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Pressor and Renal Vasoconstrictor Responses to Acute Systemic Nitric Oxide Synthesis Inhibition Are Independent of the Sympathetic Nervous System and Angiotensin II[1]

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ABSTRACT

Acute systemic, nonselective nitric oxide synthesis inhibition (NOSI) causes a marked pressor and renal vasoconstrictor response in the normal conscious chronically catheterized rat. The present studies directly address the question of how these vasoconstrictor responses are related to the combined vasoconstrictor activities of the sympathetic nervous system and angiotensin II. When the *alpha* adrenoceptors are blocked (with prazosin) the pressor and renal hemodynamic responses to NOSI are unaffected. Combined *alpha* adrenoceptor and angiotensin II receptor blockade at the same time as NOSI results in no net change in blood pressure while leaving the renal vasoconstrictor response intact. However, when the NOSI is delayed, a substantial and unblunted pressor response is seen. In contrast to the vasoconstrictor responses, the natriuretic and diuretic responses to acute NOSI are prevented by simultaneous *alpha* adrenoceptor blockade alone and combined with angiotensin II receptor blockade. These findings suggest that the hemodynamic actions of acute NOSI in the unstressed rat are independent of the sympathetic nervous system and angiotensin II. In contrast, the natriuretic/diuretic response to acute NOSI is apparently partly the result of some interaction with the sympathetic nervous system, not, as we had previously suggested, exclusively the result of a pressure natriuresis.

It is now well established that tonically produced nitric oxide (NO) plays an important vasodilatory role in the maintenance of systemic and renal vascular tone (Baylis et al., 1990; Moncada et al., 1991; Raij and Baylis 1995). When tonically produced NO is acutely inhibited, dose-dependent increases in blood pressure (BP) and falls in renal perfusion are seen due to widespread vasoconstriction (Baylis et al., 1990). This vasoconstriction is due both to withdrawal of vasodilatory NO and to amplification of any active vasoconstrictor systems. There is considerable variability in the literature regarding the relative role of the major vasoconstrictor systems in these responses to acute systemic NO synthesis inhibition (NOSI). Much of this variability is due to different levels of activity of vasoconstrictors, determined by the experimental preparation used, and often these systems are activated much above normal by acute surgery, general anesthesia, volume contraction, etc. For this reason, we used the conscious, chronically catheterized, unstressed rat preparation in a series of continuing studies to determine the *physiologic* relationship between NO and various vasoconstrictors in the regulation of BP and renal vascular tone.

A number of earlier observations suggested that angiotensin II (ANGII) plays an important role in mediating the pressor and renal vasoconstrictor responses to acute NOSI in the normal rat (Tolins and Raij 1991; Sigmon et al., 1992). However, we found that in the conscious rat, where ANGII is not controlling BP or renal hemodynamics in the baseline state, inhibition of endogenous ANGII during acute NOSI has no impact on the pressor and renal vasoconstrictor response (Baylis et al., 1993). We interpreted these observations to indicate no role for ANGII in the vasoconstrictor response to NOSI in the normal, *unstressed* animal. Unlike ANGII, the sympathetic nervous system (SNS) is tonically active in control of vascular tone and provides much of the short-term, physiologic regulation of BP. Some studies suggest that the SNS is partly responsible for the pressor and renal vasoconstrictor responses to acute NOSI (Lacolley et al., 1991; Sakuma et al., 1992; Harada et al., 1993), whereas others claim no interaction (Pegoraro et al., 1992; Pucci et al., 1992; Huang et al., 1994).

The present experiments were carried out in the conscious, chronically catheterized rat to investigate the role of the SNS

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ABBREVIATIONS: NOSI, nitric oxide synthase inhibition; GFR, glomerular filtration rate; RPF, renal plasma flow; RVR, renal vascular resistance; V, urine flow; $U_{Na}V$, urinary excretion of Na; U_KV, urinary excretion of K; FE_{Na}, fractional excretion of Na; nNOS, neuronal NOS; PAH, paraaminohippuric acid.

in the pressor and renal vascular response to acute NOSI in the normal animal, using the *alpha* adrenoceptor blocker prazosin. Because there are important interactions between the SNS and ANGII systems (Campbell and Jackson 1979; Zimmerman 1981; Purdy and Weber 1988; Blantz et al., 1990; Isaacson and Reid 1990; Qiu et al., 1994), we also used the ANGII type 1 receptor (AT_1) antagonist, losartan to determine whether an interaction between ANGII and SNS is involved in the response to acute NOSI.

