Pressor Responses to Ephedrine Are Mediated by a Direct Mechanism in the Rat

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ABSTRACT

The mechanism of the pressor response to ephedrine is controversial. In the present study, i.v. injections of ephedrine increased systemic and pulmonary arterial pressure, and i.a. injections decreased hindlimb blood flow in a dose-related manner. Responses to ephedrine were inhibited by α-receptor blocking agents and were not attenuated by blockade of the norepinephrine reuptake transporter (NET) or by catecholamine depletion using reserpine or a combination of reserpine and α-methyl-p-tyrosine, whereas responses to tyramine and amphetamine were inhibited by these treatments. The magnitude of the pressor response to ephedrine was similar in anesthetized and conscious rats. Tachyphylaxis developed to pressor responses to ephedrine and amphetamine with sequential injections; however, ephedrine tachyphylaxis differed in that subsequent responses to α-receptor agonists were attenuated. These results suggest that the systemic and pulmonary pressor and hindlimb vasoconstrictor responses to ephedrine are mediated by direct action on α-adrenergic receptors and that the release of norepinephrine from adrenergic terminals plays no significant role. These results provide support for the hypothesis that responses to ephedrine are directly mediated in the intact rat, whereas responses to amphetamine are mediated in a large part by the release of norepinephrine from adrenergic terminals.

Ephedrine is chemically and structurally related to amphetamine and methamphetamine and is a substrate for the adrenergic nerve terminal membrane monoamine transporter (Abdallah et al., 1968). Ephedrine has actions on α-and β-adrenergic receptors and displaces norepinephrine from adrenergic terminals (Abdallah et al., 1968; Rothman et al., 2003; McMahon and Cunningham, 2003). The indirect actions of ephedrine are reported to be the mechanism by which ephedrine increases arterial pressure, cardiac output, and peripheral resistance; however, direct effects have also been reported (Waldeck and Widmark, 1985; Kawasuji et al., 1996; Vansal and Feller, 1999). Ephedrine has been linked to serious cardiovascular toxicity, including hypertension, vaso- spasm, angina, coronary artery disease, arrhythmia, myocardial infarction, stroke, and death (Cockings and Brown, 1997; Zahn et al., 1999; Haller and Benowitz, 2000; Foxford et al., 2003; McMahon and Cunningham, 2003). Cardiovascular events result from both acute and chronic ephedrine use and occur in individuals with no history of cardiovascular disease (Cockings and Brown, 1997; Grzesk et al., 2004). Ephedrine, like other drugs of abuse, has stimulant and hallucinogenic actions (McMahon and Cunningham, 2003). In addition, experienced drug users report that ephedrine-induced euphoria is qualitatively similar to that induced by other amphetamine-like sympathomimetics (Martin et al., 1971). Despite a recent Food and Drug Administration ban on ephedrine-containing dietary supplements, ephedrine preparations are still available through the mail and on the Internet, and ephedrine use is not likely to change because of the high demand for these preparations (Ashar et al., 2003; Food and Drug Administration, HHS, 2004; Kim and LeBourgeois, 2004). The release of catecholamines and subsequent activation of adrenergic receptors is thought to be the primary mechanism responsible for the cardiovascular response to ephedrine and isolated tissue studies support the concept of an indirect mechanism of action (Fleckenstein and Burn, 1953; Burn and Rand, 1958; Cairoli et al., 1962; de Moraes et al., 1968). Other studies in isolated tissues have shown that ephedrine
acts by direct stimulation of adrenergic receptors (Tye et al., 1967; Waldeck and Widmark, 1985; Bao et al., 1990; Kawasuji et al., 1996). It has recently been reported that despite evidence that ephedrine has direct effects in isolated tissues, the pressor response is completely indirectly mediated in vivo in the rat (Kobayashi et al., 2003).

The effects of ephedrine-like agents such as amphetamine methamphetamine and methylenedioxymethamphetamine have been shown to be reduced by pretreatment with reserpine as well as with a combination of reserpine and α-methyl-p-tyrosine (AMPT) (Sabol and Seiden, 1998; Uehara et al., 2004). Few studies have examined the mechanisms involved in the cardiovascular responses to acute ephedrine administration in the intact rat. Since there is little information available on mechanisms in vivo, the present study was undertaken to investigate the mechanisms by which this agent increases arterial pressure in the rat. Catecholamines were depleted by administering reserpine and by administering reserpine and α-methyl-p-tyrosine together to determine whether the release of catecholamines mediates the responses to ephedrine and amphetamine. The effect of acute ephedrine administration on arterial pressure, hindlimb blood flow, and pulmonary arterial pressure as well as the adrenergic receptors involved in these responses were examined in this study.

