Insulin-Like Growth Factor–I Decreases Mean Blood Pressure and Selectively Increases Regional Blood Flow in Normal Rats (44049)

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Abstract. **The insulin-like growth factor-I (IGF-I) and its receptors are widely distributed in peripheral vascular tissue, yet their role in the regulation of blood pressure and blood flow remains unknown. This study investigated the effect of IGF-I on blood pressure and selected regional blood flow in normal Wistar rats anesthetized with chloralose/urethane. The femoral artery was cannulated and used to monitor arterial blood pressure. Electromagnetic flow probes were placed around the left common iliac artery, left renal artery, and the superior mesenteric artery, and used to measure blood flow. IGF-I (2.6 μg, 5.1 or 10.3 nmol/animal iv) was injected as a bolus into the femoral vein. Following the injection of IGF-I (10.3 nmol), we observed a significant decrease of plasma glucose (57%) and a significant decrease of mean arterial pressure (MAP) that continued to decline throughout the 60-min experimental period. IGF-I (5.1 nmol) significantly decreased blood glucose by 44% and decreased the MAP by 14% with a nadir at 15 min and recovery after 60 min. A smaller dose of IGF-I (2.6 nmol) did not significantly decrease the blood glucose but resulted in a slight but significant decrease in MAP. The heart rate was increased by 10.3 and 5.1 but not 2.5 nmol of IGF-I. IGF-I (10.3 nmol) was associated with regional vascular responses with a preferential increase in flow of the iliac and superior mesenteric vessels, measured as vascular conductance. IGF-I (5.1 and 2.6 nmol) increased preferentially renal vascular conductance. Preinfusion with L-NAME, a nitric oxide inhibitor, inhibited the effects of IGF-I on flow. We conclude that IGF-I can selectively dilate vascular beds leading to a decrease in blood pressure and that the response to IGF-I is mediated by nitric oxide.**
[P.S.E.B.M. 1996, Vol 213]

Insulin-like growth factor–I (IGF-I) shares some of the structural features of insulin (1, 2) and, when infused intravenously, causes a decrease in blood glucose by stimulating glucose uptake by the adipose and muscle tissue (3–5). In addition, it has been demonstrated that both hormones regulate vascular metabolism (6, 7). Recently, we and other investigators demonstrated that the intravenous infusion of insulin is followed by a decreased mean arterial pressure (MAP) (8–13), a response that appears to be mediated by a direct action on the vascular endothelial cells (14–16), through specific insulin receptors (17–19). IGF receptors are also present on vascular endothelial cells but with a different pattern of distribution (20, 21). Since both the insulin and the IGF-I receptors are heterotetrameric structures with considerable structural and functional homology (22, 23), we investigated whether IGF-I has any effect on cardiovascular tone and peripheral blood flow. We also investigated whether nitric oxide has a mechanistic role in these responses.

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Received February 12, 1996. [P.S.E.B.M. 1996, Vol 213]
Accepted May 24, 1996.

This work was supported by National Institutes of Health Grants GM-08167 and MH-47181.

0037-9727/96/2132-0187$10.50/0

Materials and Methods

Male Wistar rats (Harlan, Indianapolis, IN) weighing 250–300 g were housed two to a cage in a temperature-controlled environment (23°C) with a 12:12-hr light:dark cycle and were given rodent chow and water *ad libitum*.

Following an 18 to 24-hr fast, the animals were anesthetized with urethane (1 g/kg) and α-chloralose (70 mg/kg) and placed on a heating pad. A rectal thermometer was inserted, and body temperature was maintained at 37° ± 1.0°C. A tracheotomy was performed on all animals to prevent the accumulation of secretions in the upper respiratory tract. Catheters filled with heparinized saline (2000 U/ml) were inserted into a femoral artery for monitoring the cardiovascular parameters and into a femoral vein for infusions and blood sample collections. A midline abdominal incision was made, and pulsed-Doppler flow velocity transducers were placed on the left common iliac artery, left renal artery, and superior mesentery artery. The incision was closed, and the transducer wires exteriorized and connected to a pulsed-Doppler flow meter to monitor changes in regional blood flow.

