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Cardiovascular Action of Insulin-like Growth Factor-1 Is Not Mediated by Calcitonin Gene-Related Peptide Neurons

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Calcitonin gene-related peptide (CGRP) is a potent vasodilator located in the peripheral nerves including the perivascular nerves. Previous studies in our laboratory determined that the vasodilatory action of insulin is mediated in part by CGRP-containing neurons. Since insulin-like growth factor-1 (IGF-1) and insulin share molecular and receptor structural similarity as well as functional similarity, we investigated the role of the CGRP-containing neurons in IGF-1-mediated vasodilation. Wistar rats were made CGRP deficient by treatment with capsaicin (50 mg/kg) 1–3 d after birth. Vehicle-treated controls and CGRP-deficient rats were maintained for 12 to 13 wk. At this time rats were fasted overnight, anesthetized with urethane and chloralose, and prepared for cardiovascular recordings. The basal mean arterial pressure (MAP) was higher in CGRP-deficient rats when compared with controls. The infusion of IGF-1 resulted in an equivalent decrease in MAP in both the CGRP-deficient and control rats. IGF-1 infusion did not change the heart rate in control rats but decreased it in CGRP-deficient rats. IGF-1 also increased flow as determined by conductance in the iliac, renal, and superior mesenteric vascular beds in both vehicle controls and CGRP-deficient rats. We concluded that unlike insulin the IGF-1-mediated vasodilatory response is not mediated by the CGRP-dependent perivascular neurons.

Key Words: Calcitonin gene-related peptide; capsaicin; diabetic; insulin-like growth factor-1; mean arterial pressure.

Introduction

Insulin-like growth factor-1(IGF-1) is a peptide hormone, with structural as well as physiologic receptor characteristics similar to insulin *(1,2)*. In addition to its metabolic action, IGF-1 has been demonstrated to induce dilation of blood vessels leading to an increase in vascular flow and a decrease in mean arterial pressure (MAP) *(3–6)*. The increased blood flow is selective for different vascular beds *(3,4)*. The details of the mechanism of IGF-1-induced vasodilation are not completely understood; however, its actions appear to be mediated by a combination of nitric oxide (NO)-dependent mechanisms as well as decreasing sympathetic nerve activity *(5, 7–9)*. Our laboratory has been interested in determining the mechanism of insulin and IGF-1-induced vessel dilation. Previous studies have suggested that the autonomic nerves are important in the vasodilatory response to insulin *(10)*. Portions of the insulin-induced vasodilation are blockable with β-adrenergic blockade as well as ganglionic blockade *(10,11)*. We previously demonstrated that a significant portion of insulin's vasodilatory action is dependent on calcitonin gene-related peptide (CGRP)–containing neurons *(12)*. Blood vessels have an extensive perivascular nerve net and many of these nerves contain the peptide CGRP *(13–15)*. When CGRP is administered or released from nerves, it is a potent vasodilator *(15–19)*. When insulin was administered to CGRP-deficient rats, the insulin-induced vasodilatory response was decreased and MAP was decreased. This decreased responsiveness was especially true in the vascular bed supplying the skeletal muscles *(12)*.

Since insulin and IGF-1 share a number of structural and functional homologies, we investigated the importance of the perivascular CGRP-containing neurons on IGF-1-mediated vasodilation.

Results

The growth and size of the CGRP rats at 12 to 13 wk was equal to controls but fasting glucose was significantly less (Table 1). The CGRP-deficient rats when compared with controls had a significantly higher basal MAP but a lower basal heart rate (HR) (Table 2). There was no difference in the basal vascular flows in the iliac, renal, or superior mesenteric vascular beds of the CGRP-deficient rats compared with controls (Table 2). The iv infusion of IGF-1 in control rats resulted in a slight initial increase in MAP followed by an 11% decrease that was maintained for 80 min (Fig. 1). The MAP response to IGF-1 in the CGRP-deficient rats was equivalent to that observed in the controls. IGF-1 infusion in control rats resulted in a significant decrease in HR in CGRP-deficient animals when compared with controls (Fig.

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Table 1
Basal Body Weight, Plasma Glucose, and Plasma Insulin Levels in Control and CGRP-Deficient Rats[a]

	Body wt (g)	Glucose (mg/dL)	Insulin (μU/mL)
Control	216 ± 6 (18)	95.1 ± 3 (8)	12.7 ± 3 (6)
CGRP Deficient	207 ± 3 (12)	79.1 ± 3[b] (6)	9.8 ± 1 (6)

[a]Numbers in parentheses are the number of rats.
[b]$p < 0.001$ vs controls.

