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# Evidence from its cardiovascular effects that 7-nitroindazole may inhibit endothelial nitric oxide synthase in vivo

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**Abstract**

We have examined whether the cardiovascular effects of 7-nitroindazole, a reportedly selective inhibitor of neuronal nitric oxide (NO) synthase, are induced without inhibition of endothelial NO synthase. A significant increase in mean arterial blood pressure but no change in heart rate was observed after 7-nitroindazole administration (50 mg/kg i.p.) in rats anesthetized with urethane or urethane and chloralose, while both an elevation in mean arterial blood pressure and bradycardia were observed in conscious animals after 7-nitroindazole administration (50 mg/kg i.p.). No enhancements in these effects on mean arterial blood pressure and heart rate were observed in urethane-chloralose anesthetized rats treated with a higher dose of 7-nitroindazole (75 mg/kg i.p.). Use of halothane to induce anesthesia abolished the pressor effect of 7-nitroindazole in rats studied under urethane anesthesia. 7-Nitroindazole shortened the duration of the acetylcholine (3 μg or 30 μg i.v.) but not the sodium nitroprusside (2 μg i.v.) induced hypotension in urethane-anesthetized rats. Pretreatment with L-arginine (300 mg/kg i.v.) inhibited the effects of 7-nitroindazole on mean arterial blood pressure and acetylcholine induced hypotension, suggesting involvement of the L-arginine-NO pathway in the effects of 7-nitroindazole. The effects of 7-nitroindazole on blood pressure and on the depressor responses to acetylcholine and sodium nitroprusside are similar to the effects previously observed after non-selective NO synthase inhibition by L-arginine analogs. Our results suggest, therefore, that 7-nitroindazole affects basal endothelial NO formation in vivo. The suppressive action of halothane on the cardiovascular effects of 7-nitroindazole suggests that the influence of anesthetics should be taken into consideration in studies of the cardiovascular effects of NO synthase inhibitors, particularly 7-nitroindazole.

*Keywords:* Nitric oxide (NO) synthase; Arterial pressure, systemic; Acetylcholine; Sodium nitroprusside; 7-Nitroindazole

## 1. Introduction

Blood vessel tone is regulated by nitric oxide (NO) production via two constitutively expressed isoforms of NO synthase: neural and endothelial (Förstermann et al., 1993; Dinerman et al., 1993). The endothelially produced NO has accounted for the action of endothelium-derived relaxing factor (EDRF), and its vasodilatory function is well documented (Moncada et al., 1991; Calver et al., 1993). Both basal and pharmacologically stimulated (e.g. by vasodilators such as acetylcholine) endothelial NO synthesis appears to regulate blood vessel tone (Calver et al., 1993). The relative contribution of neurally derived nitric oxide to the regulation of resting vascular tone, however, is uncertain, since the $N^g$-substituted arginine analogs commonly used to reveal the role of NO in cardiovascular system inhibit both endothelial and neuronal NO synthase. Recently, an atypical competitive NO synthase inhibitor, 7-nitroindazole has been reported (Moore et al., 1993). Unlike $N^g$-nitro-L-arginine methyl ester (L-NAME) and other $N^g$-substituted arginine analogs, administration of 7-nitroindazole has been reported not to cause a hypertensive effect. Further, 7-nitroindazole administration has been reported not to alter the magnitude of the depressor effect of intravenous acetylcholine (Moore et al., 1993). Based

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on these results, it has been suggested that 7-nitroindazole does not influence endothelial NO synthase in vivo and could be a selective inhibitor of neuronal NO synthase, although in vitro endothelial NO synthase is inhibited by 7-nitroindazole (Babbedge et al., 1993). The proposed selectivity of 7-nitroindazole for neuronal NO synthase in vivo has prompted the testing of this drug for potential treatment of experimental ischemic insults (Yoshida et al., 1994; Grome et al., 1995; Kamii et al., 1995; Globus et al., 1995; Sancesario, unpublished observation) since neuronal NO production has been proposed to contribute to the development of ischemic brain injury (Huang et al., 1994). The results of such studies have been, however, discordant. A beneficial effect of 7-nitroindazole on the neuropathological outcome after ischemia has been found in some studies (Yoshida et al., 1994; Grome et al., 1995), but not in others (Kamii et al., 1995; Globus et al., 1995; Sancesario, unpublished observation). Further, increasing the dose of 7-nitroindazole was found to result in the loss of an ameliorative effect (Grome et al., 1995). In addition, the drug was not totally devoid of cardiovascular effects in such studies, although 7-nitroindazole was not reported to increase blood pressure significantly (Kelly et al., 1995). In contrast, we found a slight, but significant, increase in mean arterial blood pressure after 7-nitroindazole administration in rats (Zagvazdin et al., 1994). To further elucidate the specificity of 7-nitroindazole, we examined the depressor responses following intravenous infusion of acetylcholine (an endothelial NO dependent vasodilator) or sodium nitroprusside (an endothelial NO independent vasodilator) before and after 7-nitroindazole administration. We also examined whether pretreatment with L-arginine, the NO synthase substrate, reversed the pressor effect of 7-nitroindazole. Finally, in an attempt to find the reason for the absence of significant elevation in blood pressure after 7-nitroindazole in previous studies, we investigated whether the pressor effect of 7-nitroindazole was affected by the type of anesthetic used, in particular by halothane, since hypertension following non-selective NO synthase inhibition has been shown to be attenuated by halothane anesthesia (Wang et al., 1991, 1993a; Sigmon et al., 1995).