Before the start of the experiment a 0.3-ml blood sample was collected and mixed with 0.3 ml of phosphate-buffered saline containing 5% bovine serum albumin and heparin (2000 U/ml). An equal volume of heparinized saline (100 U/ml) at room temperature was injected as replacement. The blood samples were immediately transferred to plastic conical tubes and stored in ice. The samples were centrifuged and the plasma stored at −30°C for subsequent determinations of glucose.

When indicated, saline or recombinant IGF-I (2.6, 5.1, and 10.3 nmol/animal; Genetech Inc., San Francisco, CA) was slowly injected into the femoral vein. The injection started 5 min after the establishment of stable control recordings (time 0) and lasted 1.5 min. The total volume of each injection was 0.1 ml/100 g body wt. MAP, heart rate, and regional blood flows were monitored continuously for 60 min. In other studies, nitro-arginine methyl ester (L-NAME; 20 mg/kg iv) was infused 10 min prior to the infusion of 5.1 nmol IGF-I/animal and the measurements conducted as described above. Blood samples were collected before and 30 and 60 min following the end of the infusion. After the experiment, all animals were sacrificed by pneumothorax.

A Micro 5000 signal processing system (Modular Instruments, Melvern, PA) and the BioWindows software program (Modular Instruments) were used for the continuous measurement and recording of cardiovascular and flow responses. Vascular conductance was calculated as regional blood flow/MAP. The 0.5-, 15-, 30-, 45-, and 60-min time points, selected for statistical analysis, represent 5-sec computer averages of 12 points measured for 1 min prior to the indicated time. Plasma glucose was measured using a glucose diagnostics kit (Sigma Chemical Co, St. Louis, MO).

Data are reported as the mean + SEM. Student's *t* test was used to compare pairs of means. When comparing values in which each animal served as its own control, the paired test was employed, whereas the unpaired test was used for comparison of different groups. To determine whether several mean values changed as a function of time in the same group, one-way analysis of variance (ANOVA) was used, while two-way ANOVA was used when different groups were compared. Dunnett's test of repeated measures was used to compare data obtained at different time periods with baseline values.

Results

MAP, heart rate, and plasma glucose levels are given in Table I. IGF-I (2.6 nmol/animal) did not change the blood glucose but resulted in a small but significant ($P < 0.05$) reduction in MAP at 45 min (Fig. 1). At a dose of 5.1 nmol/animal IGF-I decreased the blood glucose 44% and resulted in a rapid decrease ($P < 0.0001$) in MAP that reached a nadir at 15 min with a subsequent return to control levels after 60 min (Fig. 1A). The maximum effect we discovered was at 10.3 nmol IGF-I/animal, which decreased ($P < 0.01$) plasma glucose by a maximum of 57% (Table I). This

Table I. Plasma Glucose, before and 30 min after IGF-I, and Basal Mean Arterial Pressure (MAP) and Heart Rate (HR) in Normal Rats

IGF-I (nmol/animal)	Plasma glucose		Basal MAP	Basal HR
	Before	30 min after		
2.6	101.1 ± 5.8 (4)[a]	107.0 ± 17 (4)	74.5 ± 3.5 (4)	376.9 ± 30.7 (4)
5.1	98.2 ± 7.3 (11)	54.9 ± 9.3[b] (11)	71.6 ± 2.2 (11)	433.6 ± 19.5 (11)
10.3	99.1 ± 7.2 (7)	42.8 ± 5.1[b] (7)	64.7 ± 3.6 (7)	424.9 ± 16.1 (7)

[a] Number in parentheses = *n*.
[b] $P < 0.01$ paired *t* analysis.