Materials and Methods

General Methods. Experiments were performed on male Sprague-Dawley rats, weighing 300 to 400 g, anesthetized with thiobutabarbital sodium (Inactin) (100 mg/kg i.p.) with supplemental doses given as needed to maintain a uniform level of anesthesia. The trachea was cannulated to maintain airway patency, and the animals breathed room air spontaneously. An external jugular vein was catheterized for the intravenous (i.v.) administration of drugs. The common carotid artery was catheterized for the measurement of systemic arterial pressure. Systemic arterial pressure was measured with a Statham transducer and recorded on a Grass model 7D polygraph, and the data were digitized by a Biopac MP100 data acquisition system.

Hindlimb Vascular Response to Ephedrine. For hindlimb blood flow measurements, a Transonic flow probe (Transonic Systems Inc., Ithaca, NY) was placed around the right iliac artery just below the aortic bifurcation, and hindlimb blood flow was measured with a Transonic Systems T-106 small animal flowmeter. A catheter in the left iliac artery was advanced to the aortic bifurcation for the i.a. administration of drugs into the right hindlimb circulation. Systemic arterial pressure and right iliac flow data were recorded on a PC using a Biopac MP100 acquisition system. Agonists were injected directly into the right hindlimb circulation in small volumes (10–30 μl) so that changes in right iliac blood flow could be measured with minimal changes in systemic arterial pressure.

Pulmonary Response to Ephedrine. For measurement of pulmonary arterial pressure, a 3F radiopaque catheter was passed from the left external jugular vein into the main pulmonary artery under fluoroscopic guidance. Pressures were recorded on a Grass model 7 polygraph, and mean pressures were derived by electronic integration. Catheter positions were verified at postmortem examination, and these methods have been described previously (Baber et al., 2003).

The duration of the response is defined as the period of time from injection of the agonist to the time at which the measured parameter (either mean arterial pressure, or hindlimb blood flow) returned to the preinjection value. A P value of <0.05 was used as the criterion for statistical significance.

Responses to Ephedrine in the Conscious Rat. Mean arterial pressure (MAP) and heart rate were recorded in conscious, unrestrained rats in their home cages using a radio telemetry system (Dataquest A.R.T. 2.2; Data Sciences International, St. Paul, MN). Briefly, under ketamine/xylazine (90 and 10 mg/kg i.p., respectively) anesthesia, the arterial pressure cannula of a battery powered radio telemetry probe (TL11M2-C50-PXT; Data Sciences International) was inserted and secured into the descending abdominal aorta rostral to the femoral bifurcation. The probe was then placed in the abdominal cavity and secured to the abdominal musculature. In all rats, a polyurethane cannula (Micro-renathane, 0.33-inch o.d. × 0.014-inch i.d.; Braintree Scientific, Braintree, MA) was inserted into the femoral vein and the free end tunneled subcutaneously to the nape of the neck and exteriorized. After surgery, fluids and penicillin (60,000 units i.m.) were administered. Buprenorphine (2.5 mg/kg i.p., b.i.d.) was administered for 2 days. The rats were allowed to recover from the surgical procedures for 7 to 10 days before beginning any experimental protocol. In these experiments, the daily weight was measured and baseline MAP and heart rate were recorded for a minimum of 20 min before the administration of any drugs. Ephedrine (10 mg/kg; 0.10 ml) or saline (0.10 ml) was administered and followed by a 0.1-ml saline flush. Cardiovascular parameters were allowed to return to baseline before administering the next dose.

The output from the telemetry probe was recorded (250 Hz) using receivers placed under the home cages. The data were sent to a consolidation matrix before being stored on a personal computer. Data acquisition was controlled using Data Sciences Datquest acquisition software. The data were averaged into 2-s bins and displayed. The magnitude of the peak changes in MAP and heart rate elicited by the drugs were calculated off-line as the difference between the baselines and peak drug response using the Datquest analysis program. Response duration was calculated off-line using the Datquest analysis program. The duration of the systemic arterial pressure response was calculated as the interval between drug administration and the point at which the systemic arterial pressure returned to within 7 mm Hg of baseline value.

Experimental Protocols. In the first set of experiments, a dose-response curve for ephedrine and amphetamine was obtained and tachyphylaxis was observed when ephedrine or amphetamine was administered as sequential injections in the same animal. Therefore, midrange doses of ephedrine (10 mg/kg i.v.) and amphetamine (3 mg/kg i.v.) were used for further evaluation of acute responses. The L-ephedrine, d-amphetamine, and dl-norepinephrine stereoisomers were used in the present study.

In a second set of experiments, animals were treated with cocaine (5 mg/kg i.v.), and blockade of reuptake after cocaine administration was assessed by analyzing responses to tyramine (100 μg/kg i.v.) and norepinephrine (3 μg/kg i.v.).

