Characterization of acute reversible systemic hypertension in a model of heme protein-induced renal injury

DAVID H. WARDEN, ANTHONY J. CROATT, ZVONIMIR S. KATUSIC, AND KARL A. NATH
Nephrology Research Unit and Departments of Medicine and Anesthesiology, Mayo Clinic/Foundation, Rochester; and University of Minnesota, Minneapolis, Minnesota 55905

Warden, David H., Anthony J. Croatt, Zvonimir S. Katusic, and Karl A. Nath. Characterization of acute reversible systemic hypertension in a model of heme protein-induced renal injury. Am. J. Physiol. 277 (Renal Physiol. 46): F58–F65, 1999.—In the glycerol model of renal injury we describe an acute rise in systemic arterial pressure which is attended by a reduced vasodilatory response to acetylcholine in vivo; vasodilatory responses to verapamil, however, were not impaired. Neither arginine nor sodium nitroprusside diminished this rise in blood pressure; N-nitro-L-arginine methyl ester (L-NAME) elevated basal mean arterial pressure and markedly blunted the rise in mean arterial pressure following the administration of glycerol. Aortic rings from the glycerol-treated rat demonstrate an impaired vasodilatory response to acetylcholine, an effect not repaired by arginine; the vasodilator responses to nitric oxide donors, sodium nitroprusside and SIN-1, were also impaired; 8-bromo-cGMP, at higher doses, evinced a vasodilatory response comparable to that observed in the control rings. This pattern of responses was not a nonspecific effect of aortic injury, since aortic rings treated with mercuric chloride, a potent oxidant, displayed an impaired vasodilatory response to acetylcholine but not to sodium nitroprusside. We conclude that in the glycerol model of heme protein-induced tissue injury, there is an acute elevation in mean arterial pressure attended by impaired endothelium-dependent vasodilatation in vitro and in vivo. We suggest that the acute scavenging of nitric oxide by heme proteins depletes the blood vessel wall of its endogenous vasodilator and permeation of heme proteins into the blood vessel wall may contribute to such sustained effects as observed in vitro.

nitric oxide; vasoconstriction; rhabdomyolysis

THE INTRAMUSCULAR INJECTION of hypertonic glycerol into rats induces myolysis, hemolysis, and attendant heme protein-induced renal injury and has long been used as a model of acute renal failure (3, 28). The mechanisms accounting for such renal injury rely on three main pathogenetic pathways, namely, renal vasoconstriction, direct tubular toxicity, and intratubular cast formation (28). Renal vasoconstriction occurs promptly and prominently in this setting and is assigned a critical role in the ensuing injury (2, 11, 13, 28). Vasoconstriction induces ischemia and a spectrum of metabolic perturbations including ATP and GSH depletion (1, 28, 29); such metabolic perturbations may be directly damaging and/or conspire with heme protein-dependent mechanisms of renal injury. For example, iron-mediated oxidant stress, which is one of the dominant pathways of nephrotoxicity in this model (3), is critically exacerbated by GSH depletion (1); heme protein-dependent cast formation in the kidney may be exacerbated by prior ischemic conditioning (28, 30). Thus vasoconstriction induced by heme proteins renders the kidney more vulnerable to the injurious effects of heme proteins, and for such reasons, understanding the mechanisms accounting for vasoconstriction and abnormal vascular behavior, in general, in this and other models, represents an important aspect of the study of acute renal failure (7, 28).

In the course of studies involving the glycerol model of acute renal failure, we noted that within seconds of the administration of glycerol there is a marked rise in mean arterial pressure (MAP) that persists for up to 2 h. Thus vasoconstriction in this model is not localized to the kidney but appears as part of a more generalized response. Our attention was drawn to this phenomenon for several reasons. Although utilized as a model of rhabdomyolysis, a condition that is often associated with hypotension and shock incurred, in part, by the sequestration of interstitial fluid (28), glycerol-induced acute renal failure is ushered in, ironically, by acute elevation in systemic arterial pressure. Additionally, a remarkable feature of the glycerol model is the acute elevation in the plasma concentrations of heme proteins that promptly occurs (28). Since heme proteins possess the capacity to potently bind nitric oxide (16, 17), such presence of heme proteins in the plasma compartment could efficiently remove nitric oxide from the vasculature, thereby depriving the vasculature of its endogenous vasodilator (5, 15). Such elevation in arterial pressure thus provides a novel in vivo model for the exploration of elevation in arterial pressure observed with heme proteins, a critical issue in current attempts to develop safe, hemoglobin-based red blood cell substitutes (6, 9, 12, 16, 24).