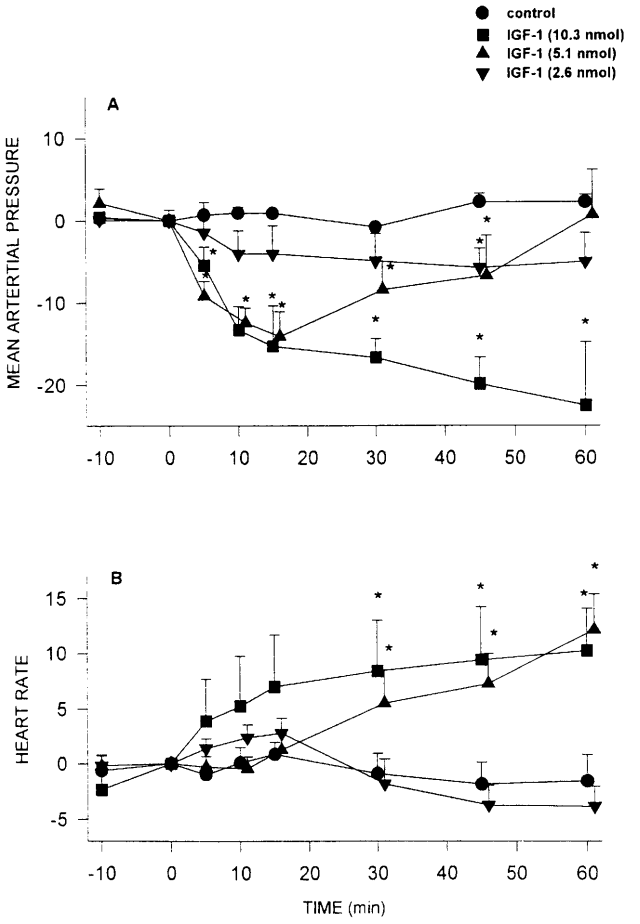

Figure 1. Effects of IGF-I (2.6 [n = 4], 5.1 [n = 11], and 10.3 [n = 7] nmol) infusion on mean arterial pressure (MAP) The time points selected for evaluation were before (−10) and 0, 5, 10, 15, 30, 45, and 60 min following IGF-I infusion. Data are expressed as mean percent change ± SEM from time 0. The significance of the differences was as follows: 2.6 nmol IGF-I versus control, $P < 0.038$; 5.1 and 10.3 nmol IGF-I versus control, $P < 0.0001$ (ANOVA). *$P < 0.05$ for selected time points. (B) The effects of IGF-I (2.6 [n = 4], 5.1 [n = 11], and 10.3 [n = 7] nmol) on heart rate. Data are expressed as mean percent change ± SEM. Significant differences: 5.1 nmol IGF-I versus control, $P < 0.005$; 10.2 nmol IGF-I versus control, $P < 0.0001$ (ANOVA). *$P < 0.05$ for selected time points.