Table 2
Basal MAP, HR, and Blood Flow in Control and CGRP-Deficient Rats[a]

			Blood flow[b]		
	MAP (mmHg)	HR (beats/min)	Iliac (kHz Ds)	Renal (kHz Ds)	Superior mesenteric (kHz Ds)
Control	87.2 ± 2.14 (10)	29 ± 16.7 (10)	0.07 ± 0.003 (10)	0.03 ± 0.005 (10)	0.02 ± 0.002 (10)
CGRP deficient	95.2 ± 2.3[c] (8)	402 ± 12.8 (8)	0.06 ± 0.004 (8)	0.04 ± 0.004 (8)	0.035 ± 0.009 (7)

[a]Data are the mean ± SE. Numbers in parentheses are the number of rats.
[b]Ds, Doppler shift.
[c]$p < 0.005$ vs control.

2). HR returned to baseline after 1 h. The conductances (calculated from blood flow/MAP) in the iliac, renal, and superior mesenteric vascular beds were also determined. IGF-1 infusion in controls and CGRP-deficient rats resulted in an increase in iliac conductance (Fig. 3A). The conductances returned toward baseline in CGRP-deficient rats after 50 min. The renal vascular response to IGF-1 in control and CGRP-deficient rats also increased in both groups (Fig. 3B). The increased vascular conductance in the control animals persisted whereas the CGRP-deficient rats returned toward baseline. IGF-1 increased the conductance in the superior mesenteric vessels of control rats and CGRP-deficient rats (Fig. 3C). Again, in the control rats the increased conductance persisted, whereas the CGRP-deficient rats returned toward baseline.

Discussion

We have demonstrated that IGF-1 infusion causes a decrease in MAP in both control and CGRP-deficient rats and that the responses to IGF-1 in our two animal models are similar. These results also confirm previous studies in our laboratory demonstrating that systemic infusion of IGF-1 decreased MAP *(3,4)*. Basal MAP was significantly higher in CGRP-deficient rats compared with controls. This would be expected because CGRP is a vasodilator and a deficiency in CGRP would lead to enhanced peripheral constriction and vascular resistance *(18,19)*. This tendency for increased MAP in CGRP-deficient rats is also supported by observations in CGRP knockout animals *(20)*. The degree of the CGRP-deficient rats' blood pressure response to IGF-1 was very similar to what was observed in controls, suggesting that CGRP deficiency did not alter the MAP response to IGF-1. The IGF-1-mediated increase in conductance in the various vascular beds was also similar in the control and CGRP-deficient rats. The conductance increased in the vascular beds supplying skeletal muscle, the kidney, and the mesentery in control rats, as also was observed in previous studies *(3,4)*. CGRP deficiency did not alter the maximum IGF-1-induced increase in conduction. However, the IGF-1-induced increased flow return to baseline was much faster in CGRP-deficient animals. This could be owing to the diminished vascular balance from vasodilatory toward constriction *(19,21,22)*. The loss of the perivascular vasodilator CGRP-containing fibers would shift the balance toward an increased sensitivity to the baroreceptor or sympathetic-mediated constriction *(21,23)*.

The HR responses were different between the control and CGRP-deficient rats following the infusion of IGF-1. IGF-1 decreased HR in CGRP-deficient rats while having

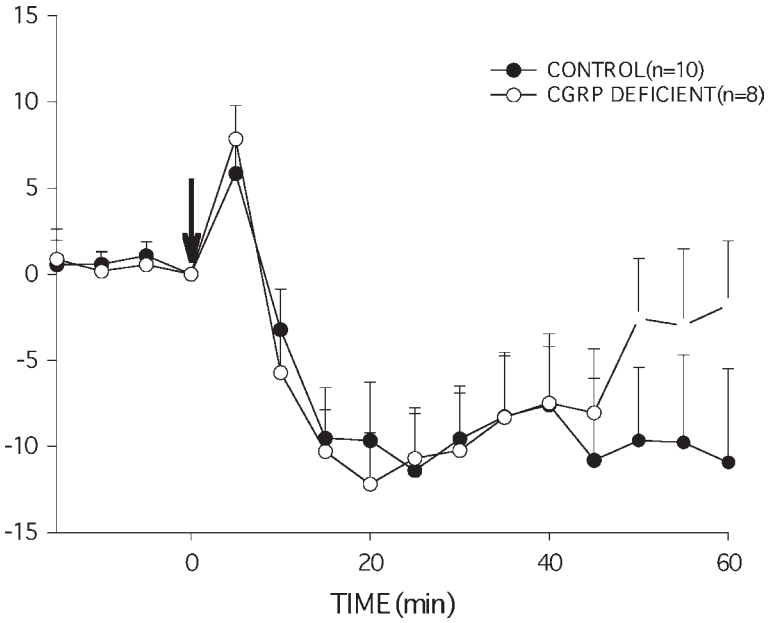

Fig. 1. Effect of iv infusion of IGF-1 (40 µg/animal) on MAP in control and CGRP-deficient rats. Arrow = time of infection.

