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Cardiac Effects of Postconditioning Depend Critically on the Duration of Reperfusion and Reocclusion Episodes

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ABSTRACT

Objective: The objective of the present study was to evaluate the effects of ischemic postconditioning on left ventricular function in isolated rat hearts.

Methods: The hearts of 24 Wistar rats were were isolated, perfused immediately, and distributed into 3 groups: GI, control (n = 8); GII, three 10-second cycles of postconditioning (n = 8); and GIII, three 30-second cycles of postconditioning (n = 8). After a 15-minute stabilization period, all hearts underwent 20 minutes of global ischemia following 20 minutes of reperfusion. At times t_0 (control), t_5, t_{10}, t_{15}, and t_{20} (0, 5, 10, 15, and 20 minutes of reperfusion, respectively), we recorded the heart rate, coronary flow, systolic pressure, $+(dP/dt)_{max}$ (maximum speed of increase in the left ventricular pressure), and $-(dP/dt)_{max}$ (maximum speed of decrease in the left ventricular pressure). Data were analyzed by a 1-way analysis of variance, followed by the Tukey test; a P value <.05 was considered statistically significant.

Results: There were no significant differences among the analyzed groups with respect to heart rate, coronary flow, systolic pressure, and $-(dP/dt)_{max}$ ($P > .05$); however, statistically significant differences in $+(dP/dt)_{max}$ between GII and GI and between GII and GIII occurred at t_{20} (GI, 1409.0 ± 415.1 mm Hg/s; GII, 1917.3 ± 403.0 mm Hg/s; GIII, 1344.8 ± 355.8 mm Hg/s) (GII versus GI, $P = .04$; GII versus GIII, $P = .02$).

Conclusion: Ischemic postconditioning with three 10-second cycles of reperfusion/reocclusion was demonstrated effective for preserving $+(dP/dt)_{max}$ in isolated rat hearts that underwent 20 minutes of ischemia following 20 minutes of reperfusion.

INTRODUCTION

Following an acute myocardial infarction, reestablishing the coronary blood flow with thrombolysis or primary angioplasty is essential to salvage viable myocardium. The infarct size is the deciding determinant of the extent and severity of remodeling and of the prognosis of patients after myocardial infarction [Pfeffer 1991; St. John Sutton 1997]. Reperfusion remains the definitive treatment for attenuating myocardial infarction, contractile dysfunction, and apoptosis. Nevertheless, reperfusion carries with it an inherent risk of intensifying myocardial death—a phenomenon termed *reperfusion-induced injury* [Braunwald 1985; Vinten-Johansen 2005].

In 2003, a phenomenon termed *ischemic postconditioning*, a cardioprotective maneuver that targets the reperfusion phase, was first described by Zhao et al [2003]. Their study described the application of several brief, transient cycles of alternating reperfusion and ischemia immediately after the sustained ischemic episode, which reduced the myocardial injury. In the in vivo dog model, at the immediate onset of reperfusion after a 60-minute occlusion of the left anterior descending coronary (LAD) artery, postconditioning was achieved by allowing reflow for 30 seconds followed by 30 seconds of reocclusion of the LAD artery, repeated a total of 3 times before the final reperfusion phase. Ischemic postconditioning reduced infarct size by 44%, an effect comparable with that obtained with ischemic preconditioning [Zhao 2003].

The temporal characteristics of postconditioning are critical to cardioprotection. In isolated rat hearts, a 30-second algorithm (a 30-second reperfusion plus a 30-second reocclusion) failed to reduce infarct size [Tsang 2005].

The objective of the present study was to evaluate the effects of ischemic postconditioning on left ventricular function in isolated rat hearts.

METHODS

Animals

Experiments were performed with 24 male Wistar rats (280-315 g) in accordance with the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publication no. 85-23; revised 1996) and with prior approval of the Animal Care Committee of São Francisco de Assis Cardiovascular Foundation, Belo Horizonte, Brazil.

