Effect of chronic IL-6 infusion on acute pressor responses to vasoconstrictors in mice

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Boesen EI, Pollock DM. Effect of chronic IL-6 infusion on acute pressor responses to vasoconstrictors in mice. Am J Physiol Heart Circ Physiol 293: H1745–H1749, 2007. First published June 8, 2007; doi:10.1152/ajpheart.00329.2007.—Interleukin (IL)-6 has been implicated as a contributing factor in the pathogenesis of hypertension, although the mechanisms involved are unclear. Studies conducted in vitro suggest that IL-6 may have a direct effect on vascular tone and may modulate constrictor responses to agonists. Whether this effect can be observed in vivo is unknown. Therefore, mice were treated with either IL-6 (16 ng/h sc) or vehicle for 14 days, and the acute blood pressure and heart rate responses to endothelin (ET)-1, angiotensin II (ANG II), and phenylephrine (PE) were assessed under isoflurane anesthesia. Blood pressure responses to ET-1 were identical in vehicle- and IL-6-infused mice, both in the presence and the absence of ganglionic blockade with chlorisondamine. The fall in heart rate during ET-1 responses was significantly attenuated in IL-6-infused mice with autonomic reflexes intact (vehicle vs. IL-6, P < 0.05 at 1 and 3 nmol/kg of ET-1), but this difference was not observed after ganglionic blockade. Both blood pressure and heart rate responses to ANG II were indistinguishable between IL-6- and vehicle-infused mice, as were responses to PE except for a significant increase in the blood pressure response and decrease in the heart rate response in IL-6-infused mice observed only at the highest dose of PE (300 μg/kg; P < 0.05). These findings show that, despite what might be predicted from studies conducted in vitro, chronic exposure to elevated plasma IL-6 concentrations in itself does not predispose the mouse to enhanced responsiveness to vasoconstrictors in vivo.

cytokines; endothelin; angiotensin II

INCREASED CIRCULATING LEVELS of the proinflammatory cytokine interleukin (IL)-6 are associated with increased risk of cardiovascular disease and all-cause mortality, particularly in older individuals (10, 26, 27, 29). Currently, a potential role for IL-6 in hypertension is emerging. Epidemiologic studies have demonstrated a positive association between plasma IL-6 and blood pressure in apparently healthy men and women (2, 5). Chronic infusion of IL-6 increases blood pressure in pregnant but not nonpregnant rats (9, 24). In addition, angiotensin II (ANG II)-induced hypertension is attenuated, and acute pressor responses to psychosocial stress reduced, in male IL-6-knockout mice (15, 16).

Despite the growing evidence implicating IL-6 in hypertension, the mechanisms involved are poorly understood. One possibility is that IL-6 may affect vascular tone and responsiveness to vasoconstrictors. Several studies have reported acute vasoconstrictor effects of IL-6 on arterial tissue (1, 11, 25). In addition, preincubation of aortic strips with IL-6 enhances contraction to phenylephrine (PE) (23), although organ culture of rat basilar artery or human temporal artery with IL-6 does not alter constrictor to endothelin (ET)B receptor activation (17, 32). Chronic IL-6 infusion in mice was reported to increase ANG II type 1 (AT1) receptor expression, enhance constriction to ANG II, increase oxidative stress, and impair endothelium-dependent vasodilation, all measured in aortic tissue in vitro (30). Impaired endothelial nitric oxide (NO)-cGMP-mediated relaxation and enhanced constriction to PE have also been reported in aortic strips isolated from IL-6-infused pregnant rats (24). While these changes noted in aortic tissue raise the possibility of similar changes occurring in resistance vessels and may well have implications for blood pressure control, effects of chronic IL-6 infusion on pressor responsiveness in vivo have not been studied. We therefore tested the hypothesis that chronic exposure to elevated levels of IL-6 would enhance pressor responses to vasoactive agents in mice.