## 2. Materials and methods

### 2.1. Experimental design and groups

Nine groups of male Sprague-Dawley rats (280–420 g) were studied. These groups differed in the anesthetics used during the arterial blood pressure and heart rate recording sessions and/or in the drug pretreatment. The groups are distinguished in these respects in Table 1. Rats in all nine groups were used for mean arterial blood pressure and heart rate monitoring to determine the cardiovascular effects of 7-nitroindazole under different anesthesia or under no anesthesia. A dose of 50 mg/kg of 7-nitroindazole in 2–3 ml of peanut oil or vehicle (an equal volume of peanut oil) was administered intraperitoneally (i.p.) in all rats, except for the animals in the fifth group, in which rats received 75 mg/kg of 7-nitroindazole i.p. Although previous studies have shown that maximal NO synthase inhibition is achieved with a dose of 50 mg/kg of 7-nitroindazole (Moore et al., 1993; Yoshida et al., 1994), a dose of 75 mg/kg of 7-nitroindazole was used in this one anesthetized group (group 5) to determine whether a maximal effect was achieved in our experiments with a 50 mg/kg dose of 7-nitroindazole. Intravenous L-arginine infusion before 7-nitroindazole administration in group 9 was used to verify the involvement of the L-arginine-NO pathway in the pressor effects of 7-nitroindazole. The eighth and ninth groups were additionally used to study the effects of 7-nitroindazole on the hypotensive responses induced by intravenous bolus injection of an endothelial NO dependent (i.e. acetylcholine) or an endothelial NO independent (i.e. sodium nitroprusside) vasodilatory agent. The responses to such bolus injections were tested before and after vehicle and then after 7-nitroindazole (group 8) or before and after 7-nitroindazole plus L-arginine administration (group 9). Animals in groups 1 and 2 were not anesthetized during the experimental session, and were catheterized for arterial blood pressure and heart rate measurements on the day prior to such measurements. In the remaining seven groups, the rats were anesthetized, surgically prepared for cardiovascular measurements, and then these measurements were made while the rats were still anesthetized. In group 3 (Table 1), rats were initially rendered unconscious by 60 s of halothane inhalation, followed by injection of urethane (1800 mg/kg i.p.) to maintain anesthesia. Rats in the remaining groups did not receive halothane. Rats in groups 4, 5 and 6 were anesthetized with urethane (1200 mg/kg i.p.) and supplemented with $\alpha$-chloralose (60–75 mg/kg i.p.) and urethane (up to 600 mg/kg i.p.) if necessary to reach and maintain a satisfactory level of anesthesia (no response to tail pinching) before and following surgery. Rats in groups 7, 8 and 9 were anesthetized with urethane alone (2000 mg/kg i.p.).

### 2.2. Surgical preparation

In the first two groups, rats were anesthetized with ether one day before the experimental session and the femoral artery was catheterized with polyethylene tubing (PE 50) for blood pressure measurement. Another polyethylene tube was implanted in the abdominal cavity for intraperitoneal 7-nitroindazole delivery. The catheters were externalized subcutaneously, secured at the back of the neck, filled with heparinized solution and sealed. To ensure normal fluid balance after surgery, the rats were given access to 20 ml of 5% dextrose solution in addition to the regular water. On the next day, these rats were placed in opaque plastic chambers. The chambers provided sufficient

space for forward, backward, and rotational movements. The arterial catheter was connected to a pressure transducer (Statham Oxnard, CA, USA) and MacLab bridge amplifier 60 min before the start of the recording session. Rats in the remaining seven groups were anesthetized as described above, intubated and mechanically ventilated with air and supplemental oxygen during the experimental recording session. The stroke rate and volume of the respirator were adjusted to maintain arterial blood gases within the physiological range ($P_aO_2$ above 110 mm Hg, $P_aCO_2$ between 34–42 mm Hg, pH between 7.36–7.43). In all rats in the anesthetized groups, a polyethylene cannula was inserted into the femoral artery for continuous arterial blood pressure and heart rate monitoring using a blood pressure analyzer BPA-100 (Micro-Med, Louisville, KY, USA) and for intermittent blood sampling of blood gases for assay (Corning blood gas/pH analyzer, Ciba Corning Diag., Medford, MA, USA). A polyethylene cannula was also inserted into the femoral vein in rats in groups 8 and 9 for L-arginine, acetylcholine and sodium nitroprusside administration. Rectal temperature was maintained at 37°C in rats in the anesthetized groups with a homeothermic heating blanket automatically controlled by a heating unit with a rectal temperature probe. A MacLab data acquisition system (AD Instruments, Milford, MA, USA) was used to collect and store data.