Isolated Perfused Rat Hearts

Hearts were excised rapidly and mounted on a Langendorff perfusion system. After a 15-minute stabilization period, all hearts were subjected to 20 minutes of global ischemia and 20

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minutes of reperfusion. Hearts were assigned randomly to one of the following groups: GI, control (n = 8); GII, 10-second postconditioning regimen (n = 8), consisting of 3 cycles of 10 seconds of reperfusion followed by 10 seconds of global ischemia immediately after the index ischemia; GIII, 30-second postconditioning regimen (n = 8), consisting of 3 cycles of 30 seconds of reperfusion followed by 30 seconds of global ischemia immediately after the index ischemia (Figure 1).

Hemodynamics

Data for heart rate, peak systolic pressure, $+(dP/dt)_{max}$ (maximum speed of increase in the left ventricular pressure),

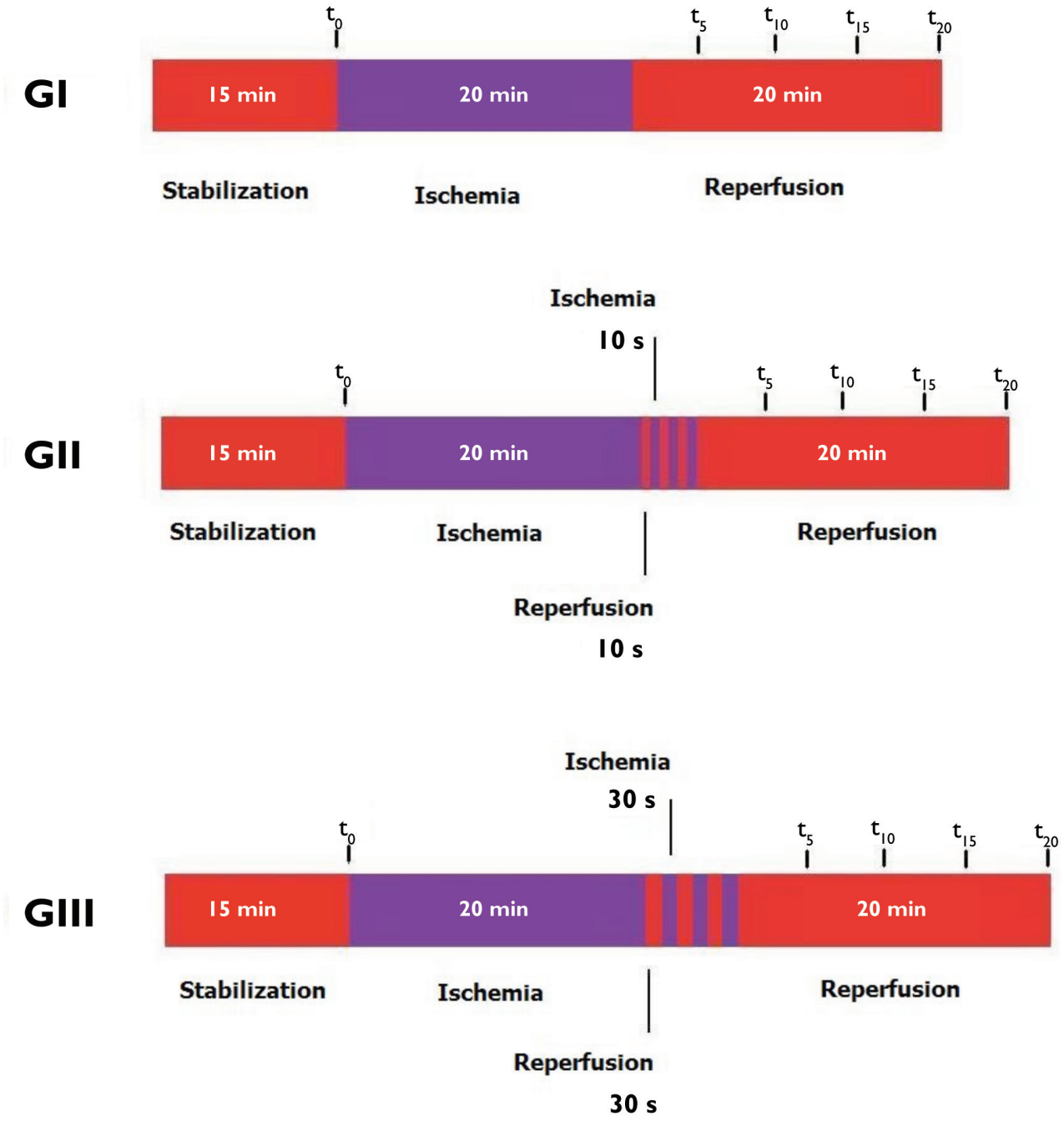

Figure 1. Experimental protocol. GI, group I (control); GII, group II (10-second postconditioning protocol consisting of 3 cycles of 10 seconds of reperfusion followed by 10 seconds global ischemia immediately after the index ischemia); GIII, group 3 (30-second postconditioning protocol consisting of 3 cycles of 30 seconds of reperfusion followed by 30 seconds of global ischemia immediately after the index ischemia. t_0, t_5, t_{10}, t_{15}, and t_{20} indicate 0 (baseline), 5, 10, 15, and 20 minutes of reperfusion, respectively.