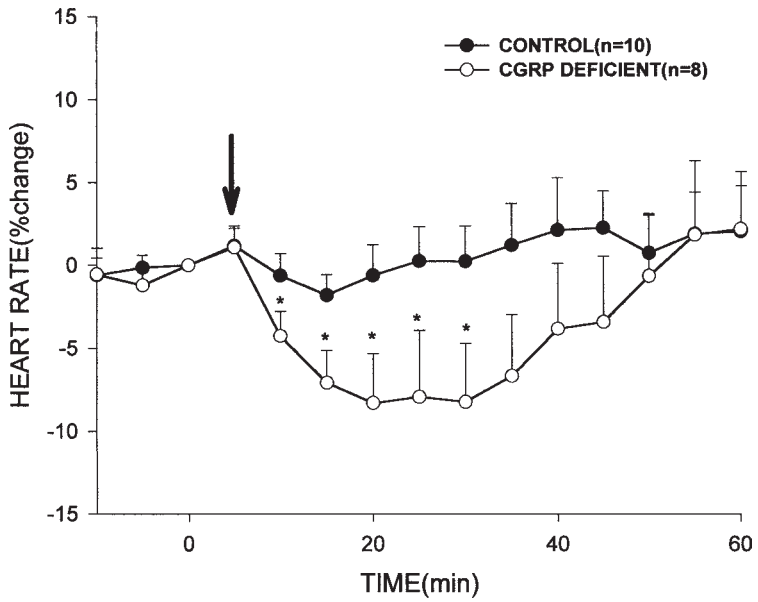

Fig. 2. Effect of iv infusion of IGF-1 (40 µg/animal) on HR in control and CGRP-deficient rats. $p < 0.01$ vs controls; *$p < 0.05$ vs control at selected time points. Arrow = time of infection.

only a small influence in control animals. CGRP has been shown to increase HR by enhancing sympathetic nervous activity *(21,24)*. The lack of CGRP thus would cause a decrease in chronotropic and inotropic effects on the heart *(24)*. Therefore, when CGRP is depleted, HR decreases. We have previously demonstrated that IGF-1 acts to decrease sympathetic nerve activity, leading to a decrease in HR *(3,4)*. Since CGRP acts to enhance HR, CGRP-deficient animals will be more susceptible to the IGF-1-induced decrease in sympathetic tone to the heart. Following this same scenario, the tendency for a faster return of MAP to baseline in CGRP-deficient animals would likely be owing to enhanced constrictor tone.

It has also been demonstrated that IGF-1 stimulates NO's production, suggesting that NO is at least partially responsible for IGF-1 vasodilatory actions *(5,25)*. Furthermore, it has been determined that CGRP's vasodilatory action is also partially mediated by NO *(25)*. Thus, a decrease in CGRP-stimulated NO production in CGRP-deficient rats may further explain a faster return to baseline than the controls.

In summary, the infusion of IGF-1 in control and CGRP-deficient animals resulted in an equivalent decrease in MAP