### 2.3. Measurement of mean arterial blood pressure and heart rate

In conscious animals, the experimental recording session started at least 60 min after placing a rat into the experimental chamber in order to provide time for environmental accustomization. In anesthetized rats, the arterial blood pressure and heart rate recordings began upon completion of the surgical preparation of the animal. Arterial blood pressure and heart rate were monitored before and during the 50–60 min after drug or vehicle administration in all rats. For analysis, mean arterial blood pressure and heart rate for the 10 min period before 7-nitroindazole or vehicle injection, for the 10 min period commencing 10 min after such injection and for the 10 min period beginning 50 min after such injection were used.

### 2.4. Measurement of acetylcholine and sodium nitroprusside induced depressor responses

After completing the animal preparation in the eighth and ninth groups of rats, two to three depressor responses following intravenous bolus infusions for each of the two doses of acetylcholine (3 μg or 30 μg) were recorded over a 25–30 min period prior to vehicle or drug administration. After flushing the catheter with physiological saline, two to three depressor responses to sodium nitroprusside (2 μg i.v.) were then recorded, still prior to vehicle or drug administration. The recording of pre-drug hypotensive responses took approximately 50 min. In group 8, vehicle was then administered and 10 min later bolus infusions of hypotensive agents were repeated in the same order as described above. The rats then received 7-nitroindazole (50 mg/kg i.p.) and 10 min later infusions of hypotensive agents were again repeated. Each subsequent infusion of hypotensive agent was conducted following restoration of baseline blood pressure. The peak magnitude of the mean arterial blood pressure decrease and the time that was necessary for restoration of arterial blood pressure from the peak response to the preexisting baseline value (duration of the hypotensive response) after bolus infusion of acetylcholine (each dose separately) or sodium nitroprusside were determined in each rat for the period before vehicle injection, for the period after vehicle injection, for the period 10–25 min after 7-nitroindazole injection (only

Table 1
Effect of 7-nitroindazole on mean arterial blood pressure and heart rate (beats/min) in rats

| Group | Treatment | Anesthesia | n | Blood pressure | | | Heart rate | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Before | After | | Before | After | |
| | | | | 10–0 | 10–20 | 50–60 | 10–0 | 10–20 | 50–60 |
| 1. | 7NI (50 mg/kg) | None | 5 | 113 ± 2 [b] | 130 ± 2 [a] | 126 ± 1 [a] | 344 ± 11 [b] | 302 ± 15 [a] | 253 ± 19 [a] |
| 2. | Vehicle | None | 5 | 114 ± 3 [b] | 117 ± 3 | 115 ± 3 | 360 ± 10 [b] | 371 ± 6 | 377 ± 7 |
| 3. | 7NI (50 mg/kg) | Halothane + urethane | 5 | 98 ± 5 | 102 ± 6 | 100 ± 5 | 320 ± 7 | 318 ± 11 | 315 ± 23 |
| 4. | 7NI (50 mg/kg) | Urethane + chloralose | 8 | 91 ± 6 | 106 ± 5 [a] | 105 ± 5 [a] | 331 ± 12 [c] | 334 ± 12 | 330 ± 16 |
| 5. | 7NI (75 mg/kg) | Urethane + chloralose | 8 | 88 ± 3 | 103 ± 3 [a] | 98 ± 6 [a] | 351 ± 13 [c] | 341 ± 16 | 339 ± 24 |
| 6. | Vehicle | Urethane + chloralose | 6 | 89 ± 6 | 88 ± 5 | 84 ± 3 | 313 ± 14 | 315 ± 15 | 306 ± 16 |
| 7. | 7NI (50 mg/kg) | Urethane | 5 | 90 ± 4 | 109 ± 3 [a] | 118 ± 6 [a] | 311 ± 16 | 297 ± 25 | 292 ± 8 |
| 8A. | Vehicle | Urethane | 5 | 88 ± 4 | 87 ± 5 | 87 ± 4 | 311 ± 12 | 315 ± 12 | 318 ± 10 |
| 8B. | 7NI (50 mg/kg) | Urethane | 5 | 87 ± 5 | 113 ± 3 [a] | 108 ± 4 [a] | 312 ± 11 | 329 ± 20 | 325 ± 13 |
| 9. | L-Arginine + 7NI (50 mg/kg) | Urethane | 5 | 85 ± 4 | 81 ± 5 | 98 ± 8 [a] | 308 ± 14 | 304 ± 18 | 324 ± 22 |

Time periods (in minutes) during which data were recorded before and after drug or vehicle treatment are indicated for both cardiovascular measures. The subgroups 8A and 8B show the effect of successive injection of vehicle and 7-nitroindazole in the same (eighth) group of rats. [a] Indicates a significant difference within group from pre-drug or vehicle baseline values. [b] Indicates a significant difference in mean arterial blood pressure or heart rate between unanesthetized and anesthetized animals before treatment, except for the groups marked with [c] which did not show such a difference. Halothane (group 3) was used only for induction of anesthesia. Values shown are the group means ± S.E.M.