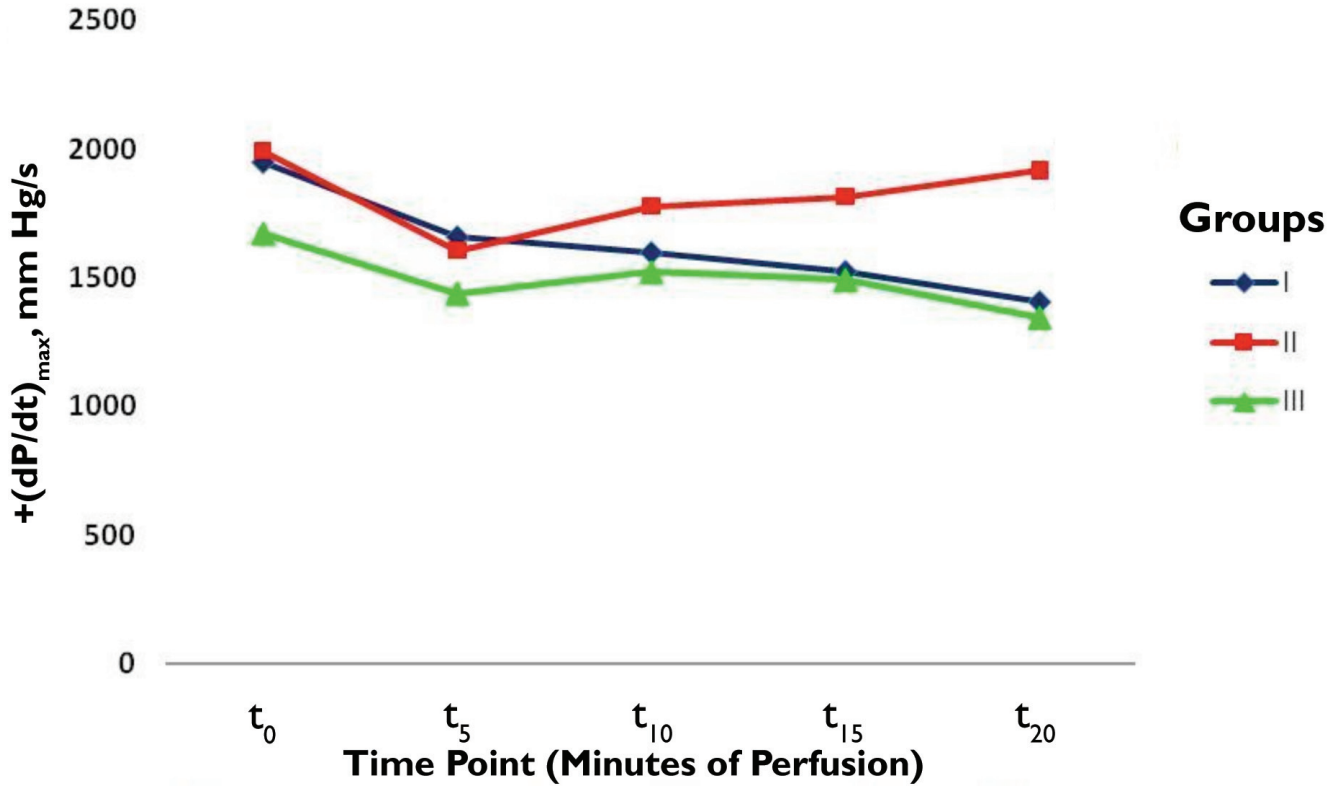

Figure 2. Effect of postconditioning on $+(dP/dt)_{max}$ (left ventricle contractile function). Group I, control; group II, 10-second postconditioning protocol; group III, 30-second postconditioning protocol. *P* = .02 (group II versus group III); *P* = .04 (group II versus group I).

and $-(dP/dt)_{max}$ (maximum speed of decrease in the left ventricular pressure) were acquired with a monitor (BESE DH 073; Bioengenharia, Belo Horizonte, Brazil). Coronary flow was evaluated by measuring the return of perfused solution from the coronary sinus and was expressed in milliliters per minute. Hemodynamic data were collected at t_0 (baseline), t_5, t_{10}, t_{15}, and t_{20} (at 0, 5, 10, 15, and 20 minutes of reperfusion, respectively).

Statistical Analysis

All values are expressed as the mean ± SD. The results were analyzed by 1-way analysis of variance, followed by the Tukey test. Differences were considered statistically significant when *P* values were <.05.

RESULTS