The present study represents the first available characterization of such elevation in systolic arterial pressure; it encompasses in vivo studies and in vitro studies of aortic rings in this disease model, and it focuses on the scavenging of nitric oxide as an underlying mechanism for such elevation in systolic arterial pressure.

METHODS

Studies in vivo. Studies involving the glycerol model of acute renal injury were approved by the respective Institutional Animal Care and Use Committees of the University of Minnesota and the Mayo Clinic. Studies were performed in male Sprague-Dawley rats in which the glycerol model of renal failure was induced by the injection of 50% glycerol.
L-arginine hydrochloride (180 mg·kg⁻¹·h⁻¹) was administered intravenously, and mean arterial pressure was fully developed, acetylcholine, 10 µg/kg and 1 h after administration of glycerol when the rise in mean arterial pressure was followed for 2 h thereafter. To test the effect of nitric oxide-independent vasodilators on vascular responses in rats subjected to hypertonic glycerol, we administered a submaximal concentration of acetylcholine to determine whether endothelial function was impaired. Additional experiments contrasted the effects of mercuric chloride (HgCl₂) on relaxation of aortic rings in vitro. For these experiments, all tissues were dissected from control aortae and prepared for experimentation as above. Functional endothelium was confirmed in all tissues by relaxation to acetylcholine. Experimental tissues were then treated with HgCl₂ at the indicated concentration by addition to the bathing solution for 20 min; tissues were then washed free of HgCl₂ by exchanging the bathing solution three times over 30 min. Control tissues were treated in identical fashion except for omission of HgCl₂.

RESULTS

Studies in vivo. Following the intramuscular administration of hypertonic glycerol, a prompt elevation in mean arterial pressure occurs. In all of the five animals so treated, a marked elevation of blood pressure occurred, which peaked some 60 min after the administration of glycerol representing a mean elevation in arterial pressure of 28 ± 4 mmHg (Fig. 1A). For up to 150 min after the administration of hypertonic glycerol, the increment in mean arterial pressure was significantly greater than in the control rats (Fig. 1B). To determine whether endothelium-dependent vasodilatation was impaired in this setting, we examined the vasodilatory response to acetylcholine at a time when vasoconstriction and elevation in mean arterial pressure was clearly manifest after the administration of glycerol, namely, 60 min after its administration. As shown in Fig. 2, the reduction in mean arterial pressure in response to acetylcholine was less in the glycerol-treated animal compared with the control animal when sequentially determined at 1, 2, and 3 min after the administration of acetylcholine. Thus the elevation in mean arterial pressure that occurs following the administration of hypertonic glycerol is attended by an impaired endothelium-dependent vasorelaxation.

To probe further the possible involvement of the nitric oxide system in elevation of mean arterial pressure following the administration of glycerol, we studied four groups of rats all subjected to intramuscular glycerol and treated with either saline (control), arginine, sodium nitroprusside, or L-NAME, begun 45 min...
prior to the administration of glycerol. Figure 3 shows the temporal profiles for mean arterial pressure in these conditions. The results of these studies demonstrate that arginine failed to affect the basal blood pressure or the rise in blood pressure following the injection of glycerol; indeed, the blood pressure profiles for the rats treated with arginine- and the saline-treated controls were virtually superimposable, prior to and after the administration of glycerol (Fig. 3); the maximum rise in mean arterial pressure was not significantly different in saline-treated and arginine-treated rats (24 ± 1 and 26 ± 2 mmHg, respectively). The administration of sodium nitroprusside did not alter the peak arterial pressure achieved after the administration of glycerol (Fig. 3); however, since sodium nitroprusside significantly lowered mean arterial pressure prior to the administration of glycerol, the increment in mean arterial pressure in response to the administration of glycerol in nitroprusside-treated rats, namely, 41 ± 2 mmHg, was significantly increased. On the other hand, the administration of L-NAME, while expectedly and significantly elevating basal blood pressure prior to the administration of glycerol, markedly blunted the rise in mean arterial pressure that occurred in response to intramuscular glycerol, namely, 6 ± 3 mmHg (Fig. 3).