acetylcholine induced responses were measured during this period), and for the period 25–50 min after 7-nitroindazole injection. In the ninth group of rats, after completing the measurement of pre-drug depressor responses to intravenous bolus infusions of acetylcholine (both doses) and sodium nitroprusside as described above for group 8, L-arginine (300 mg/kg) was administered intravenously by bolus infusion and 5 min later 7-nitroindazole (50 mg/kg i.p.) was injected. Commencing 10 min after 7-nitroindazole injection, administrations of acetylcholine and sodium nitroprusside were repeated. The peak magnitude of the arterial blood pressure decrease and the duration of the hypotensive response following infusion of each dose of acetylcholine or sodium nitroprusside were determined for each rat in group 9 for the period before L-arginine and 7-nitroindazole administration, for the period 10–25 min after 7-nitroindazole injection, and for the period 25–50 min after 7-nitroindazole injection. The time period after L-arginine and 7-nitroindazole treatment was divided in two periods because the characteristics of the acetylcholine induced hypotensive responses were obviously different between the 10–25 and 30–60 min periods after L-arginine and 7-nitroindazole injection in group 9. The period after 7-nitroindazole injection in group 8 was similarly subdivided for comparison to the two corresponding time periods in group 9.

### 2.5. Drugs

Acetylcholine chloride, sodium nitroprusside, peanut oil, urethane, α-chloralose and L-arginine hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 7-Nitroindazole was purchased from Lancaster Synthesis (Windham, NH, USA). Halothane was manufactured by Halocarbon Laboratories (River Edge, NJ, USA). Acetylcholine, sodium nitroprusside, urethane, α-chloralose and L-arginine were dissolved in 0.9% saline. The volume of i.v. drugs did not exceed 0.2 ml per infusion. 7-Nitroindazole was dissolved in 2–3 ml of peanut oil using an ultrasonic water bath heated to 70°C during 1 h.

### 2.6. Statistical analysis

Data are expressed as means ± standard error of the means. A repeated measures two way analysis of variances with preplanned comparisons was used to test statistical significance of the difference among means. A $P < 0.05$ was considered significant.

## 3. Results

### 3.1. Effect of 7-nitroindazole on mean arterial blood pressure and heart rate

There were no significant differences in mean arterial blood pressure among the seven groups of anesthetized rats before drug or vehicle treatments (except that blood pressure in group 3 was slightly higher than in group 9). In contrast, mean arterial blood pressure in the two groups of unanesthetized rats before treatment was significantly higher ($P < 0.05$) than blood pressure in all groups of

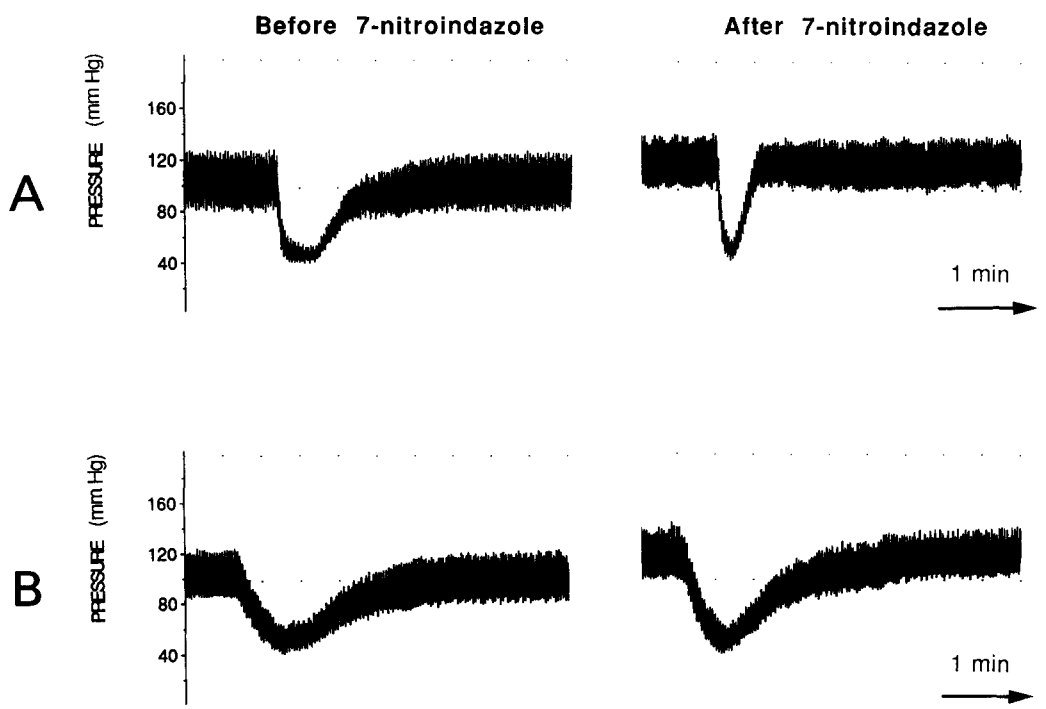


Fig. 1. (A) Representative traces showing the typical depressor responses to i.v. acetylcholine (30 μg) bolus infusion before and after 7-nitroindazole (50 mg/kg) administration. (B) Representative traces showing the typical depressor responses to i.v. sodium nitroprusside (2 μg) bolus infusion before and after 7-nitroindazole (50 mg/kg) administration.