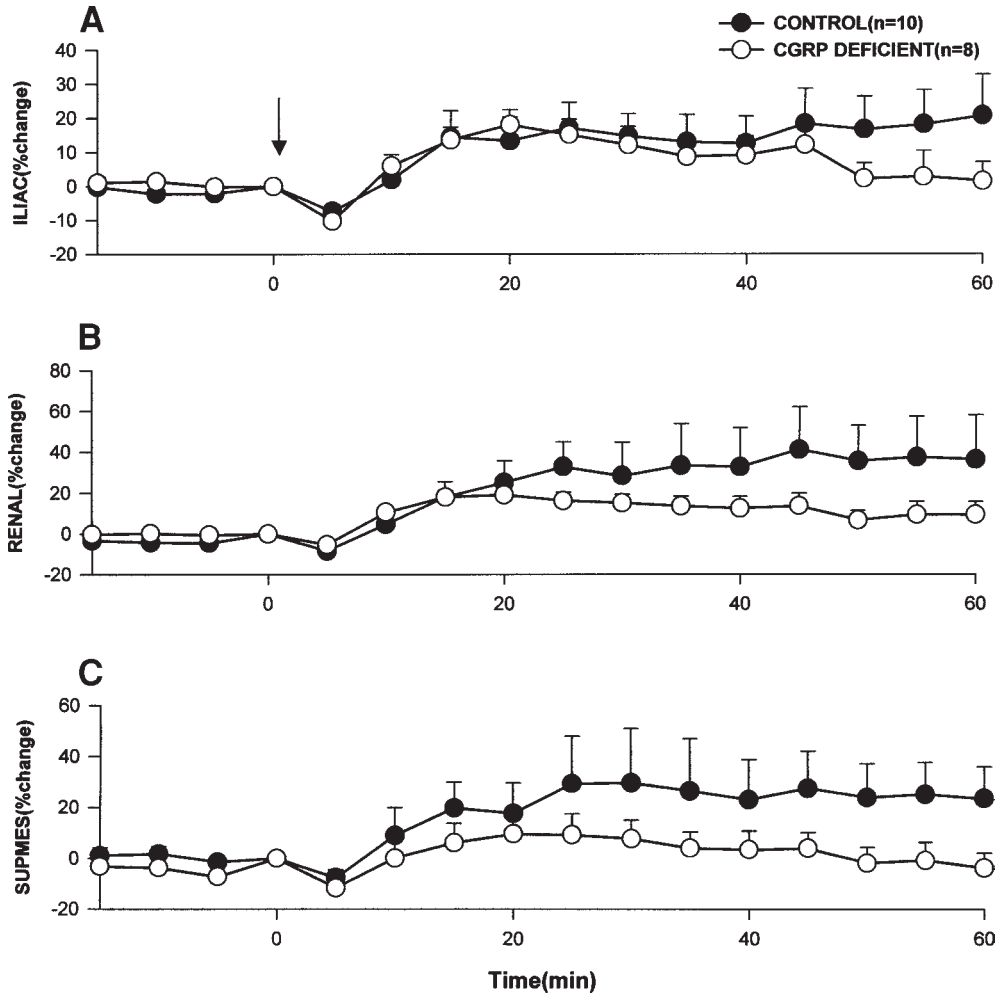

Fig. 3. Effect of iv infusion of IGF-1 (40 μg/animal) on selected vascular conductances (flow/pressure) in (**A**) iliac, (**B**) renal, and (**C**) superior mesenteric vascular beds of control and CGRP-deficient rats. $p < 0.01$ vs control in renal and superior mesenteric vessels.

or an increase in vascular conductance. The faster recovery in the CGRP-deficient animals is likely owing to enhanced sympathetic tone. Thus, we conclude that IGF-1's vasodilatory action is not mediated by the CGRP neurons.

Materials and Methods

All animal studies were conducted following institutional guidelines, and the protocols were approved by the institutional animal investigation committee. Vehicle-treated controls and CGRP-deficient male Wistar rats were used. Rats were maintained in a room with 12-h light/12-h dark conditions. Rats were made CGRP deficient by treating the animals with a single injection of capsaicin (50 mg/kg) dissolved in ethanol saline (50/50) by 1–3 d after birth. This concentration has been demonstrated to produce a significant (95%) reduction in vascular CGRP *(26,27)*. Control animals received only the vehicle solution. Pups were weaned 3 to 4 wk postpartum, placed three to four per cage, and allowed food and water ad libitum. Rats were weighed weekly and used for experiments between wk 10 and 12.

To determine the cardiovascular dynamic response to IGF-1, rats were fasted for 24 h and anesthetized with urethane (500 mg/kg) and α-chloralose (80 mg/kg). They were placed on a heating pad and maintained at a body temperature of 37ºC. A tracheotomy was performed and catheters were placed in both femoral veins and a femoral artery. The artery was used for continuous blood pressure monitoring, while the femoral vein was used to provide the anesthetic or IGF-1 infusion. The abdomen was opened and Doppler flow probes were placed around the iliac, renal, and superior mesenteric arteries to monitor blood flow. A blood sample was taken for glucose and insulin determination. Following completion of the instrumentation and establishment of a recording baseline, IGF-1 (40 μg/animal) was infused as a bolus over 1 min into the femoral vein. MAP, HR, and conductances (flow/MAP) were monitored for 2 h. Blood glucose was determined using a glucose analyzer (YSI, Yellow Springs, OH), and insulin was determined using an insulin radioimmunoassay kit (ICN, Costa Masa, CA). The cardiovascular data were analyzed using two-way analysis of variance, and post-hoc tests were used where appropriate.

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