To determine whether the vasculature in glycerol-treated rats is nonspecifically injured such that responses to nitric oxide-independent vasodilators are impaired, we performed additional studies in which we determined the efficacy of the same dose of a nitric oxide-independent vasodilator, verapamil, in reducing mean arterial pressure. There were no significant differences in mean arterial pressure in control and glycerol-treated rats (injected 3 h previously) just prior to the administration of verapamil (133 ± 2 vs. 142 ± 5 mmHg; n = 4 in each group, P = NS) and following the administration of verapamil (104 ± 3 vs. 101 ± 6 mmHg; n = 4 in each group, P = NS); the reductions in mean arterial pressures were not significantly different between control and glycerol-treated rats (29 ± 2 vs. 41 ± 5 mmHg; n = 4 in each group, P = NS). Thus the vasculature is not impaired in its response to nitric oxide-independent vasodilators in glycerol-treated rats.

Studies in vitro. We also studied the behavior of aortic rings in vitro in animals subjected to glycerol-induced acute renal failure. As demonstrated in Fig. 4, there was a markedly attenuated relaxation response to an endothelium-dependent vasodilator, acetylcholine, in rats subjected to glycerol-induced acute renal failure compared with aortic rings harvested from control animals. This blunted vasodilatory response in glycerol-treated animals was not repaired by the presence of arginine at a concentration of 250 µM in the extracellular medium. Thus the impaired vasodilatory response observed in the glycerol model is not ameliorated by the provision of excess arginine, a finding consistent with the effects of arginine administered in vivo, and one that suggests that neither deficiency nor unavailability of arginine account for the impaired vasodilatation in glycerol-induced acute renal failure.

To determine whether the provision of nitric oxide would affect this impaired vasodilatory response in rat
aortic rings in vitro, we studied the relaxation response of aortic rings to nitric oxide delivered in the form of sodium nitroprusside (Fig. 5) or in the form of SIN-1 (Fig. 6); these compounds release nitric oxide in vitro and are used as pharmacological systems that deliver nitric oxide. The vasodilatory response in aortic rings from the glycerol model to either form of delivery of nitric oxide was clearly blunted compared with the vasodilation achieved in the control rings; only in the presence of very high concentrations of nitroprusside (10^{-6} M) was the vasorelaxation response comparable to control aortic rings.

We also examined the relaxatory response of aortic rings to 8-bromo-cGMP (Fig. 7). At lower concentrations the relaxation response in aortic rings from glycerol-treated rats was less than that observed in controls; however, at higher concentrations, namely, 10^{-4} M, of 8-bromo-cGMP, the relaxation response in aortic rings from glycerol-treated rats was comparable to that observed in controls (Fig. 7). Thus, at higher but not at lower concentrations of 8-bromo-cGMP, the distal effector for vasodilatation in the nitric oxide-dependent system, namely, cGMP, elicits a comparable amplitude of vasodilatation in aortic rings from glycerol-treated and control rats. In contrast, the provision of arginine completely fails, and pharmacological nitric oxide-generating systems are attenuated, in the capacity to achieve such effects.

In an attempt to determine specificity of the changes we observed in the glycerol model, we examined the behavior of vascular responses in another model of acute renal insufficiency, one induced by HgCl_2 and one that is heme protein-independent. HgCl_2, in micromolar concentrations, is a potent generator of hydrogen peroxide and induces hydrogen peroxide-renal injury that can be significantly vitiated by scavengers of hydrogen peroxide such as catalase and pyruvate (22). As demonstrated in Fig. 8, with increasing doses of HgCl_2, there is a stepwise impairment in the vasodilatory response to acetylcholine indicating impairment, in part, of endothelium-dependent relaxation. However, unlike the aortic rings in the glycerol model, the HgCl_2-treated rings respond to sodium nitroprusside in a manner quite similar to aortic rings from control animals (Fig. 9). To underscore the role of oxidants in HgCl_2-induced injury, the presence of pyruvate, a scavenger of hydrogen peroxide, protected against the defect in acetylcholine-induced vasodilatation induced by HgCl_2 (data not shown). Thus, in a model of heme protein-independent, oxidant-mediated vascular injury induced by HgCl_2, the provision of the nitric oxide-generating agent sodium nitroprusside induces vasodilatation comparable to controls (Fig. 9), quite unlike...
the circumstance with vascular rings from glycerol-treated rats (Fig. 5).