anesthetized rats. Heart rate was also higher in the unanesthetized groups than heart rate in the anesthetized groups (with the exception of groups 4 and 5 under urethane-chloralose anesthesia). Mean arterial blood pressure increased significantly from pre-drug baseline after administration of 50 mg/kg of 7-nitroindazole in all conscious and anesthetized rats treated with this dose (groups 1, 4, 7, 8B), except in the rats in which anesthesia was induced by halothane and maintained by urethane (group 3). In contrast, mean arterial blood pressure was unchanged following vehicle administration in the vehicle-treated groups (2, 6 and 8A). Administration of 75 mg/kg of 7-nitroindazole (group 5) also elicited an increase in arterial blood pressure, but this increase was not greater than the elevation in arterial blood pressure following administration of 50 mg/kg of 7-nitroindazole. Mean arterial blood pressure at 50–60 min after 7-nitroindazole was not significantly different from mean arterial blood pressure at 10–20 min after 7-nitroindazole administration. Bradycardia was observed in the conscious rats following 7-nitroindazole injection, but heart rate did not change significantly following administration of 7-nitroindazole or vehicle in any other group of rats. These data are summarized in Table 1.

### 3.2. *Effect of 7-nitroindazole on the depressor responses induced by acetylcholine or sodium nitroprusside bolus infusion*

Acetylcholine (both doses) and sodium nitroprusside infusions transiently reduced blood pressure with a maximal effect in the first minute, while no changes in blood pressure were observed after bolus infusion of an equal volume of saline. Before 7-nitroindazole injection, the duration of the hypotensive response induced by the higher dose of acetylcholine was longer than the duration of the hypotensive response induced by the lower dose of acetylcholine. The durations of the hypotensive responses induced by sodium nitroprusside and by the higher dose of acetylcholine were not significantly different before 7-nitroindazole. The durations of the acetylcholine (both doses) and sodium nitroprusside induced hypotensive responses were unchanged after vehicle (oil) administration. The durations of the acetylcholine induced hypotensive responses (both doses) were shortened after 7-nitroindazole injection (Figs. 1 and 2). The reduction in duration of the acetylcholine-induced responses was significant during the period of 10–25 min and 25–50 min after 7-nitroindazole administration. In contrast, the duration of the sodium nitroprusside induced hypotensive response was not changed after 7-nitroindazole injection. The magnitude of the blood pressure drop following infusion of acetylcholine or sodium nitroprusside was unchanged after vehicle administration. In contrast, the magnitude of the blood pressure drop following infusion of both doses of acetylcholine and sodium nitroprusside was increased after 7-nitroindazole administration. Data are summarized in Fig. 2.

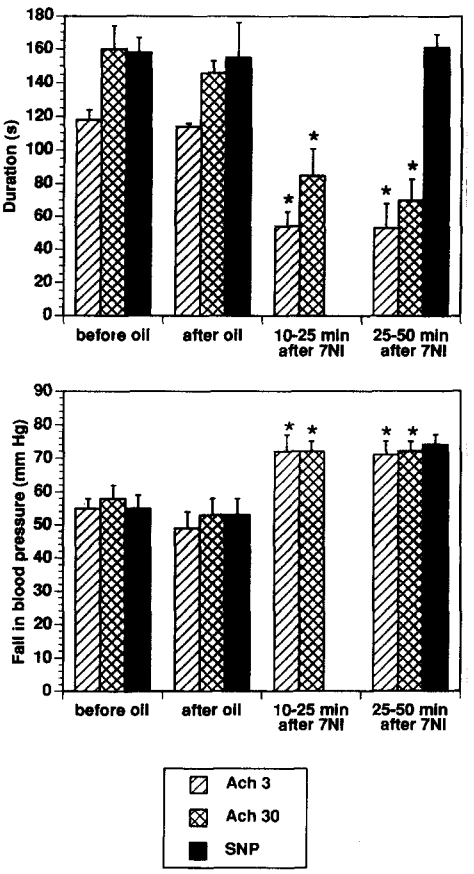


Fig. 2. Effect of vehicle (oil) or 7-nitroindazole injection on the hypotensive responses to i.v. bolus infusion of either of two doses of acetylcholine (Ach, 3 μg and 30 μg) or sodium nitroprusside (SNP, 2 μg) in urethane-anesthetized rats ($n = 5$). Hypotensive responses to sodium nitroprusside were not recorded within the 10–25 min interval after 7-nitroindazole administration due to time constraints. Vertical bars are S.E.M. * Significant difference from pre-7-nitroindazole values ($P < 0.05$).