In a third set of experiments, the effect of treatment with reserpine (2.5 mg/kg i.p.) or with reserpine and AMPT (200 mg/kg i.p.) was investigated. Reserpine was administered 18 h before the experiment, whereas AMPT was given at least 2 h before the experiment. The extent of catecholamine depletion was functionally assessed by evaluating responses to norepinephrine and to tyramine, and tissue levels were measured. The administration of reserpine caused a decrease in baseline pressure from 111 ± 2 to 97 ± 2, whereas the combination of reserpine and AMPT resulted in a baseline pressure of 106 ± 3.0. Responses to ephedrine, amphetamine, norepinephrine, and angiotensin II were not changed during experiments where an infusion of phenylephrine (1 μg/kg/min) was used to restore baseline systemic arterial pressure to control value.

In a fourth set of experiments, animals were pretreated with propranolol (0.5 mg/kg i.v.) or with phentolamine (0.5 mg/kg i.v.) before the evaluation of ephedrine responses in the systemic, hindlimb, or pulmonary vascular bed. Blockade of α- and β-receptors was assessed by evaluating responses to phenylephrine and isoprotreton. Phentolamine administration decreased baseline arterial pres-
sure to 86 ± 4 mm Hg, whereas propranolol had no significant effects on baseline systemic arterial pressure.

**High-Performance Liquid Chromatography (HPLC) Determination of Tissue Catecholamines.** After the completion of experiments measuring the systemic or hindlimb responses to ephedrine, the brain, heart, and adrenal glands were removed and homogenized in a buffer of 0.1 M citrate, 10% ethanol, and 250 mg/liter sodium octyl sulfate at pH 4. Tissues were stored in the buffer at a temperature of −80°C until analysis. An internal standard of 3,4-dihydroxybenzylamine hydrobromide was added such that its final concentration was 1 ng/100 μl of the HPLC injectate. The homogenate was centrifuged in a RC2B Sorvall centrifuge at 12,000g, and the supernatant was frozen at −80°C until assayed. Recoveries ranged from 75 to 90% in these and other studies. Our chromatographic system consisted of the following hardware and software. Dual Rainin Rabbit HP pumps equipped with a self-washing piston pump heads (capable of maximum flows of 10 ml/min) provided a flow rate of 1.5 ml/min. Injection of samples was accomplished automatically using an Alcott model 728 autosampler. This allowed us to run as many as 40 samples overnight. The HPLC column consisted of a Rainin microsorb (5 μm) C18 column (25 cm × 4.6 mm). A 1.5 cm × 4.6-mm guard column packed with microsorb C18 preceded the analytical column. Chromatographically separated monoamines were assayed by electrochemical detection using an ESA model 5100 Coulchem multielectrode array. This electrode array consisted of a model 5020 guard cell and a model 5010 dual analytical electrode cell. The guard cell voltage was +0.4 V. The two analytical cells were set at −0.04 V (detector 1) and +0.32 V (detector 2). Norepinephrine (NE), epinephrine (Epi), dopamine (Dop), 5-hydroxytryptamine (5HT, serotonin), and 5-hydroxyindoleacetic acid (5HIAA) were determined in unknown and standard samples by comparison to retention times and integrated area of the peak. Cochromatography of spiked standards of each compound were initially run with brain tissue to determine that the peaks identified as monoamines were indeed authentic NE, Epi, Dop, 5HT, and 5HIAA.

**Statistical Analysis.** Hemodynamic data are expressed as means ± S.E.M. and were analyzed using paired and unpaired t tests or analysis of variance with a Fisher post test. A p value of less than 0.05 was used as the criterion for statistical significance (*, p < 0.05).

**Results**

**Responses to Ephedrine and Amphetamine.** Responses to ephedrine and amphetamine were compared and injection of ephedrine in doses of 1 to 30 mg/kg i.v. produced dose-related increases in systemic arterial pressure when each injection was given in a separate animal (Fig. 1A, left). Injections of ephedrine in doses of 1 to 30 mg/kg i.v. also caused dose related increases in systemic arterial pressure when injections were given at 30-min intervals in the same animal (Fig. 1A). Intravenous injections of amphetamine in doses of 1 to 30 mg/kg increased systemic arterial pressure (Fig. 1B). However, pressor responses to amphetamine in doses of 1 to 30 mg/kg i.v. were only dose-related when single injections were administered to separate animals (Fig. 1B, left). Figure 1, A and B, also illustrates the effect of repeated injections of ephedrine (3 mg/kg i.v.) and amphetamine (3 mg/kg i.v.). The pressor response to ephedrine was reduced after the fourth injection, and further injections resulted in a

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**Fig. 1.** A, left, effect of i.v. injections of ephedrine on mean systemic arterial pressure when doses are administered nonsequentially as a single injection in separate animals and when all injections were administered sequentially in the same animal 20 to 30 min apart; right, effect of repeated injections of ephedrine on mean systemic arterial pressure. B, effect of intravenous injections of amphetamine on mean systemic arterial pressure. n indicates number of experiments, and the asterisk indicates that the response is significantly different than control.
measurable but diminished response, indicating the development of tachyphylaxis. The pressor response to amphetamine was reduced after the second injection, and responses to further injections were greatly attenuated.

