 days a week for 60 days) on cardiac hypertrophy was investigated in the spontaneously hypertensive rat (SHR). Sedentary SHRs (SHR-Cs) and normotensive Wistar rats were used as controls. Exercise training enhanced myocardial hypertrophy assessed by left ventricular weight/tibial length (228±7 versus 251±5 mg/cm in SHR-Cs and exercised SHRs [SHR-Es], respectively). Myocyte cross-sectional area increased ≈40%, collagen volume fraction decreased ≈50%, and capillary density increased ≈45% in SHR-Es compared with SHR-Cs. The mRNA abundance of atrial natriuretic factor and myosin light chain 2 was decreased by the swimming routine (100±19% versus 41±10% and 100±8% versus 61±9% for atrial natriuretic factor and myosin light chain 2 in SHR-Cs and SHR-Es, respectively). The expression of sarcoplasmic reticulum Ca$^{2+}$ pump was significantly augmented, whereas that of Na$^+$/Ca$^{2+}$ exchanger was unchanged (93±7% versus 167±8% and 158±13% versus 157±7%, sarcoplasmic reticulum Ca$^{2+}$ pump and Na$^+$/Ca$^{2+}$ exchanger in SHR-Cs and SHR-Es, respectively; P<0.05). Endurance training inhibited apoptosis, as reflected by a decrease in caspase 3 activation and poly(ADP-ribose) polymerase-1 cleavage, and normalized calcineurin activity without inducing significant changes in the phosphatidylinositol 3-kinase/Akt pathway. The swimming routine improved midventricular shortening determined by echocardiography (32.4±0.9% versus 36.9±1.1% in SHR-Cs and SHR-Es, respectively; P<0.05) and decreased the left ventricular free wall thickness/left ventricular cavity radius toward an eccentric model of cardiac hypertrophy (0.59±0.02 versus 0.53±0.01 in SHR-Cs and SHR-Es, respectively; P<0.05). In conclusion, we present data demonstrating the effectiveness of endurance training to convert pathological into physiological hypertrophy improving cardiac performance. The reduction of myocardial fibrosis and calcineurin activity plus the increase in capillary density represent factors to be considered in determining this beneficial effect. (Hypertension. 2009;53:708-714.)

Key Words: exercise training ■ cardiac hypertrophy ■ hypertension ■ calcium handling ■ apoptosis ■ signaling pathways

Before the late 1980s, patients with heart failure were advised to avoid physical exercise. However, it is well known that regular physical activity protects against cardiovascular disease. It is widely recognized that chronic exercise training attenuates several of the main risk factors for cardiovascular diseases, such as high blood pressure and insulin resistance.1,2 Interestingly, it has been reported that low-intensity exercise training markedly delayed the onset of decompensate heart failure and improved survival in the spontaneously hypertensive heart failure rat model.3 This effect was attained independent of any significant effect on blood pressure.3 Exercise training in selected heart failure patients has been demonstrated not only to be safe but also beneficial.4,5

Diverse stimuli, such as hypertension and myocardial infarction, induce the development of cardiac hypertrophy (CH) that constitutes one of the main cardiovascular risk factors and a poor prognostic sign associated with nearly all forms of heart failure.6 This type of CH is known as pathological. However, cardiac enlargement may represent a favorable adaptation restricted to match the increase in functional demand in response to exercise training, with preserved or enhanced cardiac function, that does not cause or contribute to disease.7,8 This type of CH is known as physiological hypertrophy (ie, athlete’s heart).

The purpose of this study was to assess the effects of chronic physical training (swimming routine) on pathological CH induced by pressure overload in the animal model of the spontaneously hypertensive rat (SHR). The results presented here support that exercise training converts the pattern of pathological into physiological hypertrophy, improving myocardial performance.