### 3.3. *Effect of 7-nitroindazole on blood pressure and the depressor response induced by acetylcholine infusion after pretreatment with L-arginine*

Pretreatment with L-arginine transiently inhibited the pressor response and reduction in the duration of the acetylcholine induced hypotensive response to 7-nitroindazole administration in rats. No increase in baseline mean arterial blood pressure (see Table 1) was observed during the 10–20 min after 7-nitroindazole administration preceded by L-arginine pretreatment. Arterial blood pressure was, however, significantly higher 60 min after 7-nitroindazole plus L-arginine pretreatment. The duration of the delay in the appearance of the pressor effect of 7-nitroindazole varied from 25 min to 45 min among rats. The duration and the peak magnitude of the acetylcholine induced hypotensive response were unchanged from predrug duration and peak magnitude 10–20 min after 7-nitroindazole injection in L-arginine pretreated rats. However, the duration of the acetylcholine induced hypotensive

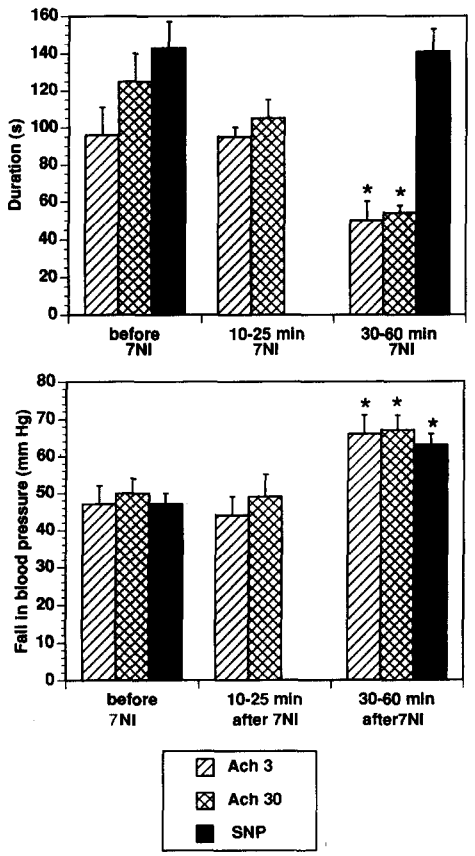


Fig. 3. Effect of 7-nitroindazole on the hypotensive response to i.v. bolus infusion of either of two doses of acetylcholine (Ach, 3 μg and 30 μg) or sodium nitroprusside (SNP, 2 μg) in urethane-anesthetized rats ($n = 5$) pretreated with i.v. L-arginine (300 mg/kg). Hypotensive responses to sodium nitroprusside were not recorded within the 10–25 min interval after 7-nitroindazole administration due to time constraints. Vertical bars are S.E.M. * Significant difference from pre-7-nitroindazole values ($P < 0.05$).

response was significantly reduced and the peak magnitude of the acetylcholine induced hypotensive response was increased 30–60 min after 7-nitroindazole administration in L-arginine pretreated rats. The duration of the depressor response induced by sodium nitroprusside was, however, unchanged, while its magnitude was increased following 7-nitroindazole administration in the L-arginine pretreated rats. Data are summarized in Table 1 and Fig. 3.

## 4. Discussion

### *4.1. Effect of 7-nitroindazole on blood pressure and heart rate*

In contrast to previous studies in rats, mice and cats (Moore et al., 1993; Yoshida et al., 1994; Kovach et al., 1994; Kelly et al., 1995), a slight but significant increase in mean arterial blood pressure was detected after intraperitoneal injection of 7-nitroindazole in both conscious and anesthetized animals, except in rats initially anesthetized with halothane. Bradycardia was observed in conscious rats after 7-nitroindazole administration, but no 7-nitroindazole mediated changes in heart rate were found in anesthetized animals, suggesting that the baroreceptor reflex was deactivated in anesthetized rats. The discrepancy between our data and previous studies in which a pressor effect of 7-nitroindazole was not observed may be related to the differences in experimental conditions, species or anesthesia. In the study conducted by Moore et al. (1993), rats were anesthetized with 10 g/kg of urethane, a much higher dose than the rats in our experiments. Their rats also had a much higher mean arterial blood pressure (155 mm Hg) before 7-nitroindazole administration than the rats in our study. It is possible that the pressor effect of 7-nitroindazole is not detectable if baseline blood pressure is as high as 155 mm Hg. Another notable difference is the artificial ventilation of rats in our experiments. We have found, however, that injection of 25 mg/kg of 7-nitroindazole causes a distinct pressor effect even in non-ventilated rats anesthetized with urethane (Zagvazdin, Sancesario, unpublished observation). We do not know whether differences in the route of 7-nitroindazole administration (i.p. versus i.v.) between our experiments and the experiments conducted by Moore et al. (1993) contributed to the different effect of 7-nitroindazole.