METHODS

Male mice obtained from Jackson Laboratories (Bar Harbor, ME) were used in these experiments, and all procedures were approved in advance by the Medical College of Georgia Institutional Animal Care and Use Committee. One experiment utilized wild-type (C57BL/6) and IL-6-knockout mice (B6.129S2-Il6tm1Kop/J). In all other experiments, C57BL/6 mice received 14-day subcutaneous infusions of either mouse recombinant IL-6 (R & D Systems, Minneapolis, MN) at a rate published previously (16 ng/h; Ref. 14) or vehicle [0.1% bovine serum albumin (BSA) in phosphate-buffered saline]. The solutions were delivered by microosmotic pumps (Alzet model 1002, Alza, Palo Alto, CA) implanted subcutaneously at the back of the neck under brief anesthesia (2% isoflurane; Baxter Pharmaceutical Products, Deerfield, IL).

Responses to ET-1. For wild-type and IL-6-knockout mice, or after 14 days of vehicle or IL-6 infusion, mice were anesthetized (isoflurane) and placed on a servo-controlled heating table to maintain body temperature. Catheters were inserted into I) the carotid artery to measure arterial pressure and heart rate, with care taken not to damage the vagus nerve, and 2) the jugular vein for infusion of 1% BSA in phosphate-buffered saline at 6 μl/min. After a 30-min stabilization period, blood pressure and heart rate responses to rapid intravenous infusions of ET-1 (0.1–3 nmol/kg; American Peptide, Sunnyvale, CA) were recorded (Powerlab data acquisition system). ET-1 doses were administered at 10-min intervals in ascending order in volumes of 50 μl, which were flushed in intravenously at a rate of 100 μl/min by infusion pump. In separate groups of similarly prepared mice a blood sample of ~100 μl was taken from the carotid artery catheter immediately before commencement of the stabilization period and replaced with an equivalent volume of 1% BSA, and the effects of ET-1 (0.03–3 nmol/kg) were studied after ganglionic blockade with chlorisondamine (10 mg/kg iv; Tocris Bioscience, Ellisville, MO).

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Responses to ANG II and PE. In separate groups of mice, a blood sample of ~100 μl was taken immediately after completion of surgery and replaced with an equivalent volume of 1% BSA. After the 30-min stabilization period, blood pressure and heart rate responses to rapid intravenous infusions of ANG II (0.1–3 μg/kg; Phoenix Pharmaceuticals, Belmont, CA) and PE (10–300 μg/kg; Sigma-Aldrich, St. Louis, MO) were measured. For each agent, doses were administered in volumes of 50 μl delivered at 200 μl/min and given in random order, with either the ANG II or the PE dose-response curve constructed first, this being alternated between mice.

Plasma IL-6 concentrations. Blood samples were centrifuged in heparinized capillary tubes, and the plasma was collected and stored at −80°C until analysis. Plasma IL-6 concentrations were measured with a commercially available kit according to the manufacturer’s directions (Quantikine Mouse IL-6 Immunoassay; R & D Systems).

Data analysis. Basal arterial pressures and heart rates were calculated by averaging values recorded during the 5 min before administration of the first dose of ET-1 or from the average values obtained over 30-s intervals immediately before administration of each dose of ANG II or PE. Representative traces of responses to agonists are shown in Fig. 1. Responses to all agonists were calculated as the area under the curve from preinfusion values for either the 10-min period following each ET-1 dose or the duration of the response to ANG II or PE.

Unpaired t-tests or Mann-Whitney U-tests were used to compare basal data between vehicle- and IL-6-infused mice in each experiment. Responses to vasoconstrictors were analyzed by repeated-measures analysis of variance testing whether responses to vasoconstrictors were dose-dependent \( (P_{\text{dose}}) \) and whether IL-6 infusion or genotype affected the responses in a manner independent of the dose of vasoconstrictor \( (P_{\text{dose*group}}) \) or dependent on the dose of vasoconstrictor \( (P_{\text{dose*group}}) \). Post hoc contrasts were used to compare between groups where \( P_{\text{dose*group}} < 0.05 \). \( P \leq 0.05 \) was considered statistically significant. Data are presented as means ± SE except where indicated.

RESULTS

Infusion of IL-6 for 14 days significantly increased plasma IL-6 concentration, but basal mean arterial pressures and heart rates were similar between vehicle- and IL-6-infused mice (Table 1).