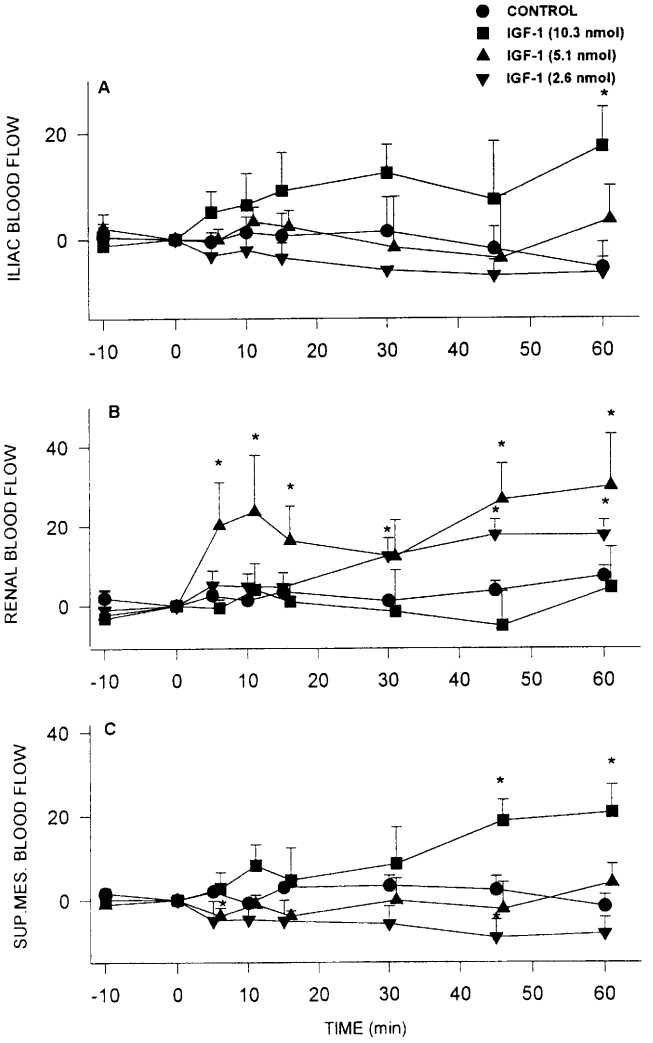

Figure 2. Effects of IGF-I (10.3 [n = 7], 5.1 [n = 11], and 2.6 [n = 4] nmol) infusion on iliac (A), renal (B), and superior mesenteric (C) blood flow (BF). The time points that were selected for evaluation were before (−10) and 0, 5, 10, 15, 30, 45, and 60 min following IGF-I infusion. Data are expressed as mean percent change ± SEM from time 0. (A) 5.1 nmol IGF-I versus control and 10.3 nmol IGF-I versus control, $P < 0.03$; (B) 2.6 nmol IGF-I versus control, $P < 0.001$; 5.1 nmol IGF-I versus control, $P < 0.003$; (C) 2.6 nmol IGF-I versus control, $P < 0.001$; 5.1 nmol IGF-I versus control and 10.3 nmol IGF-I versus control, $P < 0.01$ (ANOVA). *$P < 0.05$ for selected time points.

largest dose of IGF-I resulted in a rapid decrease ($P < 0.0001$) in MAP that continued to decrease throughout the 60-min experimental period.

The heart rate response to these IGF-I studies can be seen in Figure 1B. While a 2.6-nmol dose of IGF did not change the heart rate, doses of 5.1 and 10.3 nmol/animal significantly increased ($P < 0.005$) the heart rate after 30 min.

IGF-I at the lowest concentration (2.6 nmol) increased ($P < 0.001$) flow in the renal but actually decreased ($P < 0.001$) flow in the superior mesenteric vascular bed (Fig. 2). IGF-I at the intermediate concentration (5.1 nmol) caused a selective increase ($P < 0.003$) in the renal blood flow with no changes in the iliac and superior mesenteric blood flow. IGF-I at the highest concentration (10.3 nmol) caused an increase ($P < 0.03$) in iliac blood flow (Fig. 2) but did not alter renal blood flow. Blood flow in the superior mesenteric artery was increased ($P < 0.05$) after 45 and 60 min. Conductance (flow/MAP) calculated from the same data shows that following 2.6 nmol IGF-I conductance was again selectively increased 20% after 30 min ($P < 0.001$) in the renal but not in the iliac or superior mesenteric vessels (Fig. 3). The conductance following the infusion of IGF-I at 5.1 nmol was increased 15%–20% in the iliac ($P < 0.02$) and superior mesenteric but resulted in a 40% increase ($P < 0.0001$) in the renal vascular bed. IGF-I at 10.3 nmol significantly increased ($P < 0.001$) iliac conductance approximately 30%. The renal conductance was not altered by IGF-I