The use of halothane may be another reason for the lack of a pressor effect of 7-nitroindazole in some previous studies (Yoshida et al., 1994; Kelly et al., 1995; Montecot et al., 1995). Halothane is known to attenuate the hypertensive response following NO synthase inhibition by L-arginine analogs or diphenyleneiodonium, while chloralose and urethane do not (Wang et al., 1991; Wang and Pang, 1993; Sigmon et al., 1995). In our experiments, induction of anesthesia by halothane abolished the pressor response induced by 7-nitroindazole administration. In contrast, neither urethane nor α-chloralose anesthesia affected pressor response following NO synthase inhibition by 7-nitroindazole in our experiments. Our findings are consistent with recent reports from the University of Paris in which the pressor effect of 7-nitroindazole was detected in rats anesthetized only with chloralose, but not in rats anesthetized with halothane the day prior to blood pressure measurements (Cholet et al., 1995; Montecot et al., 1995). Although the mechanism by which halothane anesthesia impairs the pressor effects of NO synthase inhibitors may be complex, several studies indicate that halothane affects endothelial NO (Wang et al., 1993a; Blaise et al., 1994; Sigmon et al., 1995). Therefore, elimination of the pressor effect of 7-nitroindazole by halothane anesthesia may involve interference by halothane with basal endothelium-derived NO regulation of vascular tone. It appears that halothane has a prolonged inhibitory action of this kind, since in our study and in previous studies (Yoshida et al., 1994; Kelly et al., 1995) halothane was only used to induce anesthesia. In our study, 7-nitroindazole was in-

jected 30–40 min after induction of anesthesia by halothane. Other studies indicate that the inhibitory action of halothane on the pressor effect of 7-nitroindazole may last more than 2 h (Kelly et al., 1995; Montecot et al., 1995).

Difference in species may also explain the lack of a significant effect of 7-nitroindazole on arterial blood pressure observed in some studies. In some species, the pressor effect following administration of NO synthase inhibitors seems to be smaller than in rats. For example, we did not see a significant increase in blood pressure after intraperitoneal injection of 7-nitroindazole (50 mg/kg i.p.) in pigeons under ketamine anesthesia, while L-NAME (30 mg/kg i.v.) induced only a slight increase (17 mm Hg) in systemic blood pressure (Zagvazdin et al., 1996). No elevation in blood pressure was observed in cats under chloralose-urethane anesthesia after 50 mg/kg of 7-nitroindazole (Kovach et al., 1994). However, inhibition of NO synthase by L-NAME (30 mg/kg) in cats also resulted in no significant effect on blood pressure under either chloralose-urethane anesthesia (Sandor et al., 1994) or ketamine-pentobarbitone anesthesia (Van Gelderen et al., 1991). In contrast, 30 mg/kg of L-NAME or less caused a severe (40 mm Hg or more) hypertensive response in rats under urethane (Chyu et al., 1992; Zagvazdin, Sancesario and Reiner, unpublished observation) or barbiturate (Van Gelderen et al., 1991; Gardiner et al., 1990) anesthesia. Therefore, it is possible that the pressor effect of 7-nitroindazole was not observed in cats (Kovach et al., 1994) because of a relatively small vascular responsiveness to endothelial NO synthase inhibition or it is possible that a prominent increase in pulmonary vascular resistance, leading to reduction in cardiac output, may negate the expected rise in arterial blood pressure (Van Gelderen et al., 1993).

Overall, the effect of the so called selective neuronal NO synthase inhibitor 7-nitroindazole on arterial blood pressure appears to be similar to the effect of other so called non-selective NO synthase inhibitors, although the blood pressure elevation after administration of a maximally effective dose of 7-nitroindazole was typically less than 20 mm Hg (see Table 1). Hypertension following administration of L-arginine analogs are likely due largely to inhibition of basal NO synthesis within resistance vessels (Greenblatt et al., 1993; Calver et al., 1993; Huang et al., 1995). Thus, the difference in the pressor effect of 7-nitroindazole and L-arginine-based NO synthase inhibitors may indicate a smaller degree of inhibition of endothelial NO synthase by 7-nitroindazole. Inhibition of neural NO synthesis by 7-nitroindazole also may contribute to the pressor effect (for review see Umans and Levi, 1995), however, several studies did not find evidence for a substantial contribution of the nervous system to the hypertension following NO synthase inhibition (Chyu et al., 1992; Pegoraro et al., 1992; Huang et al., 1995). Although we can not exclude the possibility that the observed elevation in arterial blood pressure after 7-nitroindazole administration was caused by an unknown mechanism distinct from the inhibition of endogenous NO formation, the fact that L-arginine pretreatment inhibited (though temporarily) the pressor effect supports the probability that NO synthase inhibition was responsible. Thus, our findings suggest the possibility that 7-nitroindazole does to some extent inhibit formation of NO by endothelial NO synthase in vivo. At the very least, our findings do not support the absolute selectivity of 7-nitroindazole inhibition for neural NO synthase.