Materials and Methods
Male SHRs at 4 months of age were randomly assigned to sedentary (SHR-C; n=13) and swimming-trained (SHR-E; n=9) groups. Age-
Cardiac Gross Morphology, Histology, and Gene Expression

Morphological data from each experimental group are summarized in the Table. Exercise training exacerbated CH in SHRs, as revealed by the increase in the left ventricular mass/tibial length ratio and the LV mass index. No significant changes were detected in systolic blood pressure or body weight compared with the sedentary SHR. A significant increase in LV diastolic diameter was detected in the SHR-E at the end of the 60-day swimming protocol. The geometry of the LV chamber was modified by the exercise routine from a concentric toward an eccentric type of CH, as revealed by the decrease in the relation between the thickness of the LV free wall and the radius of the cavity.

Exercise training induced an average increase of 40% in mean cardiac myocyte cross-sectional area, whereas collagen volume fraction was decreased by ~50%, making its abundance not different to that of normotensive rats (Figure 1A and 1B). Interestingly, these histological changes in the myocardium of the exercised rats were accompanied by a significant increase in capillary density (Figure 1C). Myocardial capillary density showed a tendency toward a smaller value in the SHR-Cs compared with the nonhypertrophied myocardium of the Wistar rats, although it did not reach statistical significance.

Because it is well known that pathological CH is characterized by the induction of genes normally expressed during fetal development, such as atrial natriuretic factor (ANF) and myosin light chain 2, the mRNA abundance of these 2 genes was assessed in the myocardium of sedentary and exercised SHRs by real-time RT-PCR. Swimming training significantly lowered the myocardial expression of both ANF and myosin light chain 2 (Figure 1D). The SERCA2a and the Na+/Ca2+ exchanger are 2 proteins involved in

### Table. General Characteristics of the Experimental Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>SHR-C (n=13)</th>
<th>SHR-E (n=9)</th>
<th>Wistar (n=5)</th>
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<td>Body weight, g</td>
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<td>304±7†</td>
<td>403±16*</td>
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<td>LW, mg</td>
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<td>931±24*†</td>
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<td>183±3†</td>
<td>118±3*</td>
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<td>430±11</td>
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<td>LVWT, mm</td>
<td>1.88±0.03</td>
<td>1.86±0.01†</td>
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<tr>
<td>LVDD, mm</td>
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<td>6.91±0.14*</td>
<td>7.29±0.35*</td>
</tr>
<tr>
<td>H/R</td>
<td>0.59±0.02</td>
<td>0.53±0.01*†</td>
<td>0.44±0.02*</td>
</tr>
</tbody>
</table>

LWW indicates LV weight; RW, right ventricular weight; TL, tibial length; LVMI, LV mass index; SBP, systolic blood pressure; HR, heart rate; LWWT, LV wall thickness; LVDD, LV diastolic diameter; H/R:LWWT, LV cavity radius.

*P<0.05 vs SHR-C, by ANOVA. †P<0.05 vs Wistar, by ANOVA.

and sex-matched normotensive Wistar rats (n=5) were used as nonhypertrophied controls. The study was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the 60-day experimental protocol was approved by the La Plata School of Medicine Animal Welfare Committee.

For detailed Materials and Methods, please see the online data supplement, available at http://hyper.ahajournals.org.

### Results

Left ventricular (LV) structural, molecular, and functional remodeling was studied at 60 days of exercise training in SHRs in the compensated stage of CH. Age- and sex-matched sedentary SHRs, as well as normotensive rats (Wistar), were use as hypertrophic and normotrophic controls, respectively.