Materials and Methods

Studies were conducted on 20 male Sprague-Dawley rats, aged 3 to 6 months, obtained from Harlan Sprague-Dawley Inc. (Indianapolis, IN). In all rats a preliminary surgery was conducted in which catheters were placed in the left femoral artery and vein and in the urinary bladder. All surgery was conducted under general anesthesia with short-acting barbiturate anesthetic. At the end of the surgery vascular catheters and bladder catheters were primed and plugged, and rats were returned to individual cages. Full sterile technique was used throughout. Details of this chronic catheterization method have been published previously (Baylis et al., 1990, 1993, 1994; Qiu et al., 1994). Rats were allowed free access to rat chow (~24% protein and ~0.4% Na) and drinking water and were handled and trained to accustom them to the activity in the laboratory. A period of 7 days elapsed between surgery and the acute experiments. When more than one study was conducted on the same animal, at least 2 days rest was allowed between experiments.

Renal function studies were conducted as follows. Rats were placed in a restraining cage and the arterial catheter was connected to a pressure transducer and recorder for BP measurement. The arterial line was also used for occasional sampling of blood. An i.v. infusion of [^{3}H]inulin (2–5 μCi/ml; New England Nuclear, Boston MA) and paraaminohippuric acid (PAH; 1%; Merck, Sharp and Dohme, West Point, PA) was given in 0.9% NaCl at 5 μl/min/100 g rat body weight. The bladder pin was removed for collection of urine and a tube with side arm was attached to the bladder catheter for collection of urine. After an 80 min equilibration period, two 20-min control urine collections were made with midpoint arterial blood samples. The bladder catheter was flushed with air 2 min before the end of the period to ensure complete collection of urine. Midpoint blood samples (about 150 μl) were centrifuged, the plasma was removed for analysis, and the red cells were reconstituted with sterile 0.9% NaCl and restored to the rat. After completion of control measurements, one of the following five experiments was conducted. In the first series of experiments (group 1), rats received the NO synthesis inhibitor nitro L-arginine methyl ester (L-NAME; 10 mg/kg i.v. bolus; Sigma, St. Louis, MO), and repeat measurements were made 10 min after administration of the drug. This dose of L-NAME has been previously shown by us to give the maximal rise in BP in the conscious chronically catheterized rat (Baylis et al., 1990). In the second series (group 2), rats received the same dose of L-NAME and were given the *alpha*-1 adrenoceptor antagonist prazosin (0.1 mg/kg, i.v.; Pfizer Inc., Groton CT) immediately after the L-NAME (within 60 sec), and repeat measurements were made 10 min later. This dose of prazosin produces complete blockade of the pressor response to the *alpha*-1 adrenoceptor agonist methoxamine (60 μg/kg, i.v. bolus) (Qiu et al., 1994; Baylis, 1995). In the third series, group 3 rats received the ANGII AT_1 antagonist, losartan (3 mg/kg, i.v.; Du Pont Merck, Wilmington, DE), followed by the same dose of L-NAME + prazosin as in group 2. This dose of losartan completely prevents the pressor response to 5 ng, i.v. bolus ANGII (Baylis et al., 1993). Drugs were given in rapid succession within approximately 2 min. Group 4 rats received combined prazosin and losartan alone with i.v. 0.9% NaCl (as a vehicle for L-NAME) given approximately 25 min later, and repeat measurements were made 10 min later. In the final series, group 5 rats received prazosin and then losartan in rapid succession, and then approximately 25 min later, i.v. L-NAME was administered, and repeat measurements were made 10 min later. At the end of the final clearance period in each of the five series, red blood cells were reconstituted and restored to the rat. Vascular and bladder catheters were primed and plugged, and rats were returned to their home cages, after which they were either used for additional experiments or sacrificed.