Responses to ET-1. Both vehicle- and IL-6-infused mice demonstrated dose-dependent increases in mean arterial pressure and decreases in heart rate in response to ET-1 \( (P_{\text{dose}} = 0.0001; \text{Fig. 2, A and B}) \). Although mean arterial pressure responses were similar in magnitude in vehicle- and IL-6-infused mice, IL-6-infused mice displayed significantly smaller reductions in heart rate, particularly at the higher doses of ET-1 \( (P < 0.05 \text{ for } 1 \text{ and } 3 \text{ nmol/kg}; \text{Fig. 2B}) \). This resulted in maximal reductions in heart rate of 112 ± 29 vs. 54 ± 19 beats/min at 3 nmol/kg in vehicle- and IL-6-infused mice, respectively. To determine whether the absence of endogenous IL-6 affected the responses to ET-1, dose-response curves to ET-1 were also constructed in wild-type and IL-6-knockout mice. Both genotypes demonstrated dose-dependent changes in mean arterial pressure and heart rate in response to ET-1, but there were no significant differences in the responses between the two genotypes (Fig. 3).

Table 1. Baseline characteristics in anesthetized mice

<table>
<thead>
<tr>
<th></th>
<th>Arterial Pressure, mmHg</th>
<th>Heart Rate, beats/min</th>
<th>Plasma IL-6 Concentration, pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>84±3</td>
<td>585±11</td>
<td>ND</td>
</tr>
<tr>
<td>IL-6</td>
<td>85±1</td>
<td>571±13</td>
<td>ND</td>
</tr>
<tr>
<td>Wild type</td>
<td>86±2</td>
<td>618±15</td>
<td>ND</td>
</tr>
<tr>
<td>IL-6−/−</td>
<td>86±2</td>
<td>575±23</td>
<td>ND</td>
</tr>
<tr>
<td>ET-1 (before ganglion blockade)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>80±5</td>
<td>610±34</td>
<td>8.0 (6.3–19.7)</td>
</tr>
<tr>
<td>IL-6</td>
<td>77±6</td>
<td>622±22</td>
<td>90.9 (45.0–340)*</td>
</tr>
<tr>
<td>ET-1 (after ganglion blockade)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>53±2</td>
<td>462±5</td>
<td>ND</td>
</tr>
<tr>
<td>IL-6</td>
<td>43±2</td>
<td>458±8</td>
<td>ND</td>
</tr>
<tr>
<td>ANG II and PE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>84±1</td>
<td>595±6</td>
<td>20.8 (3.7–34.6)</td>
</tr>
<tr>
<td>IL-6</td>
<td>85±1</td>
<td>616±6*</td>
<td>88.9 (55.5–157.9)*</td>
</tr>
</tbody>
</table>

Values are from \( n = 4–10 \) mice and represent means ± SE except for initial plasma interleukin (IL)-6 concentrations, which are median values with range given in parentheses. ET, endothelin; ANG II, angiotensin II; PE, phenylephrine; ND, not determined. *\( P < 0.05 \) vs. corresponding vehicle group. †\( P < 0.001 \) vs. corresponding vehicle group.
Responses to ET-1 during ganglion blockade. To determine whether baroreflexes were masking an alteration in the direct effects of ET-1 on the heart rate of IL-6-infused mice, responses to ET-1 were recorded in separate groups of vehicle- and IL-6-infused mice after ganglion blockade with chlorisondamine. Treatment with chlorisondamine significantly reduced the responses to ET-1 in IL-6-infused mice, expressed as means ± SE for area under the curve (AUC) from baseline. Responses were obtained in separate groups of mice either with autonomic reflexes intact (A and B; n = 8 and 6 for vehicle and IL-6 groups, respectively) or after ganglionic blockade with chlorisondamine (C and D; n = 4 and 5 for vehicle and IL-6 groups, respectively). Repeated-measures ANOVA was used to test whether responses to ET-1 were dose-dependent \( (P_{\text{dose}}) \) and whether IL-6 infusion affected the responses in a manner independent of the dose of ET-1 \( (P_{\text{dose/group}}) \) or dependent on the dose of ET-1 \( (P_{\text{dose/group}}). \) \* \( P < 0.05 \) for IL-6 vs. vehicle at the given dose. NS, nonsignificant.