**Figure 1.** Exercise training induced in the SHR a significant increase in cardiomyocyte cross-sectional area (CSA; ~40% vs sedentary SHR; A) and a significant decrease (~50%) in collagen abundance (CVF), making it not different from that detected in the normotrophic normotensive rats (B). Collagen was quantified as the percentage of red area in the histological slides. C shows that physical training succeeds in increasing myocardial capillary density. D. The relative expression of 2 molecular markers of pathological CH was evaluated by real-time RT-PCR in the myocardium of SHR-C, SHR-E, and Wistar rats. A significant reduction in the mRNA abundance of ANF and MLC-2 was detected in the hypertrophied myocardium of the SHR subjected to the swimming routine. Corresponding data of normotensive (Wistar) rats were included for the sake of facilitating the comparison. *P<0.05 vs SHR-C; †P<0.05 vs Wistar, by ANOVA. MLC-2 indicates myosin light chain 2.
calcium cycling in which expression has been reported to be altered (downregulation of SERCA and upregulation of Na\(^+\)/Ca\(^{2+}\) exchanger) in several models of experimental and human pathological CH and cardiac failure. Physical training induced a significant increase in the expression of SERCA2a, whereas no changes were detected in the expression of Na\(^+\)/Ca\(^{2+}\) exchanger (Figure 2A and 2B).

**Exercise Training Downregulates Calcineurin Activity**

Periodic swimming significantly decreased calcineurin A (CnA) β expression, a good indicator of calcineurin activity,\(^{10,11}\) in the hypertrophied myocardium of the SHR-E to levels not different from those detected in the myocardium of normotensive rats (Figure 3A). On the other hand, no effect of endurance training was evident on the phosphatidylinositol 3-kinase (PI3-K)/Akt pathway (Figure 3B and 3C).

**Apoptosis Is Inhibited by Exercise Training**

Because apoptosis is increased in hypertensive CH and it has been demonstrated to play a role in the transition from hypertrophy to heart failure,\(^{12-15}\) we aimed to determine whether exercise training was able to induce an inhibitory effect on the apoptosis cascade. To this purpose, we assessed by protein immunoblotting the activation of procaspase-3, as well as the cleavage extent of poly(ADP-ribose) polymerase by caspase-3 in the myocardium of exercised and sedentary SHRs and in that of normotensive rats. Endurance training significantly decreased the extent of procaspase-3 cleaved into fragments of 17 kDa (Figure 4A), as well as the amount of fragments of 85 kDa from the precursor poly(ADP-ribose) polymerase-1 (Figure 4B), although not to the levels detected in normotensive rats, indicating a decreased activation of both effectors of the apoptotic pathway.

**Endurance Training Improves Cardiac Function**

At the beginning of the experimental protocol, no difference was observed between the experimental groups with respect to LV systolic function evaluated echocardiographically. However, a slight but significant increase in midventricular shortening was detected in the trained SHR at the completion of the 60-day swimming routine (Figure 5).

**Discussion**

In this study, we analyzed the myocardial effects of endurance training (periodic swimming) in an experimental model of pathological CH induced by pressure overload. Our results demonstrate that periodic exercise training is capable of transforming hypertension-induced pathological CH into physiological CH at the structural, molecular, and functional levels, at least when initiated in the compensated phase of CH. These data are in agreement with recent studies supporting the idea that low-intensity exercise protocols, such as periodic swimming, are effective in decreasing cardiac pathologies.

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**Figure 2.** Exercise training induced a significant increase (~80%) in the myocardial expression of the sarcoplasmic reticulum Ca\(^{2+}\) pump (SERCA2a; A) without altering that of the Na\(^+\)/Ca\(^{2+}\) exchanger (NCX), as revealed by Western blot analysis. Average data are depicted in the bar graphs, and representative blots are shown on top of the bars for both panel figures. *P < 0.05 vs SHR-C; †P < 0.05 vs Wistar, by ANOVA.

**Figure 3.** A, Myocardial CnAβ expression quantified by Western blot analysis was upregulated in the hypertrophied myocardium of the sedentary SHR, whereas a significant decrease in its expression was detected after the completion of the training period. Because the expression of CnAβ reflects well the level of activation of the phosphatase and the myocardial CnAβ expression of the SHR-E was not significantly different from that of the normotensive normotrophic rats, it is possible to conclude that endurance training normalized the activity of this intracellular signaling pathway. The expression of the prohypertrophic kinases PI3-K p110α (B) and phospho-Akt (P-Akt; C) was significantly higher in the hypertrophied myocardium of the SHR-C compared with the normotensive rats and remained unchanged at the end of the swimming protocol. Average data are depicted in the bar graphs, and representative blots are shown on top of the bars for each panel figure. *P < 0.05 vs SHR-C, by ANOVA; n = 5 for each group.
exercise training may result in beneficial adaptations, even in the presence of heart failure.\(^3,16\) However, excessive exercise can have deleterious effects on cardiac remodeling and function, as reported by Schultz et al.\(^17\)