The following analyses and calculations were made. Urine volume was measured gravimetrically, and then urine was analyzed for [^{3}H]inulin activity and concentrations of PAH, Na, and K. Blood samples were analyzed for hematocrit, plasma [^{3}H]inulin activity, and PAH, Na, and K concentrations. These measurements allow calculation of glomerular filtration rate (GFR), renal plasma flow (RPF), renal vascular resistance (RVR), urine flow (V), urinary excretion of Na and K ($U_{Na}V$ and U_KV respectively), and fractional excretion of Na (FE_{Na}). Details of these analyses and calculations have been published by us previously (Baylis et al., 1990, 1993, 1994; Qiu et al., 1994). When all experiments were completed, rats were euthanized and the bladder and kidneys were inspected to establish that they were free of infection.

Within-group analysis was by paired t test and between-group analysis was by unpaired t test or one way ANOVA. All data are shown as mean ± 1 S.E. and $p < .05$ is considered to be statistically significant.

Results

Body weights were 408 ± 13, 383 ± 7, 390 ± 6, 421 ± 12 and 424 ± 12 g in groups 1 to 5, respectively. As summarized in Table 1, acute systemic NOSI with L-NAME in group 1 rats produced a large rise in BP, renal vasoconstriction and significant falls in GFR and RPF. A natriuretic and diuretic effect was seen without any change in U_KV. These observations are consistent with previous reports by us (Baylis et al., 1990, 1993, 1994).

Group 2 rats were subjected to acute systemic NOSI combined with simultaneous *alpha*-1 adrenoceptor antagonism. The pressor response was indistinguishable from the response to NOSI alone and, unexpectedly, the renal hemodynamic responses to NOSI were slightly enhanced by *alpha*-1 adrenoceptor antagonism (Table 1 and Fig. 1). In contrast, the natriuretic and diuretic response to NOSI was completely prevented by concomitant *alpha*-1 adrenoceptor antagonism, and a fall occurred in U_KV. We did not conduct studies with *alpha*-1 adrenoceptor antagonism alone in this series of experiments, although earlier work by us shows little change in RVR but a fall in BP, GFR and $U_{Na}V$ when prazosin is given in this preparation (Fig. 1). Group 3 rats received combined, simultaneously administered NOSI, *alpha*-1 adrenoceptor antagonism and ANGII AT_1 receptor antagonism with losartan. When both *alpha*-1 adrenoceptors and ANGII AT_1 receptors were blocked, there was no net pressor response with acute systemic NOSI, whereas the renal hemodynamic effects were similar to those seen with NOSI alone. As with group 2, the natriuretic and diuretic responses to NOSI were prevented (Table 1 and Fig. 1).

As a control for the effects of losartan and prazosin, group 4 rats received simultaneous *alpha* adrenoceptor and ANGII blockade, which produced substantial falls in BP and RVR and a slight rise in RPF but a fall in GFR (as in all other groups) because of a fall in filtration fraction (FF). Both V and U_KV were also reduced by inhibition of *alpha* adrenoceptor and ANGII systems. We have also previously reported the effect of acute ANGII AT_1 inhibition (+ losartan) (Baylis et

TABLE 1

Summary of BP and renal function in young adult conscious chronically catheterized male Sprague-Dawley rats studied in control and then during one of the following experimental (Exp) maneuvers.

Group 1 rats received nonselective NO synthesis inhibitor L-NAME (i.v., 10 mg/kg), group 2 received L-NAME + concomitant *alpha* adrenoceptor blockade with prazosin (i.v., 0.1 mg/kg), and group 3 received L-NAME, prazosin and angiotensin II AT_1 receptor blockade with losartan (i.v. 3 mg/kg) all given simultaneously. Group 4 received prazosin and losartan together and group 5 received prazosin and losartan initially and then L-NAME after a 25-min delay.