**Effects of Ephedrine and Amphetamine on Responses to Phenylephrine and Norepinephrine.** Tachyphylaxis to the pressor response to ephedrine (3 mg/kg i.v.) or amphetamine (3 mg/kg i.v.) occurred after sequential injections of each agent. The effect of ephedrine or amphetamine induced tachyphylaxis on responses to norepinephrine (0.3–3 μg/kg i.v.) and phenylephrine (1–10 μg/kg i.v.) were investigated (Fig. 2). Injections of norepinephrine and phenylephrine were administered after five sequential injections of ephedrine or amphetamine to induce tachyphylaxis. The increase in arterial pressure in response to norepinephrine was not changed by ephedrine tachyphylaxis; however, the area under-the-curve of the norepinephrine response at lower doses (0.3 and 1 μg/kg i.v.) was reduced (Fig. 2A). However, amphetamine tachyphylaxis enhanced the increase in arterial pressure and the area under the curve of the response to norepinephrine (Fig. 2A). After the development of ephedrine tachyphylaxis, the increase in arterial pressure in response to phenylephrine was diminished, as was the area under the curve of the response (Fig. 2B). After administration of amphetamine the increase in mean arterial blood pressure in response to phenylephrine was not changed; however, the area under the curve of the responses was increased (Fig. 2B). Ephedrine or amphetamine induced tachyphylaxis had no effect on the pressor response to angiotensin II or on the vasodepressor response to bradykinin (Table 1).

**Effects of Cocaine, Reserpine, and AMPT.** The effects of cocaine, reserpine, and reserpine plus AMPT on pressor responses to ephedrine and amphetamine were compared, and these data are summarized in Figs. 3 through 5. After administration of cocaine (5 mg/kg i.v.), the increase in systemic arterial pressure in response to ephedrine (Fig. 3A, left) was not changed, whereas responses to amphetamine (Fig. 3A, right) and tyramine (Fig. 3B, left) were decreased.

### Table 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (µg/kg i.v.)</th>
<th>Treatment</th>
<th>Change in Arterial Pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiotensin II</td>
<td>0.1</td>
<td>Control</td>
<td>30 ± 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ephedrine</td>
<td>28 ± 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Amphetamine</td>
<td>29 ± 5</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>Control</td>
<td>47 ± 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ephedrine</td>
<td>48 ± 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Amphetamine</td>
<td>52 ± 2</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>10</td>
<td>Control</td>
<td>−20 ± 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ephedrine</td>
<td>−22 ± 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Amphetamine</td>
<td>−18 ± 4</td>
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</table>
significantly. The pressor response to norepinephrine (Fig. 3B, right) was enhanced by cocaine whereas the pressor responses to angiotensin II (Fig. 3C, left) and depressor responses to acetylcholine (Fig. 3C, right) were not altered by cocaine administration. The increase in systemic arterial pressure in response to i.v. injections of angiotensin II and on the decrease in mean arterial pressure in response to i.v. injections of acetylcholine. * indicates number of experiments, and the asterisk indicates that the response is significantly different than control.

![Fig. 3: Effect of cocaine on pressor responses to various agents.](image)

Responses to Ephedrine Are Directly Mediated

**Fig. 3. A**, effect of cocaine (5 mg/kg i.v.) on the increase in mean arterial pressure in response to i.v. injections of ephedrine and amphetamine. **B**, effect of cocaine (5 mg/kg i.v.) on the increase in mean arterial pressure in response to intravenous injections of tyramine and norepinephrine. **C**, effect of cocaine (5 mg/kg i.v.) on the increase in mean arterial pressure in response to i.v. injections of angiotensin II and on the decrease in mean arterial pressure in response to i.v. injections of acetylcholine. * indicates number of experiments, and the asterisk indicates that the response is significantly different than control.

**Fig. 4. A**, effect of reserpine (2.5 mg/kg i.v.) and reserpine plus AMPT (200 mg/kg i.v.) on the increase in mean systemic arterial pressure and the area under the curve of the response to i.v. injections of ephedrine. **B**, increase in mean systemic arterial pressure and the area under the curve of the response to i.v. injections of amphetamine. **C**, increase in mean systemic arterial pressure and the area under the curve of the response to i.v. injections of tyramine. **D**, increase in mean systemic arterial pressure and the area under the curve of the response to i.v. injections of norepinephrine. * indicates number of experiments, and the asterisk indicates that the response is significantly different than control.