The heart rate decreased in the 3 groups after 20 minutes of reperfusion (t_{20}) compared with the baseline (t_0) measurement; there were no significant differences (GI, 232.5 ± 36.8 beats/min; GII, 241.8 ± 46.7 beats/min; GIII, 249.4 ± 40.4 beats/min; *P* > .05) (Table 1). There were also no significant differences between the groups with respect to coronary flow (GI, 18.5 ± 4.6 mL/min; GII, 21.4 ± 4.4 mL/min; GIII, 22.1 ± 9.0 mL/min; *P* > .05), systolic pressure (GI, 132.6 ± 49.3 mm Hg; GII, 140.8 ± 43.1 mm Hg; GIII, 112.6 ± 33.2 mm Hg; *P* > .05) and $-(dP/dt)_{max}$ (GI, 1490.6 ± 512.0 mm Hg/s; GII, 1770.4 ± 406.6 mm Hg/s; GIII, 1399.1 ± 327.4 mm Hg/s; *P* > .05) (Tables 2-4).

Table 1. Heart Rate*

Group	Time Point				
	t_0	t_5	t_{10}	t_{15}	t_{20}
I, beats/min	259.0 ± 47.0	273.0 ± 61.6	261.9 ± 43.0	244.8 ± 39.6	232.5 ± 36.8
II, beats/min	243.4 ± 32.9	255.3 ± 45.3	245.0 ± 38.7	241.8 ± 46.7	246.5 ± 35.3
III, beats/min	263.3 ± 44.5	265.9 ± 33.0	258.1 ± 39.4	255.1 ± 44.5	249.4 ± 40.4

*Data are expressed as the mean ± SD. Group I, control; group II, 10-second postconditioning protocol; group III, 30-second postconditioning protocol. t_0, t_5, t_{10}, t_{15}, and t_{20} indicate 0 (baseline), 5, 10, 15, and 20 minutes of reperfusion, respectively. There were no significant differences between the groups.