	BP	RVR	GFR	RPF	FF	V	$U_{Na}V$	FE_{Na}	U_KV
	mm Hg	*mm Hg (ml/min)*	*ml/min*	*ml/min*		*μl/min*	*μeq/min*	*%*	*μeq/m*
Group 1									
Control (n = 8)	119 ± 2	5.5 ± 0.5	3.01 ± 0.08	12.6 ± 1.0	0.251 ± 0.021	21.3 ± 4.0	2.40 ± 0.53	0.564 ± 0.114	2.64 ± 0.24
Exp (+NAME)	154 ± 3	13.0 ± 0.8	2.40 ± 0.18	6.8 ± 0.4	0.359 ± 0.017	67.2 ± 13.7	5.34 ± 1.35	1.629 ± 0.381	2.45 ± 0.21
p, c vs. exp	<0.001	<0.001	<0.005	<0.001	<0.001	<0.01	<0.05	<0.001	N.S.
Group 2									
Control (n = 7)	121 ± 1	5.6 ± 0.3	2.99 ± 0.17	11.9 ± 0.6	0.254 ± 0.013	15.9 ± 2.0	1.77 ± 0.28	0.432 ± 0.070	2.28 ± 0.20
Exp (NAME + prazosin)	153 ± 2	15.6 ± 0.9	1.89 ± 0.09	5.5 ± 0.3	0.345 ± 0.013	27.0 ± 6.2*	1.69 ± 0.65*	0.624 ± 0.207*	1.74 ± 0.13*
p, c vs. exp	<0.001	<0.001	<0.001	<0.001	<0.005	N.S.	N.S.	N.S.	<0.01
Group 3									
Control (n = 6)	121 ± 1	5.9 ± 0.3	2.75 ± 0.14	11.1 ± 0.6	0.251 ± 0.017	17.9 ± 2.6	1.47 ± 0.24	0.390 ± 0.067	2.11 ± 0.19
Exp (NAME + prazosin + losartan)	130 ± 5[a]	12.9 ± 1.0	1.96 ± 0.16	5.6 ± 0.3	0.356 ± 0.021	15.6 ± 1.6*	1.17 ± 0.22*	0.494 ± 0.129*	1.83 ± 0.14[a]
p, c vs. exp	N.S.	<0.001	<0.01	<0.001	<0.005	N.S.	N.S.	N.S.	N.S.
Group 4									
Control (n = 7)	117 ± 2	4.8 ± 0.5	3.58 ± 0.15	13.6 ± 1.07	0.271 ± 0.020	14.5 ± 1.4	1.56 ± 0.32	0.308 ± 0.067	3.32 ± 0.36
Exp (prazosin + losartan)	88 ± 2	3.4 ± 0.3	3.07 ± 0.08	14.9 ± 0.9	0.211 ± 0.017	8.3 ± 0.4	1.32 ± 0.23	0.297 ± 0.049	2.46 ± 0.13
p, c vs. exp	<0.001	<0.001	<0.005	<0.05	<0.001	<0.01	N.S.	N.S.	<0.05
Group 5									
Control (n = 7)	114 ± 1	4.4 ± 0.3	3.36 ± 0.22	14.5 ± 1.0	0.234 ± 0.008	14.7 ± 1.6	1.71 ± 0.23	0.369 ± 0.062	3.35 ± 0.34
Exp (prazosin + losartan + NAME delayed)	131 ± 2	8.3 ± 0.8	2.74 ± 0.15	9.4 ± 1.1	0.302 ± 0.014	28.8 ± 6.7	3.55 ± 0.77	0.930 ± 0.223	2.73 ± 0.15
p, c vs. exp	<0.005	<0.001	<0.01	<0.005	<0.025	<0.05	N.S.	<0.05	N.S.

Values are mean ± S.E. p values are paired differences within each group. * Denotes a significant difference ($p < 0.05$) in experimental period of group 1 vs. group 2 or group 1 vs. group 3. [a] Denotes a difference in experimental period between groups 2 and 3. FE_{Na}, fractional excretion of sodium.