Heart, and adrenal glands of rats treated with reserpine and AMPT.

**Hindlimb Vascular Response.** In the hindlimb vascular bed of the rat, direct i.a. injections of ephedrine (100–1000 μg) and amphetamine (10–30 μg) caused dose-related decreases in hindlimb blood flow, and with smaller doses of the amines tachyphylaxis was not observed. The decreases in hindlimb blood flow in response to ephedrine were not altered by reserpine or by treatment with reserpine and AMPT (Fig. 5A). In contrast, the decreases in hindlimb blood flow in response to i.a. injections of amphetamine (Fig. 5B) and tyramine (Fig. 5C) were significantly reduced by pretreatment with reserpine and with reserpine and AMPT. Decreases in hindlimb blood flow in response to i.a. injections of norepinephrine were enhanced (Fig. 5D).

**Responses to Ephedrine in the Conscious and Anesthetized Rat.** Responses to ephedrine were compared in anesthetized and conscious freely moving rats, and these data are presented in Fig. 6. The magnitude of the increase in
mean arterial blood pressure after the systemic injection of ephedrine (10 mg/kg) in the anesthetized rat was similar to that observed in the conscious rat (Fig. 6A). However, the duration of the pressor response to ephedrine was significantly shorter in the conscious animal (Fig. 6B). The administration of ephedrine increased heart rate in the anesthetized animal, but it resulted in a significant decrease in heart rate in the conscious rat (Fig. 6C).

**Effect of Phentolamine on Systemic and Hindlimb Responses.** The effect of the phentolamine on the pressor response to ephedrine is shown in Fig. 7A. After administration of phentolamine (0.5 mg/kg i.v.), the magnitude of the pressor response to ephedrine was decreased significantly (Fig. 7A, right). In contrast, the increase in heart rate in response to ephedrine was not decreased by phentolamine (Fig. 7A, middle). Similar results were obtained with the α-1 selective receptor antagonist prazosin (Table 4). Phentolamine attenuated the pressor response to phenylephrine (Table 4). The role of α-adrenergic receptors in mediating the hindlimb vasoconstrictor response to ephedrine was investigated (Fig. 7A, right). When ephedrine (100–1000 μg i.a.) was administered after phentolamine (0.5 mg/kg i.v.), the decrease in hindlimb blood flow was abolished and an increase in hindlimb blood flow was observed (Fig. 7A, right).

**Effect of Propranolol on Systemic Hindlimb Responses to Ephedrine.** The effect of the propranolol on the response to ephedrine is shown in Fig. 7B. Propranolol (0.5 mg/kg i.v.) did not affect the magnitude of the pressor response (Fig. 7B, left); however, the heart rate response was decreased. The duration of the pressor response to ephedrine was also significantly increased by propranolol (Fig. 7B, middle). Propranolol attenuated the effects of the β-adrenergic receptor agonist isoproterenol (Table 4). The role of β-adrenergic receptors was evaluated and the decrease in hindlimb blood flow in response to ephedrine (100–1000 μg i.a.) was not changed after propranolol (0.5 mg/kg i.v.) (Fig. 7B, right). Subsequent administration of phentolamine inhibited the decrease in hindlimb blood flow in response to ephedrine.

**Pulmonary Vascular Responses to Ephedrine.** The effect of ephedrine (1–10 mg/kg i.v.) on pulmonary arterial pressure is shown in Fig. 8. Ephedrine produced dose-related increases in pulmonary arterial pressure. The magnitude of the increases in pulmonary pressure was significantly decreased after treatment with phentolamine (0.5 mg/kg i.v.) (Fig. 8, left) and were not changed by propranolol (0.5 mg/kg i.v.) (Fig. 8, middle). However, the duration of the pulmonary pressor response was enhanced by propranolol (Fig. 8, right). The effect of ephedrine after catecholamine depletion was investigated. The increases in pulmonary pressure in response to ephedrine after the administration of reserpine plus AMPT were not changed (Fig. 8B, left). The pulmonary pressor response to tyramine was inhibited (Fig. 8B, middle), and responses to norepinephrine were enhanced (Fig. 8B, right) after catecholamine depletion with reserpine and AMPT.

**Discussion**

The present results show that systemic and pulmonary pressor responses and hindlimb vasoconstrictor responses to ephedrine are mediated by direct activation of α-receptors and that release of norepinephrine from adrenergic terminals plays no significant role in the rat. Systemic pressor and hindlimb vasoconstrictor responses to ephedrine were attenuated by phentolamine and were increased in duration by propranolol, suggesting that responses are mediated by α-receptors and modulated by β-receptors. Responses to ephedrine were not attenuated by cocaine in a dose that blocked the response to tyramine. The hypothesis that responses to ephedrine are not mediated by an indirect mechanism is supported by experiments with reserpine and AMPT, showing that ephedrine induced systemic and pulmonary pressor responses and hindlimb vasoconstrictor responses were not reduced by catecholamine depletion. In contrast, the increase in systemic arterial pressure and the decrease in hindlimb blood flow in response to amphetamine were abolished after catecholamine depletion with reserpine or with a combination of reserpine and AMPT.