It is widely recognized that exercise training protects individuals from a variety of cardiovascular diseases.\(^3,16,18,19\) However, the mechanisms underlying this beneficial effect are not completely understood. It is interesting to note that, in our experimental protocol, endurance training exerted its beneficial effect without modifying systolic blood pressure, a result that is in agreement with previous reports.\(^20,21\)

The cardiac response elicited by pressure overload differs greatly at the structural and functional levels from that induced by endurance training. Pathological CH is characterized by cardiac fibrosis; decrease vascularization; enhanced apoptosis; re-expression of fetal genes; down-regulation of metabolic genes, especially those involved in fatty acid metabolism; LV dysfunction; and increase mortality (for review, see References 22–24). Both physiological and pathological CHs are associated with alterations in cardiac geometry; pressure overload usually determines concentric hypertrophy (increased wall thickness with relatively small cavities), whereas volume-overloaded hearts present eccentric hypertrophy (proportional increase in wall thickness and chamber dimensions). The latter is the pattern seen with endurance exercise training, such as long distance running or swimming.\(^8\) Despite the fact that differences between physiological and pathological CHs were well known, until relatively recently, it was unclear whether these 2 forms of hypertrophy were induced by different intracellular signaling cascades. Because usually the stimuli for pathological CH are chronic, whereas those for physiological CH are intermittent, the duration of the stimulus was thought to be critical in determining the phenotypic response. However, Perrino et al\(^25\) demonstrated in an interesting murine model of intermittent pressure overload that it was the nature of the triggering stimulus and the intracellular signaling pathway activated, as opposed to the duration, that established the type of CH. At present, \(\geq 2\) cascades playing distinct roles in physiological and pathological CHs have been characterized, the PI3-K/Akt and the calcineurin pathways, respectively (reviewed in References \(23,24\)). We demonstrated that endurance training was able to normalize calcineurin activity without interfering with the PI3-K/Akt pathway. This result is of great importance, because calcineurin appears to largely mediate pathological but not physiological CH.\(^10,11,26–31\) Calcineurin is a calcium/calmodulin-dependent serine/threonine phosphatase that dephosphorylates members of the nuclear factor of activated T cells transcription factor family permitting their nuclear translocation and activation of transcription. Transgenic mice overexpressing an activated form of calcineurin or NFAT3 in the myocardium developed CH that rapidly progressed to heart failure.\(^28\) On the contrary, CnAB-deficient mice displayed an impaired hypertrophic response to pathological stimuli, such as pressure overload and angiotensin II or isoproterenol infusion.\(^26\) Furthermore, in NFAT-luciferase reporter transgenic mice subjected either to physiological (exercise training or growth hormone-IGF1 infusion) or to pathological (pressure overload or myocardial infarction) stimuli, calcineurin/NFAT activity was upregulated only in the pathological models.\(^29\)

The PI3-K/Akt signaling pathway is one of the main signaling cascades involved in normal postnatal cardiac growth.\(^32\) Its upregulation has been demonstrated to induce both physiological and pathological CHs.\(^32–34\) The phenotype determined may be related, at least in part, to the degree of Akt upregulation; overstimulation of this pathway would lead to pathological CH.\(^35\) On the other hand, it is relevant that the PI3-K/Akt pathway promotes cell survival by inhibiting apoptosis at multiple points.\(^36\) This makes the strategy of endurance training even more interesting as a therapeutic tool to induce pathological CH.