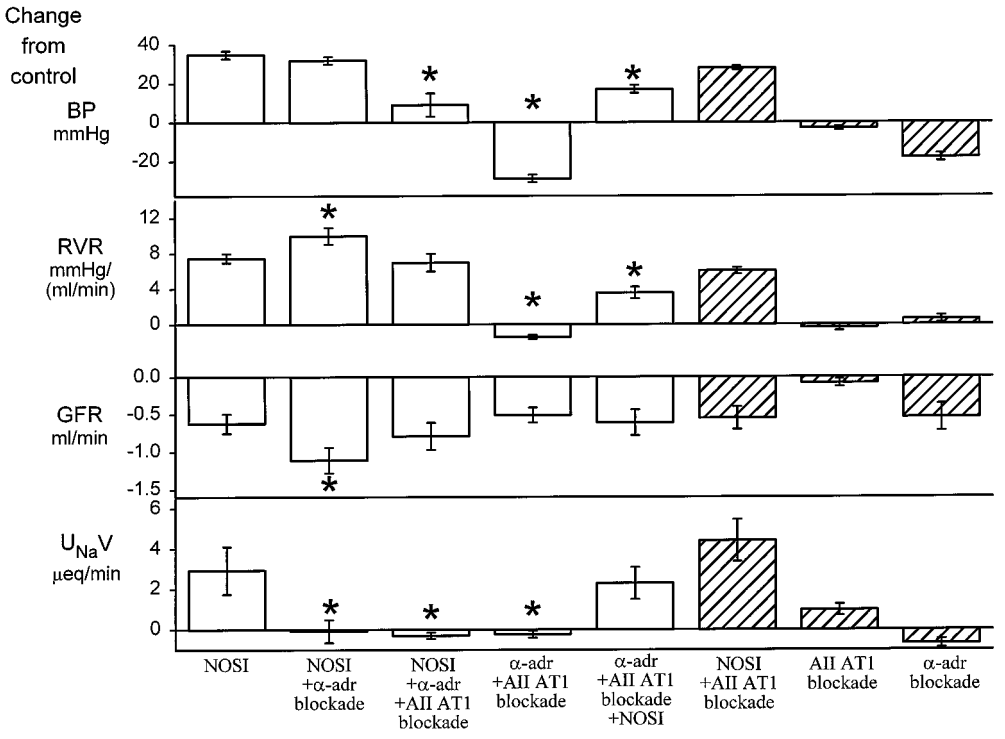

Fig. 1. Change in mean arterial BP, RVR, GFR, and $U_{Na}V$, in response to several maneuvers versus the control, baseline values. First five vertical panels summarize data obtained from present studies; group 1 rats received acute NOSI alone (L-NAME, 10 mg/kg i.v.). Group 2 rats received NOSI + concomitant *alpha* adrenoceptor blockade (prazosin, 0.1 mg/kg i.v.). Group 3 rats received combined NOSI and simultaneous *alpha* adrenoceptor and ANGII AT_1 receptor blockade (losartan, 3 mg/kg i.v.). Group 4 received simultaneous *alpha* adrenoceptor and ANGII AT_1 receptor blockade. Group 5 received combined *alpha* adrenoceptor and ANGII AT_1 receptor blockade followed by NOSI after a 25-min delay. Hatched columns (6–8) summarize data published previously by us, showing response to NOSI + ANGII AT_1 blockade with losartan, response to ANGII AT_1 blockade alone (Baylis et al., 1993), and response to *alpha* adrenoceptor blockade alone (Baylis 1995). *Indicates a difference in response compared with group 1(NOSI alone).

al., 1993) on the baseline characteristics of the conscious chronically catheterized rat (Baylis et al., 1993). Although ANGII AT_1 inhibition alone had no impact on BP or renal hemodynamics, it did elicit a small rise in $U_{Na}V$ (Fig. 1).