**TABLE 2**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Treatment</th>
<th>Change in Arterial Pressure</th>
<th>mm/Hg</th>
</tr>
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<tr>
<td>Phenylephrine</td>
<td>1</td>
<td>Control</td>
<td>16 ± 2</td>
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</tr>
<tr>
<td></td>
<td>3</td>
<td>Control</td>
<td>35 ± 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Reserpine</td>
<td>39 ± 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>Control</td>
<td>48 ± 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>Reserpine</td>
<td>45 ± 7</td>
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<tr>
<td>Angiotensin II</td>
<td>0.1</td>
<td>Control</td>
<td>30 ± 3</td>
<td></td>
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<tr>
<td></td>
<td>0.3</td>
<td>Control</td>
<td>48 ± 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>Reserpine</td>
<td>45 ± 7</td>
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**TABLE 3**

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<tr>
<th>Tissue</th>
<th>Treatment</th>
<th>NE</th>
<th>EPI</th>
<th>5-HIAA</th>
<th>DA</th>
<th>5-HT</th>
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<td>Brain</td>
<td>Control</td>
<td>0.090 ± 0.005</td>
<td>0.0043 ± 0.0006</td>
<td>0.025 ± 0.001</td>
<td>0.064 ± 0.004</td>
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<td>Reserpine + AMPT</td>
<td>0.011 ± 0.001</td>
<td>0.0015 ± 0.0003</td>
<td>0.015 ± 0.003</td>
<td>0.009 ± 0.001</td>
<td>0.011 ± 0.002</td>
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<tr>
<td>Heart</td>
<td>Control</td>
<td>0.129 ± 0.010</td>
<td>0.0038 ± 0.0005</td>
<td>0.0013 ± 0.0003</td>
<td>0.0028 ± 0.0012</td>
<td>0.023 ± 0.003</td>
</tr>
<tr>
<td></td>
<td>Reserpine + AMPT</td>
<td>0.012 ± 0.002</td>
<td>0.0022 ± 0.0004</td>
<td>0.0005 ± 0.0001</td>
<td>0.0007 ± 0.0003</td>
<td>0.003 ± 0.0006</td>
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<tr>
<td>Adrenal gland</td>
<td>Control</td>
<td>22.13 ± 0.830</td>
<td>12.44 ± 0.855</td>
<td>0.011 ± 0.001</td>
<td>0.010 ± 0.009</td>
<td>0.058 ± 0.017</td>
</tr>
<tr>
<td></td>
<td>Reserpine + AMPT</td>
<td>7.24 ± 0.80</td>
<td>18.86 ± 3.16</td>
<td>0.010 ± 0.002</td>
<td>0.0059 ± 0.0028</td>
<td>0.052 ± 0.001</td>
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</table>

*P < 0.05.
The mechanism of action of ephedrine action is controversial. Isolated tissue studies suggest that ephedrine acts through an indirect mechanism. Ephedrine induced contraction of the nicitating membrane, constriction of the pupil, forelimb, and coronary vasoconstriction are mediated by an indirect mechanism (Fleckenstein and Burn, 1953; Burn and Rand, 1958; Cairoli et al., 1962; de Moraes et al., 1968). However, direct stimulation of adenergic receptors in the guinea pig trachea, portal vein, and reserpinized rat atrium has been reported previously (Tye et al., 1967; Waldeck and Widmark, 1985; Bao et al., 1990; Kawasuji et al., 1996). Ephedrine has been shown to bind to $\mu_1$, $\mu_2$, and $\mu_3$ receptors expressed on Chinese hamster ovary cells (Vansal and Feller, 1999). It has been reported that despite evidence that ephedrine has direct effects in isolated tissues, the pressor response was shown to be indirectly mediated in vivo in the rat (Kobayashi et al., 2003). In contrast, the present results demonstrate that responses to ephedrine are directly mediated, whereas responses to amphetamine are dependent on an indirect mechanism.