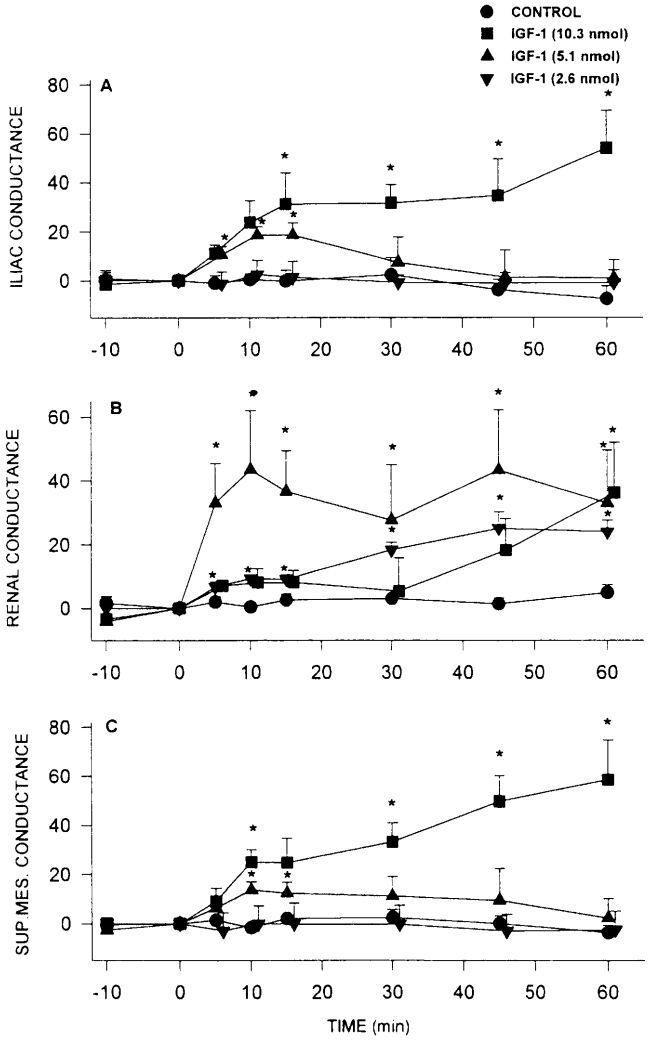

Figure 3. Effects of IGF-I (10.3 [n = 7], 5.1 [n = 11], and 2.6 [n = 4] nmol infusion on iliac (A), renal (B), and superior mesenteric (C) conductance (G). The time points that were selected for evaluation were (−10) and 0, 5, 10, 15, 30, 45, and 60 min following IGF-I infusion. Data are expressed as mean percent change ± SEM from time 0. (A) 5.1 nmol IGF-I versus control, $P < 0.02$; 10.3 nmol IGF-I versus control, $P < 0.0001$; (B) 2.6 nmol IGF-I versus control, $P < 0.001$; IGF-I 5.6 nmol versus control, $P < 0.0001$; (C) 5.1 nmol IGF-I versus control, $P < 0.05$; IGF-I 10.3 nmol versus control, $P < 0.0001$ (ANOVA). *$P < 0.05$ for selected time points.

at 10.3 nmol but the conductance in the superior mesenteric was increased 40% ($P < 0.001$). In an attempt to evaluate the mechanism of the IGF-I–induced response, we observed that preinfusion with L-NAME increased the basal MAP (Table II) but blocked the decrease in blood pressure induced by 5.1 nmol IGF-I/animal as well as inhibiting IGF-I's effect on vascular conductances in all the vascular beds (Fig. 4).

Discussion

This investigation demonstrated that recombinant IGF-I decreases the MAP in normal rats in a dose-related fashion and increases blood flow in skeletal muscle, in the splanchnic bed, and especially in the renal vasculature. An increased blood flow following IGF-I infusion has been demonstrated in humans (24–26) and also in the mammary glands of lactating goats (27), but not in the placenta of sheep (28). The evidence from our studies indicates that the renal vasculature is selectively sensitive to the action of IGF-I because at the lowest molar concentration IGF-I effectively increased renal flow and conductance while having a minimal effect on the iliac flow and actually decreasing flow in the superior mesenteric artery. These facts are consistent with previous studies showing that IGF-I can acutely increase renal plasma flow and glomerular filtration rate (29, 30). While these results suggest that the IGF-I has a direct hemodynamic effect on the kidney, the decrease in renal blood flow we observed after the injection of highest doses (10.3 nmol) of IGF could be attributed to a reflex sympathetic-induced vasoconstriction in response to the much larger decrease in blood pressure and/or the resulting hypoglycemia (31, 32). This sympathetic discharge is also consistent with this as well as also explaining the increased heart rate noted in these animals. Also, these findings of increased renal blood flow in response to IGF-I may explain, at least in part, the IGF-I–mediated renal hypertrophy (29, 33, 34).

IGF-I in intermediate and high doses increased mesenteric blood flow and increased iliac blood flow in

Table II. The Mean Arterial Pressure (MAP) and Heart Rate (HR) Response to IGF-I or IGF-I Plus L-NAME at Basal and 30 min Postinfusion

	MAP		HR	
	Basal	Plus 30 min	Basal	Plus 30 min
Controls (saline)	71.6 ± 1.9 (5)[a]	70.8 ± 2.2 (5)	446.2 ± 10.5 (5)	458.8 ± 11.7 (5)
IGF-I (5.1 nmol/animal)	71.6 ± 2.2 (11)	65.8 ± 3.7[b] (11)	433.6 ± 19.5 (11)	454.8 ± 18.3 (11)
IGF-I (5.1 nmol/animal) + L-NAME (20 mg/kg)	118.3 ± 3.9 (6)	117.9 ± 3.8 (6)	451.8 ± 18.4 (6)	459.9 ± 17.3 (6)

[a] Number in parentheses = n.
[b] $P < 0.05$.