In the final group (group 5), rats received an identical dosage of losartan, prazosin, and L-NAME as that given to group 3 rats but the sequence of administration was different. In group 5 the combined vasoconstrictor blockers were given first, BP was allowed to fall and remain low for approximately 25 min and then i.v. L-NAME was given. In these rats a small rise in BP occurred with NOSI compared with the control value, and this was blunted relative to rats given NOSI alone (+16 ± 1 versus +35± 2% $p < .001$, group 5 versus group 1; Table 1). Of note, however, is that the combination of losartan and prazosin lowered BP versus the control value (to 87± 3 mm Hg at ~25 min after coadministration of the drugs), and that the absolute magnitude of the subsequent acute rise in BP with NOSI in group 5 rats was actually equal or higher than that seen with NOSI alone (Fig. 2), a fact that was obscured by comparison with the control period data. The rise in RVR (versus control) was also attenuated compared with group 1 rats given NOSI alone ($p < .001$), which probably reflects the offsetting decline in RVR seen with losartan and prazosin alone (group 4). In contrast, the falls in GFR and RPF were similar in groups 1 and 5 (Fig. 1 and Table 1), because the increase in FF was also attenuated in group 5 ($p < .05$). An increase in FE_{Na} persisted after NOSI in group 5 rats, although the natriuretic response was not statistically significant ($p = 0.06$).

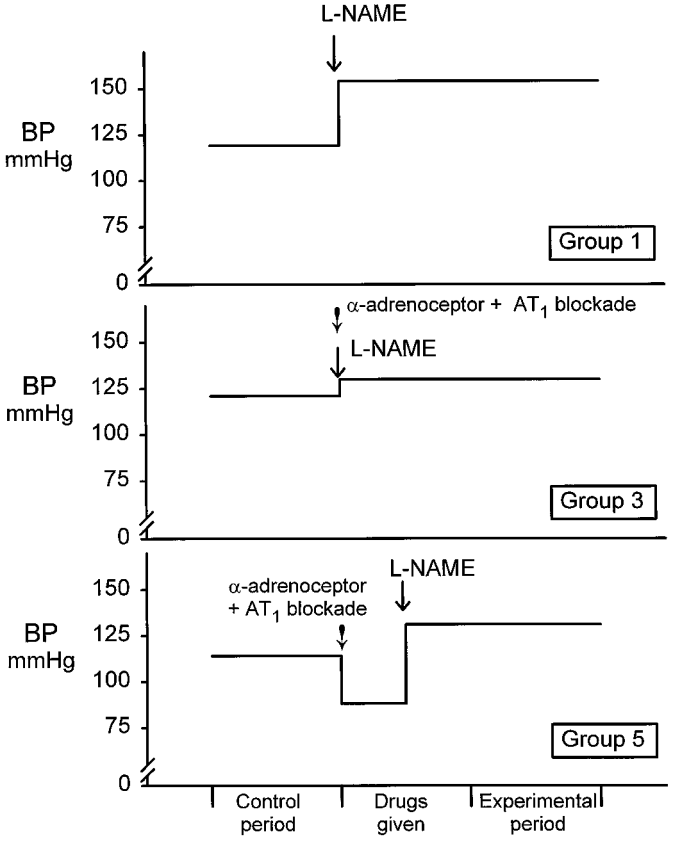

Fig. 2. Time course of mean BP during control, drug administration, and experimental observation period in groups 1, 3, and 5.

Discussion

The present studies confirm many earlier observations that acute systemic, nonselective NOSI, with compounds such as L-NAME, increases BP and RVR via widespread vasoconstriction. The NO that controls vascular tone is generated from the NOS in vascular endothelium and also from the widely distributed neuronal NOS (nNOS) (Moncada et al., 1991; Raij and Baylis, 1995). Observations in "knockout" mice suggest that NO originating from the NOS in vascular