Fig. 2. Acute responses to ET-1 in anesthetized wild-type (A) and IL-6-knockout (B) mice. Data shown are mean arterial pressure (A and C) and heart rate (B and D) responses to acute intravenous bolus administration of ET-1 in vehicle- (■) and interleukin (IL)-6 (●)-infused mice, expressed as means ± SE for area under the curve (AUC) from baseline. Responses were obtained in separate groups of mice either with autonomic reflexes intact (A and B; n = 8 and 6 for vehicle and IL-6 groups, respectively) or after ganglionic blockade with chlorisondamine (C and D; n = 4 and 5 for vehicle and IL-6 groups, respectively). Repeated-measures ANOVA was used to test whether responses to ET-1 were dose-dependent \( (P_{\text{dose}}) \) and whether IL-6 infusion affected the responses in a manner independent of the dose of ET-1 \( (P_{\text{dose/group}}) \) or dependent on the dose of ET-1 \( (P_{\text{dose/group}}). \) \* \( P < 0.05 \) for IL-6 vs. vehicle at the given dose. NS, nonsignificant.

Responses to ANG II. ANG II induced dose-dependent increases in mean arterial pressure and reductions in heart rate (Fig. 4, A and B). These responses were not significantly different between vehicle- and IL-6-infused mice.

Responses to PE. PE induced dose-dependent increases in mean arterial pressure and reductions in heart rate in both groups (Fig. 4, C and D). Responses were similar in the groups at the lower doses, but at the highest dose of PE (300 \( \mu \text{g/kg} \)) the rise in mean arterial pressure and fall in heart rate were significantly greater in IL-6-infused mice \( (P < 0.05). \)

DISCUSSION

A number of studies have investigated whether inflammatory cytokines, including IL-6, TNF-\( \alpha \), and IL-1\( \beta \), might modulate vascular responsiveness to vasoconstrictors. Most such studies to date have examined responsiveness of isolated vessels (7, 17, 23, 24, 30, 32) or of specific vascular beds in situ (1, 8) after either acute or chronic exposure to the cytokine of interest. To our knowledge, the present study represents the first to examine the effects of chronic exposure to IL-6 on whole animal cardiovascular responses to vasoconstrictors.

It was somewhat surprising that, despite previous reports of enhanced constrictor responses to ANG II and PE in rats and mice chronically infused with IL-6 (24, 30), we saw no effect of IL-6 treatment on pressor responses to these agents, except for a small enhancement of the response to PE at the highest dose used. The functional significance of this effect is not clear, since the absolute peak increase in arterial pressure in response to PE at 300 \( \mu \text{g/kg} \) was not significantly different between vehicle- and IL-6-infused mice (39 ± 1 vs. 42 ± 2 mmHg). Although there were differences between the present study and the previous studies in terms of the treatment protocol and species used, the fold increase in plasma IL-6 concentration measured at day 14 in our study was at least as large as, if not

Fig. 3. Acute responses to ET-1 in anesthetized wild-type (■) and IL-6-knockout (●) mice. Data shown are mean arterial pressure (A and C) and heart rate (B and D) responses to acute intravenous bolus administration of ET-1, expressed as means ± SE for AUC from baseline. Statistics are as in Fig. 2.

Fig. 4. Acute responses to ANG II and PE in anesthetized mice. Data shown are mean arterial pressure (A and C) and heart rate (B and D) responses to acute intravenous bolus administration of ANG II (A and B) and PE (C and D) in vehicle- (■) and IL-6- (●) infused mice, expressed as means ± SE for AUC from baseline. Statistics are as in Fig. 2.

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larger than, those reported by the other studies (24, 30). Our findings strongly suggest that chronic exposure to increased circulating IL-6 concentrations does not alter pressor responsiveness to ANG II, PE, or ET-1, therefore suggesting that the effects noted previously in aortic tissue may not carry over to include effects on the resistance vasculature.

It was of interest to examine the effects of chronic IL-6 infusion on responses to ET-1 since ET-1, and perhaps IL-6, may be involved in hypertension in humans (2, 5, 28) and both have been implicated in experimental ANG II-induced hypertension (16, 21). Neither the absence of endogenous IL-6 nor chronic IL-6 infusion had any significant effect on blood pressure responses to ET-1. However, unexpectedly, the reflex-mediated fall in heart rate observed in response to ET-1 was significantly diminished in mice infused with IL-6. This reduced effect on heart rate in IL-6-infused mice does not appear to be due either to a difference in the concurrent pressor response, as the rise in blood pressure was not different with or without ganglion blockade, or to an enhanced direct chronotropic effect of ET-1. A generalized change in baroreflex sensitivity also appears an unlikely explanation since heart rate fell during ANG II and PE responses to a similar extent in both IL-6 and vehicle groups. One possibility is that IL-6 produces a change in heart rate control that can only be observed during the more sustained changes in blood pressure elicited by ET-1 and does not come into play during the more transient pressor responses to PE and ANG II. Rapid (minutes), although incomplete, resetting of baroreflexes has been described (4, 22). Thus it may be possible that IL-6-infused mice display more rapid resetting of baroreflex control of heart rate than vehicle-infused mice. Determining whether this is the case would require a distinct set of experiments and is beyond the scope of the present study.