Reserpine depletes catecholamines from central and peripheral adrenergic terminals by interfering with vesicular transmitter storage (Sabol and Seiden, 1998). Therefore, the actions of indirect-acting agents are inhibited by reserpine, whereas responses to direct-acting amines are not reduced and may be enhanced (Sabol and Seiden, 1998). The literature suggests that there are two distinct norepinephrine pools within the adrenergic terminal, a cytoplasmic and a

**Fig. 5.** A, effect of reserpine (2.5 mg/kg i.p.) and reserpine plus AMPT (200 mg/kg i.p.) on the decrease in hindlimb blood flow and the area under the curve the response to i.v. injections of ephedrine. B, decrease in hindlimb blood flow and the area under the curve of the response to i.v. injections of amphetamine. C, decrease in hindlimb blood flow and the area under the curve of the response to i.v. injections of tyramine. D, decrease in hindlimb blood flow and the area under the curve of the response to i.v. injections of norepinephrine. $n$ indicates number of experiments, and the asterisk indicates that the response is significantly different than control.

**Fig. 6.** A, change in mean systemic arterial pressure in response to i.v. injection of ephedrine in the conscious rat. B, duration of the increase in mean systemic arterial pressure in response to i.v. injection of ephedrine in the conscious rat. C, change in heart rate in response to i.v. injection of ephedrine in the conscious rat. $n$ indicates number of experiments, and the asterisk indicates that the response is significantly different than control.
vesicular pool (Simpson, 1980). An exchange diffusion model suggests that only cytoplasmic catecholamines are released by amphetamine-like drugs (Fischer and Cho, 1979). Since reserpine is selective for vesicular stores, many studies have used coadministration of AMPT, an inhibitor of tyrosine hydroxylase, with reserpine to deplete both stores of catecholamines (Abrahams et al., 1996; Yuan et al., 2002). In the present experiments, responses to phenylephrine and angiotensin II were unchanged by treatment with reserpine or reserpine and AMPT (Table 4). The present results are the first to show that ephedrine has significant direct pressor and vasoconstrictor effects in vivo after treatment with reserpine and AMPT. The systemic, pulmonary, and hindlimb vascular responses to ephedrine were not reduced by reserpine and AMPT, whereas responses to amphetamine and tyramine were abolished. The present data are not in agreement with the concept that responses to ephedrine are indirectly mediated (Kobayashi et al., 2003). Pretreatment with reserpine and with reserpine and AMPT at doses that depleted catecholamines as measured by HPLC and that inhibited responses to tyramine and amphetamine did not reduce the magnitude or the area under the-curve of the response to ephedrine. The observation that responses to amphetamine are reduced by reserpine (Sabol and Seiden, 1998; Yuan et al., 2002) and reserpine and AMPT (Abrahams et al., 1996) is in agreement with the observation that responses to amphetamine are reduced by catecholamine depletion (Abrahams et al., 1996; Sabol and Seiden, 1998). In the present study, both reserpine and reserpine and α-methyl-p-tyrosine inhibited responses to amphetamine. Although norepinephrine is thought to be stored in distinct pools, there is evidence that these pools are in flux (Simpson, 1980). Additionally, amphetamine can alter the integrity of the vesicular pool, allowing transmitter leakage into the cytoplasmic pool where it is available for release (Sulzer and Rayport, 1990). The present

![Fig. 7. A, effect of phentolamine (0.5 mg/kg i.v.) on the increase in mean systemic arterial pressure (left), the increase in heart rate (middle), and the decrease in hindlimb blood flow (right) in response to i.v. injections of ephedrine. B, effect of propranolol (0.5 mg/kg i.v.) on the increase in mean systemic arterial pressure (left), the increase in heart rate (middle), and the decrease in hindlimb blood flow (right) in response to i.v. injections of ephedrine. n indicates number of experiments, and the asterisk indicates that the response is significantly different than control.]

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Treatment</th>
<th>Change in Arterial Pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylephrine</td>
<td>3 (μg/kg i.v.)</td>
<td>Control</td>
<td>30 ± 2</td>
</tr>
<tr>
<td></td>
<td>10 (μg/kg i.v.)</td>
<td>Control</td>
<td>40 ± 2</td>
</tr>
<tr>
<td>Ephedrine</td>
<td>10 (mg/kg i.v.)</td>
<td>Control</td>
<td>50 ± 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phenolamine</td>
<td>24 ± 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prazosin</td>
<td>18 ± 2</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>0.1 (μg/kg i.v.)</td>
<td>Control</td>
<td>-11 ± 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pranopanol</td>
<td>-30 ± 3</td>
</tr>
</tbody>
</table>

* P < 0.05.
results show that responses to ephedrine and amphetamine are mediated by different mechanisms.

The present data are not in agreement with a recent report showing that the pressor response to ephedrine in the rat is entirely due to the release of norepinephrine from adrenergic terminals (Kobayashi et al., 2003). The reason for the difference in results is uncertain but could be due to the doses of ephedrine administered as well as to the use of different catecholamine depletion regimens. It is possible that 6-hydroxydopamine causes nonspecific depression of vascular responses (Purdy et al., 1981). The i.v. doses of ephedrine used in the present study are within the range of the doses used in ephedrine-dependent individuals and in animal studies (Miller et al., 1998; Young and Glennon, 1998; Miller and Waite, 2003). However, it is possible that ephedrine may act through an indirect mechanism in some organ systems, and in the present study catecholamine depletion reduced the heart rate increase in response to ephedrine.