endothelium plays the primary role in control of vascular tone (Huang et al., 1995), because nNOS-deficient mice are normotensive (Huang et al., 1994b). In addition, administration of the reportedly nNOS-selective inhibitor, 7-nitroindazole, may not influence BP when given systemically (Moore et al., 1993; Beierwaltes,1997), although intrarenal nNOS inhibition substantially increases BP (Mattson and Bellehumeur, 1996). However, a large amount of functional evidence suggests that NO generated from neuronal NOS is important in regulation of systemic and renal vascular resistance via modulation of the SNS. For example, NO generated from nNOS within the central nervous system plays an important inhibitory role on central sympathetic outflow, by a cyclic GMP-dependent action (Cabrera and Bohr, 1995). Local inhibition of NO production in both nucleus tractus solitarius and the rostral ventrolateral medulla causes dose-dependent increases in BP and a generalized rise in sympathetic activity, including the renal efferents (Harada et al., 1993; Tseng et al., 1996). When a nonselective NOS inhibitor such as L-NAME is given systemically, the drug rapidly crosses the blood-brain barrier and produces central inhibition of nNOS (Traystman et al., 1995). Systemic NOSI increases renal sympathetic nerve activity, an effect reversible with L-arginine and preventable by spinal transection (Sakuma et al., 1992; Kumagai et al., 1994). Thus, substantial evidence suggests that the pressor response to systemic NOSI is partly due to a centrally mediated increase in sympathetic tone (Sakuma et al., 1992). In addition to an interaction between NO and central control of the SNS, peripheral nitroxidergic nerves may function as vasodilatory counterbalances to adrenergic vasoconstrictor nerves in a number of locations, including the renal arteries (Okamura et al., 1995).

In the present study we used the conscious, chronically catheterized rat preparation, in which the basal activity of the SNS is low. Nevertheless, as shown by us previously, acute systemic blockade of the *alpha* adrenoceptors with prazosin in this normal preparation leads to falls in BP of approximately 15% (Qiu et al., 1994; Baylis, 1995), demonstrating the expected BP dependence on the SNS. As we report here, acute systemic *alpha* adrenoceptor blockade with prazosin coincident with L-NAME has no impact on the pressor response to acute NOSI, suggesting that the acute hypertensive effect of NOSI is independent of the SNS. The increase in RVR is slightly potentiated by prazosin, probably because of additive renal vasoconstrictor effects of NOSI alone (present study) and prazosin alone (Baylis, 1995). This finding agrees with several other reports in which SNS inhibition with adrenergic receptor antagonists or ganglion blockers does little to attenuate the pressor response to acute systemic NOSI (Pegoraro et al., 1992; Pucci et al., 1992; Huang et al., 1994a). However, others found that the SNS plays a major role in the pressor response to NOSI (Lacolley et al., 1991) and that NOSI potentiates the vasoconstrictor responses to stimulation of the SNS (Tabrizchi and Triggle, 1991; Conrad and Whittemore, 1992). Some of this variability is probably attributable to differences in the duration of NOSI. Inhibition of the SNS has no impact on the pressor response to acute NOSI (at 45 min), whereas at 8 h and 6 days of NOSI, inhibition of the SNS attenuates the high BP (Sander et al., 1997).

There is also the potential for interactions between the SNS and ANGII in mediating some of the vasoconstrictor actions of NOSI. We showed previously that the hypertension associated with chronic NOSI was attenuated by combined *alpha* adrenoceptor and ANGII blockade (Qiu et al., 1994). In the present study, combined simultaneous systemic inhibition of *alpha*-1 adrenoceptors and ANGII AT_1 receptors ablated the pressor response to acute NOSI without altering the rise in RVR (group 3). Of course, combined systemic *alpha*-1 adrenoceptor and ANGII AT_1 receptor blockade alone lead to a substantial fall in BP, as shown in group 4, and this reduction may have been sufficient to offset the tendency of NOSI to raise BP, leading to no net change. To resolve this question, we gave the same drugs but in a different time sequence in group 5, so that BP initially fell with combined systemic *alpha*-1 adrenoceptor and ANGII AT_1 receptor antagonism. In that instance, when NOSI was superimposed, BP rose by the same acute increment as with NOSI alone (Fig. 2, compare groups 1 and 5), suggesting that there is no contribution of the SNS or ANGII to the acute pressor response to NOSI in the conscious unstressed preparation. Previous work from our laboratory suggests that amplification of endogenous endothelin activity makes a contribution (~50%) to the acute hypertensive response to NOSI in the conscious rat (Qiu et al., 1995). Given the present findings, we anticipate that the balance of the pressor response to acute NOSI in the conscious rat results directly from removal of NO-dependent vasodilation, rather than amplification of vasoconstrictors.