DISCUSSION

Prior studies of the glycerol model that include systemic hemodynamic measurements provide no clear consensus on the systemic arterial pressure profile shortly after the administration of glycerol: some studies observe no significant elevation in arterial pressure (13), others have measured blood pressure but have not commented on whether significant increments in mean arterial pressure occur (2), and finally, there is one available report which, although containing data demonstrating statistical increments in mean arterial pressure, neither draws attention to, nor discusses, the biological significance of these changes in mean arterial pressure (11). Our study is the first to demonstrate an invariant elevation in mean arterial pressure in the early phase following the administration of glycerol and to highlight what we regard as the pathobiological significance of these changes in light of current interest in the nitric oxide system in blood pressure regulation (5, 15). The inconsistent findings with regard to the elevation in mean arterial pressure in previously published studies are difficult to explain, but this may reflect the modulatory vasoactive effects of different anesthetics employed in these studies. The utilization of in vivo and in vitro preparations, as incorporated in
The glycerol model leads to the release of myoglobin from injured muscle as well as pronounced hemolysis (28). The plasma in rats subjected to the glycerol model rapidly turns pink in color, a change that reflects the release of hemoglobin from red cells. The heme prosthetic group in heme proteins possesses a remarkable capacity to bind nitric oxide (16, 17). In view of the capacity of heme proteins to avidly bind nitric oxide, we pursued the possibility that such binding of nitric oxide by extracellular heme proteins, hemoglobin and myoglobin, would deprive the vasculature of its endogenous vasodilator, nitric oxide, thereby contributing to the rapid rise in mean arterial pressure. In support of this possibility are our findings that demonstrate an impaired vasodilatory response to the endothelium-dependent vasodilator acetylcholine both in vivo and in vitro. Evidence in support of a deficiency of nitric oxide was provided by studies in which L-NAME was administered prior to the administration of glycerol. L-NAME-treated rats, predictably, mounted a prominent hypertensive response compared with rats treated with saline prior to the administration of glycerol; such elevation in arterial pressure following the administration of L-NAME reflects the deficiency of nitric oxide in vasculature (5). If net deficiency or unavailability of nitric oxide underlies the hypertensive response following the administration of glycerol, then this hypertensive response induced by hypertonic glycerol should be markedly blunted if nitric oxide is already depleted in tissues by prior administration of L-NAME. Indeed, this is what we observed in this circumstance: the elevation of mean arterial pressure induced by hypertonic glycerol pretreated with L-NAME was approximately one-fourth of that observed following the administration of glycerol in rats pretreated with saline.

These findings are consistent with the thesis that the rise in mean arterial pressure reflects, at least in part, deficiency of nitric oxide in the vasculature. The administration of arginine either in vivo or in vitro failed to repair the impaired vasodilatory response observed; thus the simple provision of substrate for nitric oxide synthase fails to mitigate the impaired vasodilatory response; additionally, these findings indicate that the deficiency of arginine per se was not a critical factor underlying these changes. Nitric oxide donors, such as nitroprusside and SIN-1, also failed to evoke a vasodilatory response in glycerol tissues comparable to that observed in control tissues for most of the range of concentrations with which these nitric oxide donors were employed in vitro. Interestingly, in prior studies, the vasodilatory effects of nitric oxide donors such as sodium nitroprusside are attenuated in the presence of heme proteins, which are in all likelihood effects that reflect the scavenging by heme proteins of nitric oxide generated by nitroprusside (23). In our studies, only with very large concentrations of one of these nitric oxide donors (nitroprusside, see Fig. 5) was a comparable vasodilatory response elicited in aortic tissue from the glycerol-treated rats. These latter observations suggest that, at least for the lower and middle range of concentrations employed for these agents, either the vascular smooth muscle was incapable of responding to nitric oxide-instigated vasodilation or that any nitric oxide that was administered by these donors was consumed or inactivated. We sought to differentiate between these possibilities by examining the effect of the analog of cGMP, 8-bromo-cGMP, in aortic rings. cGMP is the effector mechanism for nitric oxide-instigated vasodilatation, and is generated by guanylate cyclase after the latter is activated by nitric oxide. The vasodilatory response in aortic rings to the analog of cGMP, 8-bromo-cGMP, from glycerol-treated rats was comparable to the response observed in control rings at the highest concentrations but was significantly less at lower concentrations; the comparability of responses to cGMP at the highest concentration tested indicates preservation of responsivity to cGMP at such concentration. However, at lower concentrations, impaired responsivity to cGMP by vascular smooth muscle cells may also contribute to the abnormal hemodynamic response observed in the glycerol model.