### *4.2. Effect of 7-nitroindazole on depressor responses induced by acetylcholine and sodium nitroprusside bolus infusions*

In our study, 7-nitroindazole produced a reduction in the duration of the acetylcholine induced hypotension, while the duration of the depressor response to bolus infusion of sodium nitroprusside was unchanged. The same effects have previously been observed in rats after administration of so called non-selective NO synthase inhibitors (Van Gelderen et al., 1991; Chyu et al., 1992; Wang et al., 1993b). According to Aisaka et al. (1989) intravenous administration of acetylcholine elicits an immediate vasodilation of resistance vessels by a mechanism that does not involve de novo synthesis of endothelial NO, whereas the succeeding longer-lasting vasodilation does depend upon endothelial NO. In our study, the magnitude of the immediate drop in blood pressure following acetylcholine infusion was not reduced after 7-nitroindazole administration, while the duration of the subsequent hypotension was reduced. It appears, that inhibition of endogenous NO synthesis by 7-nitroindazole administration interferes with the hypotensive effect of acetylcholine principally by shortening the duration of the depressor response. The reduction in the duration of the acetylcholine-induced hypotension brought about by 7-nitroindazole was inhibited by pretreatment with L-arginine, further suggesting that inhibition of endothelial NO synthesis was responsible for the shortening of the depressor response. The short-term nature of the antagonistic effect of L-arginine on the cardiovascular actions of 7-nitroindazole may be due to a difference in pharmacodynamics between L-arginine and 7-nitroindazole.

In a previous study in rats (Moore et al., 1993), the magnitude of the depressor effect of acetylcholine, a classical endothelium dependent vasodilator, was not reduced after administration of 7-nitroindazole. This fact was interpreted as additional evidence of an inability of 7-nitroindazole to inhibit endothelial NO synthase in vivo. In our experiments, the magnitude of the acetylcholine-induced drop in blood pressure was actually potentiated after 7-nitroindazole administration, suggesting that the magnitude of the acetylcholine-induced vasodilation was not inhibited by 7-nitroindazole. However, non-attenuation

or potentiation of the amplitude of the acetylcholine induced blood pressure fall has been reported in many studies on rats following inhibition of NO synthase by L-arginine analogs, and it has been proposed that the magnitude of the hypotension elicited by acetylcholine is not dependent on endothelial NO release (Gardiner et al., 1990; Van Gelderen et al., 1991; Chyu et al., 1992; O'Shaugnessey et al., 1992; Wang et al., 1993b; Nafrialdi et al., 1994). Therefore, the observation of no change or an increase in the magnitude of the acetylcholine-induced hypotensive response may not be interpreted as evidence for the inability of 7-nitroindazole to inhibit endothelial NO synthase. Further, the magnitude of the hypotensive response to sodium nitroprusside was also increased after 7-nitroindazole injection in our study. The same effect has been observed following administration of L-NAME (Gardiner et al., 1991; Chyu et al., 1992) and could be explained by development of a specific supersensitivity to nitrovasodilators due to removal of the basal NO tone (Moncada et al., 1991). The increase in baseline arterial blood pressure caused by 7-nitroindazole appears to be the basis of the greater magnitude of the hypotensive response to both vasodilators (i.e. acetylcholine and sodium nitroprusside) after 7-nitroindazole administration. Thus, the effects of the so called selective neuronal NO synthase inhibitor 7-nitroindazole on the hypotensive response following acetylcholine and sodium nitroprusside infusion appears to be similar to the effects of so called non-selective arginine-based NO synthase inhibitors, indicating again a possible action of 7-nitroindazole on endothelium-dependent NO formation.

### *4.3. Summary and conclusion*

Our results fail to provide evidence for a lack of inhibition of endothelial NO synthase by 7-nitroindazole in vivo. This point has important implications. It may provide an explanation for the inconsistencies in the beneficial effect of 7-nitroindazole on the neuropathological outcome following experimental ischemic insult. It has been suggested that inhibition of endothelial NO synthase has a deleterious effect on the neuropathological outcome after ischemia, while inhibition of neuronal NO synthase may ameliorate ischemic damage (Huang et al., 1994). In some studies of focal brain ischemia, administration of 25 mg/kg of 7-nitroindazole has been found to be neuroprotective (Yoshida et al., 1994; Grome et al., 1995). Increasing the dose to 50 mg/kg, however, did not have a neuroprotective effect (Grome et al., 1995). It is possible that with the higher dose of 7-nitroindazole, the harmful effect of vascular NO synthase inhibition emerges, while the putative beneficial effect of neuronal NO synthase inhibition may prevail following administration of 25 mg/kg of 7-nitroindazole. In some other studies treatment with 7-nitroindazole resulted in lesser damage than treatment with L-NAME following focal (Kamii et al., 1995) or global (Globus et al., 1995) ischemia, although the beneficial effect of 7-nitroindazole was insignificant. In summary, our results suggest that 7-nitroindazole is not totally ineffective in inhibiting endothelial NO synthase in vivo, although the degree of inhibition by 7-nitroindazole appears to be less than by L-arginine analogs. The suppressive action of halothane on the cardiovascular effects of 7-nitroindazole suggests that the influence of anesthetics should be taken into consideration in studies of cardiovascular effects of NO synthase inhibitors.

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