Ephedrine increases systemic and pulmonary arterial pressure and decreases hindlimb blood flow in the anesthetized rat by directly stimulating α-adrenergic receptors, whereas β-receptors modulate the responses. Phentolamine attenuated responses to phenylephrine and significantly reduced the increase in arterial pressure but not the increase in heart rate in response to ephedrine. Phentolamine also reduced the decrease in hindlimb blood flow and the increase in pulmonary arterial pressure in response to ephedrine. Therefore, it appears that the increase in systemic and pulmonary arterial pressure and the decrease in hindlimb blood flow in response to ephedrine are predominantly due to direct activation of α-adrenergic receptors. Propranolol, in a dose that decreased responses to isoproterenol, attenuated the increase in heart rate but not the pressor response to ephedrine, indicating that the increase in heart rate is due to the stimulation of β-receptors and is in agreement with previous studies (Schindler et al., 1992). In addition, propranolol increased the duration of the systemic and pulmonary pressor responses to ephedrine. The pressor response to ephedrine involves concurrent α and β-receptor activation, with α-receptor induced vasoconstriction opposing the β-receptor mediated vasodilation (Lambrecht et al., 2002). Thus, β-receptor inhibition allows unopposed stimulation of α-receptors, which could result in a longer duration of the response to ephedrine. Moreover, when α-receptors were blocked, a β-receptor mediated vasodilator mechanism was unmasked in the hindlimb vascular bed, providing further evidence in support of the concept that the response to ephedrine results from activation of both α- and β-adrenergic receptors.
The present study demonstrates that the response to ephedrine in the rat systemic vascular bed exhibits tachyphylaxis when multiple doses are given at short intervals (20 min) in the same animal. However, tachyphylaxis to ephedrine was different from tachyphylaxis induced by repeated doses of amphetamine. The present observation that the pressor response to ephedrine was reduced but not abolished after repeated administration is consistent with studies that reported different patterns of tachyphylaxis with ephedrine and amphetamine (de Moraes and Carvalho, 1967; Takasaki et al., 1972). In the present study, pressor responses to phenylephrine and norepinephrine were reduced after the development of ephedrine tachyphylaxis but not reduced by amphetamine tachyphylaxis. These findings are in agreement with isolated tissue studies showing that α-receptors are inhibited by repeated administration of ephedrine (Furukawa and Morishita, 1975; Morishita and Furukawa, 1975). The present data provide support for the hypothesis that tachyphylaxis to the pressor response to ephedrine may involve α-receptor inhibition, whereas tachyphylaxis to amphetamine may involve catecholamine depletion, in that norepinephrine and phenylephrine pressor responses are preserved during amphetamine tachyphylaxis.

Responses to ephedrine were compared in anesthetized and in conscious freely moving rats, and the magnitude of the pressor response in the anesthetized and conscious rat was similar. However, the duration of the pressor response in the conscious rat was significantly shorter and ephedrine induced a decrease in heart rate in contrast to the increase in heart rate observed in the anesthetized rat. These differences are most likely due to anesthesia induced alterations in baroreflex function (Schwartz et al., 1988).

In summary, the present results demonstrate that systemic and pulmonary pressure and hindlimb vasoconstrictor responses to ephedrine are mediated by direct α-adrenergic receptor stimulation, whereas β-receptors modulate the responses. The increase in systemic and pulmonary arterial pressure and the decrease in hindlimb blood flow in response to ephedrine were not reduced by catecholamine depletion. In contrast, the increase in systemic arterial pressure and the decrease in hindlimb blood flow in response to amphetamine were abolished after treatment with reserpine and with a combination of reserpine and AMPT. These results provide support for the hypothesis that ephedrine has significant direct receptor-mediated effects in the intact rat, whereas responses to amphetamine are indirectly mediated, suggesting that responses to structurally similar phenylethylamines may be mediated by different mechanisms. These data also demonstrate that responses to phenylephrine and norepinephrine are decreased by ephedrine tachyphylaxis, whereas these responses were unchanged by amphetamine tachyphylaxis. These data suggest that pressor response to ephedrine is mediated by direct α-adrenergic receptor stimulation. The present data show that responses to ephedrine and amphetamine are mediated by different mechanisms and that cardiovascular responses to ephedrine-like drugs are complex.

References
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Simpson LL (1988) Evidence that there are subcellular pools of norepinephrine and...
that there is flux of norepinephrine between these pools. *J Pharmacol Exp Ther* 214:410–416.


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