Another possible explanation for the apparently reduced heart rate sensitivity of IL-6 mice with regard to ET-1 is that IL-6 somehow specifically modulates the effects of ET-1 on the baroreflex. Unfortunately, little appears to be known regarding the effects of peripheral ET-1 on the baroreflex control of the cardiovascular system. One study reported that exposure of the endothelium-denuded carotid sinus to high concentrations (100 nM) of ET-1 reduced baroreceptor activity at high carotid pressures, whereas lower concentrations (0.01–1 nM) appeared to enhance activity (6). The net effect of endogenously produced ET-1 in an intact carotid sinus, however, may depend both on these apparently concentration-dependent direct effects of ET-1 on the neurons or vascular wall and on indirect effects mediated by ETg receptor-endothelium-derived factors such as NO, which has been shown to inhibit baroreceptor activity (19), or prostaglandins, which enhance baroreceptor activity (20). Further studies are needed to increase our understanding of this complex and seemingly underinvestigated area of cardiovascular control.

A number of limitations exist regarding the ability to assess the effects of IL-6 infusion on vascular responsiveness in vivo. It is possible that chronic infusion of IL-6 may have had additional effects that masked changes in vascular reactivity to the agonists used in this study. One such possible effect is changes to the baroreflex mechanism as discussed above. An additional possibility is that if IL-6 infusion increased endogenous levels of ANG II, ET-1, or circulating catecholamines, this might alter responsiveness to exogenous administration of these or other agents. Reports of plasma norepinephrine concentrations in IL-6-knockout mice are conflicting (15, 31), and the effect of chronic IL-6 infusion on plasma catecholamines is currently unknown. Plasma ET-1 concentration is not altered by the IL-6 infusion protocol used in this study (unpublished data); however, we cannot rule out an effect of IL-6 infusion on local production of ET-1, or on plasma or tissue ANG II concentrations.

The basal blood pressures of the mice in our study, while no doubt slightly lower than those of conscious mice, are comparable to or greater than values reported by others in anesthetized mice (3, 12). Importantly, care was taken to maintain body temperature, and our mice displayed basal heart rates in the physiological range. As expected, blood pressure and heart rate fell during ganglionic blockade. While it is possible that the low blood pressure following ganglionic blockade in itself may have affected responses to ET-1, we did not attempt to restore basal blood pressures by infusion of another pressor agent as this could have also affected responses to ET-1 in some way.

The dose of IL-6 chosen for infusion in this study was based on that used in a previous study also conducted in mice (14) and yielded similar plasma IL-6 concentrations. The IL-6 infusion rate used in the previous study was designed to generate a rise in plasma IL-6 concentration to approximate the roughly fourfold elevation of plasma IL-6 seen in obesity (13). The plasma concentrations of IL-6 observed in the present study were somewhat higher than the concentration (10–30 pg/ml) previously reported during ANG II-induced hypertension in mice (16) but were much lower than those observed in sepsis (18).

Although the findings of our study do not preclude a potential for differential effects on vascular responsiveness of specific vascular beds, the results of the present study strongly suggest that chronic elevation of plasma IL-6 concentrations in normotensive mice does not lead to a generalized increase in vascular responsiveness. Whether IL-6 enhances pressor responsiveness in the setting of hypertension remains an open question; however, our data indicate that a chronically elevated plasma IL-6 concentration in itself is insufficient to alter whole animal cardiovascular responses to pressor agents. Moreover, our data caution against using effects observed on responsiveness of the aorta in vitro to predict cardiovascular responses at the whole animal level.

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REFERENCES

INTERLEUKIN-6 AND ACUTE RESPONSES TO VASOCONSTRICTORS