The present observations suggest that the SNS makes only minor contributions to the renal vasoconstrictor response of acute systemic NOSI. There was slight attenuation of the rise in RVR in group 5, delayed NOSI rats for reasons that are unclear. However, simultaneous *alpha* adrenoceptor inhibition, alone or in combination with ANGII AT_1 receptor blockade, has no attenuating effect on the increased RVR. This is consistent with our recent report that renal nerve activity does not contribute to the renal vasoconstriction, because chronic bilateral renal denervation had no impact on the response to acute NOSI in the conscious chronically catheterized rat (Baylis et al., 1997). The observations are those predicted in a normal conscious, unstressed animal in whom renal nerve activity is low and not controlling renal vascular tone under basal conditions. Several other workers agree that acute SNS inhibition does little to attenuate renal vasoconstrictor responses to acute systemic NOSI (Pegoraro et al. 1992; Pucci et al., 1992; Huang et al., 1994b). In contrast, others report that renal nerve activity, interacting with ANGII, mediates some of the renal actions of acute NOSI (Vallon et al., 1995). It is certainly likely that when renal sympathetic nerve activity is increased, secondary to some form of stress, withdrawal of vasodilatory NO should potentiate the renal efferent sympathetic nerve activity induced renal vasoconstriction. This may explain the study in urethane anesthetized rats, suggesting that the SNS plays a major role in the renal vasoconstrictor response to acute systemic NOSI (Lacolley et al., 1991). Therefore, in the conscious unstressed rat the increased RVR with acute NOSI is not due to the SNS or ANGII. Endogenous endothelin apparently makes a minor contribution, largely secondary to the pressor effect (Qiu et al., 1995). In fact, the rise in BP with acute systemic NOSI is responsible for much of the increased RVR, because intrarenal NOSI in the anesthetized rat (Deng and Baylis, 1993) and low-dose NOSI in the conscious rat

(Baylis et al., 1996) without a rise in BP, produce relatively small increases in RVR as a result of inhibition of local, tonically produced NO.

Acute systemic NOSI leads to significant increases in urine flow and sodium excretion. Due to the abrupt concomitant rise in BP, we had previously suggested that this might be due to a pressure natriuresis (Baylis et al., 1990). However, the present observations suggest that this may be incorrect, because combined, simultaneous NOSI + *alpha* adrenoceptor inhibition in group 2 rats largely prevented the natriuresis while having no impact on the increase in BP. These findings agree with our other recent studies in the conscious rat preparation where chronic renal denervation prevents the diuretic/natriuretic responses to NOSI without inhibiting the abrupt rise in BP (Baylis et al., 1997). Taken together, these observations suggest that the increased sodium excretion and urine flow seen with acute systemic NOSI is not exclusively the result of a pressure natriuresis but is in some way dependent on the SNS/renal nerve activity. Similarly, in the present work we found that the natriuresis/diuresis associated with acute systemic NOSI is also prevented with combined, simultaneous *alpha* adrenoceptor inhibition + ANGII AT_1 blockade, where the pressor response to NOSI is also abolished (group 3). In previous studies we found that the natriuresis with NOSI persisted during concomitant ANGII AT_1 receptor blockade, suggesting that the ANGII system was not involved (Baylis et al., 1993). Vallon and colleagues (Vallon et al., 1995) suggested that *alpha*-2 adrenoceptors and ANG II interact to cause this natriuretic response, although because prazosin can also block *alpha*-2B adrenoceptors (Bylund and Ray-Prenger, 1989) and concomitant ANGII and *alpha* adrenoceptor completely inhibit the natriuretic response to NOSI in the conscious rat, it is unlikely that this mechanism is operating here. The findings summarized above suggest that the SNS is heavily implicated in the increase in sodium excretion following acute systemic NOSI.

In summary, these experiments suggest that the SNS and ANGII, neither alone nor in combination, play a substantial role in the pressor or renal hemodynamic responses to acute NOSI in the conscious, unstressed rat. The SNS is apparently involved in mediation of the natriuretic response to acute NOSI, although the mechanism of the increased sodium excretion with acute NOSI remains obscure.

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