**Figure 4.** Endurance training exerted an inhibitory effect on proapoptotic signaling. It significantly decreased the extent of procaspase-3 cleaved into fragments of 17 kDa (A), as well as the amount of fragments of 85 kDa from the precursor poly(ADP-ribose) polymerase-1 (B), although not to the levels detected in normotensive rats, indicating a decreased activation of both effectors of the apoptotic pathway. Average data are depicted in the bar graphs, and representative blots are shown on top of the bars for each panel figure. \(\ast P<0.05\) vs SHR-C; \(\ast\ast P<0.05\) vs Wistar, by ANOVA; \(n=5\) for each group.

**Figure 5.** At the beginning of the experimental protocol, no significant differences in LV systolic function, assessed echographically by the midwall (MS) shortening, were present between the experimental groups. However, an improvement in cardiac performance was detected in the trained SHR after completion of the swimming routine. MS at day 60 was slightly but significantly higher in SHR-E vs SHR-C. \(\ast P<0.05\) vs SHR-C, by ANOVA.
regression, because it does not interfere with the PI3-K/Akt pathway, whereas it downregulates the pathological cascade of calcineurin/NFAT. Increased circulating levels of ANF have been positively correlated with the severity of heart failure.\textsuperscript{37,38} Importantly, our swimming protocol reduced the expression of ANF, at least at the mRNA level, as indicated by the real-time RT-PCR experiments, supporting the idea that endurance training impacts beneficially on cardiac performance, even in the presence of pathological CH.

Relatively few studies in experimental models of heart failure have addressed the effect of exercise training on the myocardial expression of calcium-handling proteins with considerable variability with respect to their findings.\textsuperscript{3,39–42} However, we are not aware of this kind of study in any model of pathological CH. In our experimental setting we detected an upregulation of the expression of SERCA2a induced by the swimming routine. Although we do not have direct evidence supporting a cause-effect relationship between SERCA2a upregulation and cardiac function, we think that it is likely involved in the enhancement in cardiac function detected by echocardiography. Moreover, our data are in agreement with previous reports demonstrating that the improvement in intracellular Ca\textsuperscript{2+} regulation underlies the benefits of exercise training on ventricular function in heart failure.\textsuperscript{41,42} We chose to measure fractional shortening at the LV midwall level because it has been shown to be an accurate and convenient index of LV systolic function superior to endocardial fractional shortening in hypertensive humans and animals.\textsuperscript{43,44,45} Another factor that is probably contributing to better contractility detected in the trained SHR is the downregulation of calcineurin activity induced by exercise training, because it has been reported that this phosphatase exerts negative inotropic effect.\textsuperscript{31,46,47} The normalization of interstitial fibrosis, the increase in capillary density, and the decreased activity of the apoptosis cascade in the SHR-E may be also involved in the improvement in cardiac function evidenced in the echocardiographic study. Interestingly, in a transgenic mice model of CH due to cardiac-specific inducible Akt1 expression, it was demonstrated that the imbalance between myocyte growth and coronary angiogenesis plays a critical role in the contractile dysfunction.\textsuperscript{48} This finding led the authors to propose that it may be advantageous to stimulate angiogenesis as part of a general strategy to prevent or reverse heart failure. In our experimental conditions, exercise training did increase myocardial capillary density in the SHR.

### Perspectives

In the present work we provide new insights into the molecular mechanisms underlying the beneficial effects of endurance training in pathological CH. We demonstrate in an animal model of hypertension-induced pathological CH that exercise training decreases myocardial interstitial collagen abundance, increases myocardial capillary density, and upregulates SERCA2a expression improving LV systolic function. We speculate that these beneficial changes were, at least in part, related to the downregulation of calcineurin activity, because this signaling pathway has been demonstrated to underlie the development of pathological and not physiological CH, although with some controversial results.\textsuperscript{23,29} Figure 6 schematically summarizes the results described above.

In this scenario, our results lend support to the idea that endurance training can positively transform pathological into physiological CH. This finding could have clinical relevance in the design of therapeutic strategies for the prevention of heart failure as the consequence of hypertension-induced CH progression.