Table 2. Coronary Flow*

Group	Time Point				
	t_0	t_5	t_{10}	t_{15}	t_{20}
I, mL/min	20.9 ± 3.5	20.6 ± 3.4	20.9 ± 3.5	18.9 ± 3.9	18.5 ± 4.6
II, mL/min	22.4 ± 3.5	23.3 ± 3.8	23.1 ± 4.3	20.5 ± 3.5	21.4 ± 4.4
III, mL/min	23.5 ± 8.1	23.1 ± 7.5	22.6 ± 8.4	21.8 ± 8.6	22.1 ± 9.0

*Data are expressed as the mean ± SD. Group I, control; group II, 10-second postconditioning protocol; group III, 30-second postconditioning protocol. t_0, t_5, t_{10}, t_{15}, and t_{20} indicate 0 (baseline), 5, 10, 15, and 20 minutes of reperfusion, respectively. There were no significant differences between the groups.

Table 3. Systolic Pressure*

Group	Time Point				
	t_0	t_5	t_{10}	t_{15}	t_{20}
I, mm Hg	142.9 ± 37.8	131.6 ± 43.6	133.8 ± 48.8	136.1 ± 50.4	132.6 ± 49.3
II, mm Hg	147.0 ± 37.8	127.6 ± 32.5	134.4 ± 37.1	139.1 ± 45.2	140.8 ± 43.1
III, mm Hg	123.3 ± 18.6	111.3 ± 24.4	117.5 ± 29.5	120.0 ± 36.4	112.6 ± 33.2

*Data are expressed as the mean ± SD. Group I, control; group II, 10-second postconditioning protocol; group III, 30-second postconditioning protocol. t_0, t_5, t_{10}, t_{15}, and t_{20} indicate 0 (baseline), 5, 10, 15, and 20 minutes of reperfusion, respectively. There were no significant differences between the groups.

Table 4. Left Ventricle Diastolic Function (maximum speed of decrease in left ventricular pressure): $-(dP/dt)_{max}$*

Group	Time Point				
	t_0	t_5	t_{10}	t_{15}	t_{20}
I, mm Hg/s	1745.3 ± 354.0	1605.1 ± 437.2	1611.3 ± 518.3	1631.4 ± 574.5	1490.6 ± 512.0
II, mm Hg/s	1805.5 ± 401.4	1586.8 ± 309.8	1683.9 ± 312.0	1775.8 ± 434.5	1770.4 ± 406.6
III, mm Hg/s	1620.4 ± 251.0	1404.9 ± 373.0	1516.1 ± 359.0	1488.9 ± 363.3	1399.1 ± 327.4

*Data are expressed as the mean ± SD. Group I, control; group II, 10-second postconditioning protocol; group III, 30-second postconditioning protocol. t_0, t_5, t_{10}, t_{15}, and t_{20} indicate 0 (baseline), 5, 10, 15, and 20 minutes of reperfusion, respectively. There were no significant differences between the groups.

Table 5. Left Ventricle Contractile Function (maximum speed of increase in left ventricular pressure): $+(dP/dt)_{max}$*

Group	Time Point				
	t_0	t_5	t_{10}	t_{15}	t_{20}
I, mm Hg/s	1947.9 ± 311.3	1659.8 ± 421.3	1599.5 ± 447.1	1526.0 ± 424.9	1409.0 ± 415.2
II, mm Hg/s	1994.1 ± 460.7	1606.5 ± 310.8	1780.1 ± 401.9	1815.4 ± 521.5	1917.3 ± 403.1†
III, mm Hg/s	1673.6 ± 250.0	1439.1 ± 395.8	1521.9 ± 435.5	1492.1 ± 437.6	1344.8 ± 355.8

*Data are expressed as the mean ± SD. Group I, control; group II, 10-second postconditioning protocol; group III, 30-second postconditioning protocol. t_0, t_5, t_{10}, t_{15}, and t_{20} indicate 0 (baseline), 5, 10, 15, and 20 minutes of reperfusion, respectively.

†P = .02, group II versus group III; P = .04, group II versus group I.