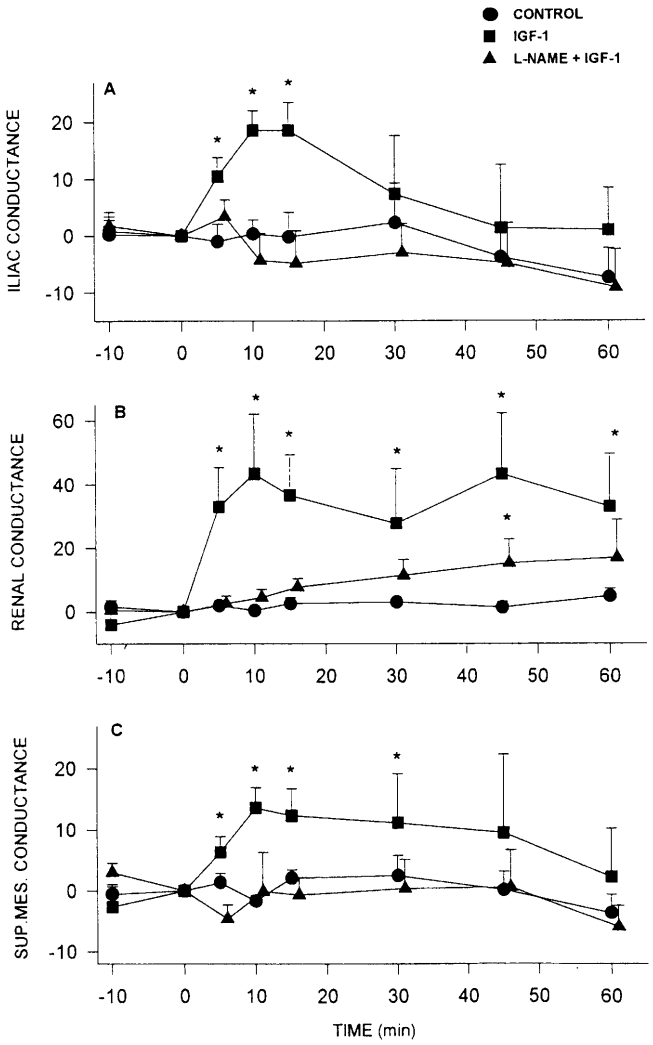

Figure 4. Effects of IGF-I (5.1 nmol) in the presence or absence of L-NAME (20 mg/kg) on iliac (A), renal (B), and superior mesenteric (C) conductance. The time points that were selected for evaluation were before (−10) and 0, 5, 10, 15, 30, 45, and 60 min following IGF-I infusion. L-NAME was infused 30 min prior to IGF-I infusion. Data are expressed as mean percent change ± SEM from time 0. (A) IGF-I versus IGF-I + L-NAME $P < 0.02$; (B) IGF-I versus IGF-I + L-NAME $P < 0.001$; (C) IGF-I versus IGF-I + L-NAME $P < 0.01$ (ANOVA). *$P < 0.05$ for selected time points versus L-NAME.

normal rats, in agreement with the observations that IGF-I, like insulin, increases blood flow in peripheral vasculature of rats (8, 11–13, 33, 35) and humans (19, 25, 32, 36–38). IGF-I receptors have been identified in blood vessels of skeletal muscle as well as the viscera. While IGF-I could act directly on the receptors of smooth muscle, it is most likely that the vasodilatation is the result of an endothelial-mediated increased nitric oxide production (16, 34, 39, 40), and our findings confirm this observation. Although IGF-I may mediate all of its actions *via* its own receptor, it is possible that some of its effect could be due to a spillover to the insulin receptor (41, 42). This may help explain our observation that the highest doses of IGF-I dilated the iliac vessels, known to be acutely responsive to insulin (36, 37), while the renal flow was increased by the lowest dose of IGF-I (29, 30).

We conclude that the observed cardiovascular responses to IGF-I are distinct but have some responses similar to insulin. We also conclude that IGF-I may play a role in selectively modulating vascular resistance especially in the kidney and that the action of IGF-I is mediated by nitric oxide.

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