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Endurance training in the SHR: conversion of pathologic into physiologic cardiac hypertrophy


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Short title: Exercise training and cardiac hypertrophy in SHR

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Expanded Material and Methods

Animal care and swimming-training protocol
Rats were housed in a temperature – controlled room (22°C) with a 12-h dark-light cycle, with free access to standard laboratory chow and tap water. SHR. The training sessions were performed during the rats’ dark cycle and consisted of 90-min swimming sessions five days/week for 60 days in a swimming apparatus containing warmed water (30-32 °C). Exercise duration was increased gradually until rats could swim for 90 min. Sedentary SHR were placed in the swimming apparatus for 10 min twice a week to mimic the water stress associated with the experimental protocol. This swimming protocol has been characterized by Medeiros et al.{Medeiros A, 2000 #191} as low to moderate intensity and long duration due to improvement in muscle oxidative capacity.

Heart rate (HR) and systolic blood pressure (SBP) by the tail-cuff method were recorded weekly. At the end the 60 day protocol animals were euthanized under deep ether anesthesia, the hearts removed and the left ventricle (LV) with the septum weighed (LVW) and normalized by tibia length (TL) to determine cardiac hypertrophy.

Echocardiographic examination
Rats were monitored echocardiographically under light anesthesia (35 mg/kg pentobarbital sodium IP) by 2-dimensional M-mode echocardiography with a 7-MHz transducer at the beginning and at the end of protocol. Measurements were performed according to the American Society of Echocardiography leading-edge method.{Lang, 2005 #217} LV mass index (LVMI) was calculated as previously described.{Escudero, 2004 #218}

Morphological studies
Ventricular tissue was fixed in buffered 10% formaldehyde and paraffin-embedded. LV coronal sections (4 µm thick) at the equator were stained with hematoxylin -eosin for determining cardiomyocyte cross-sectional area (CSA) or picrosirius red (Direct Red 80 Aldrich) for quantifying collagen volume fraction (CVF) as previously described.{Camilion de Hurtado, 2002 #94} Capillary density expressed as the average number of capillaries per mm² was determined by immunohistochemistry as previously described.{Xu, 2005 #219} Antibodies to tissue transglutaminase (Dako) and to von Willebrand factor (Dako) were used to visualize endothelial cells, and smooth muscle cells were detected with antibodies to α-smooth muscle actin (Dako). For negative controls, primary antibodies were replaced with mouse IgG. Vessels positive for tissue transglutaminase or von Willebrand factor, with no smooth muscle and a diameter of <10 µm were considered capillaries and were counted in at least 5 different microscopic fields (X450) of each section.

Real time RT-PCR
Atrial natriuretic factor (ANF) and myosin light chain-2 (MLC-2) mRNA expression was assessed by real-time RT-PCR and normalized to GAPDH following the procedure described previously.{Ennis, 2005 #106} Primer sequences are depicted in the Supplemental Table S1.

Western Blotting
Left ventricles were homogenized and protein concentration was determined by the Bradford method. Samples were size-fractionated on 4-12% Bis-Tis gels
(Invitrogen), and electrotransferred to PFDV membranes. After blocking, membranes were incubated with the specific antibodies to: calcineurin Aβ, caspase-3, PARP-1 and PI3 kinase p110α (Santa Cruz Biotechnology); NCX (Chemicon); phospho Akt (Cell Signaling) and SERCa2A (Affinity Bioreagents). Actin (Sigma) was assayed as loading control. Bands were visualized using the ECL-Plus chemiluminescence detection system (Amersham). Autoradiograms were analyzed by densitometric analysis (Scion Image). Calcineurin activity was estimated by immunoblot by determining the expression of the isoform Aβ of its catalytic subunit (CnAβ) that has been demonstrated to correlate well with the activity of the phosphatase.\cite{Haq,2001 #90; Taigen, 2000 #188}

Statistics
Results are expressed as mean ± SEM. The Student t test or 1-way ANOVA followed by the Student-Newman-Keuls test. Significance level was set at p < 0.05.

Table S1. Primer sequences used in real-time RT-PCR.

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