On the basis of these findings, we suggest the following mechanism may contribute, at least in part, to the vascular behavior we observed in vivo and in vitro. The presence of heme proteins in plasma siphons off nitric oxide from the vasculature with attendant rise in arterial pressure. Such a mechanism would account for the impaired vasodilatory response to acetylcholine in vivo and the attenuation in the elevation of arterial pressure in response to hypertonic glycerol in rats pretreated with L-NAME. The failure of arginine and nitroprusside to mitigate the elevation of arterial pressure in vivo indicates that the availability of such additional sources of nitric oxide do not swamp the capacity of heme proteins to bind nitric oxide, at least
under the conditions employed and permissible in vivo. Our in vitro studies indicate that the impaired vasodilatory response is preserved in vitro. The presence of this impaired vasodilation in vitro may arise from the permeation of heme proteins in the blood vessel wall (9, 14, 16, 19). When hemoglobin is released from red cells in the circulation, it dissociates from its tetrameric form into dimers of molecular mass of ~32 kDa (28). Such species enter the subendothelial and intercellular spaces of the vasculature and thus can provide ongoing scavenging of nitric oxide in blood vessel walls (14, 19). It is possible that other mechanisms may also contribute to the elevation in arterial pressure we observed in this model including the inactivation of nitric oxide synthase by heme proteins, the generation of reactive oxygen species, or the production of vasoactive substances such as endothelin.

Our findings in the glycerol model are consistent with a large literature centered on the vasoconstrictive effects of hemoglobin in vivo and in vitro. Such studies indicate that the scavenging of nitric oxide by hemoglobin is a critical determinant of the vasoconstriction that ensues (16, 17, 25). Such effects of hemoglobin pose a problem in the development of safe, hemoglobin-based, red blood cell substitutes. Many of the available preparations are associated with systemic hypertension and vasoconstriction, which may adversely affect tissue perfusion (6, 9, 16, 17, 25). Polymerized hemoglobin, in contrast to hemoglobin in the dimeric or tetrameric form, is less vasoconstrictive (9, 10, 16), in part because of absence of permeation of polymerized hemoglobin into blood vessel walls; dimeric and tetrameric hemoglobin may permeate the endothelial surface of blood vessels, enter the vasculature, and thus divert nitric oxide away from smooth muscle cells more effectively compared with such hemoglobin species restricted to the luminal compartment of the vasculature (14, 19). Similarly, hemoglobin prepared in liposomes exerts less vasoconstriction, a beneficial attribute that may also be due to inability of such hemoglobin preparations to permeate blood vessel walls (9, 16).

The nitric oxide system has been studied in renal impairment associated with heme proteins. For example, infusion of myoglobin leads to a decrease in renal blood flow and in creatinine clearance in conjunction with decreased urinary excretion of nitrate and nitrite and cGMP (26). In contrast to the vascular effects reported in the present study, these renal effects are attenuated by the administration of arginine (26). Similarly, in the glycerol model of acute renal failure, the administration of arginine seems to protect against renal dysfunction, whereas the administration of an inhibitor of nitric oxide synthase worsens renal function (18). These latter studies indicate that impairment in the nitric oxide system induced by heme proteins in these settings contributes to the renal dysfunction that is observed.

In summary, in a disease model studied for more than 40 years (8), the present study provides the first available characterization of an overlooked, acute, reversible, systemic hypertension that swiftly follows the administration of glycerol; utilizing in vivo studies and in vitro studies of aortic rings, physiological evidence is provided that scavenging of nitric oxide provides, at least in part, an underlying mechanism for such elevation in arterial pressure.

We gratefully acknowledge the expert secretarial assistance provided by Sharon Heppelmann.

These studies were supported by a National Institutes of Health FIRST Award Grant HL-48238 (to D. H. Warden) and by National Institutes of Health Grants DK-47060 and HL-55552 (to K. A. Nath). Portions of the data in this report have been published in abstract form (J. Am. Soc. Nephrol. 3: 548, 1992; and J. Invest. Med. 44: 304, 1996).

Address for reprint requests and other correspondence K. A. Nath, Mayo Clinic 200 First St., SW, 542 Guggenheim Bldg., Rochester, MN 55905.

Received 9 October 1998; accepted in final form 18 March 1999.

REFERENCES