Left ventricle contractile function, $+(dP/dt)_{max}$, was preserved after 20 minutes of reperfusion (t_{20}) compared with the baseline measurement (t_0) in the 10-second postconditioning protocol (GII). Differences between GII and GI and between GII and GIII were statistically significant at t_{20} (GI, 1409.0 ± 415.1 mm Hg/s; GII, 1917.3 ± 403.0 mm Hg/s; GIII, 1344.8 ± 355.8 mm Hg/s) (GII versus GI, P = .04; GII versus GIII, P = .02) (Table 5 and Figure 2).

DISCUSSION

The prerequisite for rescuing viable myocardium and reducing mortality and morbidity due to an acute myocardial infarction is the early restitution of coronary flow. Reperfusion is not a completely benign process, however, and can induce myocyte death, a phenomenon known as *lethal reperfusion injury* [Braunwald 1985], which is now known to involve the processes of necrosis and apoptosis.

Zhao et al [2003] demonstrated that a phenomenon termed *ischemic postconditioning*, a cardioprotective maneuver that targets the reperfusion phase, reduced infarct size by 44% in an in vivo dog model. This phenomenon has subsequently been confirmed in vivo (in rabbits [Yang 2004] and rats [Kin 2004]) and in vitro (in rabbits [Yang 2005], rats [Tsang 2004], and mice [Heusch 2006]).

The temporal characteristics of postconditioning are critical to cardioprotection. Kin et al [2004] demonstrated a reduction in infarct size with an in vivo rat model that consisted of 30 minutes of left ventricular coronary occlusion and 3 hours of reperfusion, followed by postconditioning with either 3 or 6 cycles of 10 seconds of reperfusion/reocclusion. Nevertheless, if postconditioning was delayed 1 minute, the infarct-sparing effect was lost. The early moments of reperfusion when postconditioning is applied are critical for exerting its potent cardioprotection.

In this investigation, a postconditioning protocol of 3 cycles of 30 seconds of reperfusion and 30 seconds of occlusion at the onset of reperfusion demonstrated nonsignificant trends for changes in cardiac frequency, coronary flow, systolic pressure, $+(dP/dt)_{max}$, and $-(dP/dt)_{max}$, compared with the control group ($P > .05$). In isolated rat hearts, a 30-second algorithm (30 seconds of reperfusion and 30 seconds of reocclusion) failed to reduce infarct size [Pfeffer 1991] and preserve contractile function, as shown in our data. The postconditioning protocol of 3 cycles of 10 seconds of reperfusion and 10 seconds of occlusion at the onset of reperfusion preserved the $+(dP/dt)_{max}$ (left ventricle contractile function) at the end of reperfusion (GII versus GI, $P = .04$; GII versus GIII, $P = .02$). Therefore, 2 aspects of the temporal characteristics of the postconditioning algorithm appear to be important: (1) The ischemia duration in the postconditioning protocol may be species dependent, and (2) it is not the quantity of cycles that is important but their duration.

The existing data suggest that preconditioning and postconditioning provide the same physiological and cellular aspects of protection [Vinten-Johansen 2005] and activate the same key pathways [Tsang 2005]. The most important triggers, mediators, and signaling mechanisms in postconditioning are adenosine, nitric oxide, ATP-sensitive potassium channels, reperfusion injury signaling kinases, and mitochondrial permeability transition pores [Vinten-Johansen 2005].

Ischemic postconditioning not only is a powerful endogenous mechanism of myocardial protection (infarct size reduction [Zhao 2003], attenuation of endothelial dysfunction [Zhao 2003], reduction in neutrophil adherence to coronary vascular endothelium [Vinten-Johansen 2005], reduction in superoxide anion generation [Halkos 2004], reduction of reperfusion arrhythmias [Galagudza 2004; Halkos 2004], reduction in myocardial edema [Tsang 2005], and reduction in apoptosis [Vinten-Johansen 2005]) but also is a new paradigm against myocardial ischemia-reperfusion injury.

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