Phenylpropanolamine Constricts Mouse and Human Blood Vessels by Preferentially Activating \( \alpha_2 \)-Adrenoceptors

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ABSTRACT

Phenylpropanolamine (\( dl \)-norephedrine) was one of the most widely used therapeutic agents to act on the sympathetic nervous system. Because of concerns regarding incidents of stroke, its use as a nasal decongestant was discontinued. Although considered an \( \alpha_1 \)-adrenergic agonist, the vascular adrenergic pharmacology of phenylpropanolamine was not fully characterized. Unlike most other circulations, the vasculature of the nasal mucosa is highly enriched with constrictor \( \alpha_2 \)-adrenoceptors. Therefore, experiments were performed to determine whether phenylpropanolamine activates vascular \( \alpha_2 \)-adrenoceptors. Mouse tail and mesenteric small arteries and human small dermal veins were isolated and analyzed in a perfusion myograph. The selective \( \alpha_1 \)-adrenergic agonist phenylephrine caused constriction of tail and mesenteric arteries and human veins. The selective \( \alpha_2 \)-adrenergic agonist UK14,304 [5-bromo-\( N \)-(4,5-dihydro-1H-imidazol-2-yl)-6-quinoxalinamine] caused constriction in tail arteries and in human veins, but not mesenteric arteries. The lack of constriction to UK14,304 was also observed in endothelium-denuded mesenteric arteries. Phenylpropanolamine constricted both types of artery but was 62-fold more potent in tail arteries. In mesenteric arteries, constriction to phenylpropanolamine was not affected by the selective \( \alpha_2 \)-adrenergic antagonist, rauwolscine (\( 10^{-7} \) M) but was abolished by the selective \( \alpha_1 \)-adrenergic antagonist, prazosin (\( 3 \times 10^{-7} \) M). In contrast, constriction to phenylpropanolamine in tail arteries and in human veins was inhibited by rauwolscine but not prazosin. Therefore, phenylpropanolamine is a preferential \( \alpha_2 \)-adrenergic agonist. At low concentrations, it constricts blood vessels that express functional \( \alpha_2 \)-adrenoceptors, whereas at much higher concentrations, phenylpropanolamine also activates vascular \( \alpha_1 \)-adrenoceptors. This action likely contributed to phenylpropanolamine’s therapeutic activity, namely constriction of the nasal vasculature.

Phenylpropanolamine (\( dl \)-norephedrine) was one of the most widely used therapeutic agents to act by modulating the sympathetic nervous system. First synthesized in 1912, phenylpropanolamine was introduced as a nasal decongestant in the 1930s (Lasanga, 1988). Its therapeutic use, therefore, predated major discoveries in sympathetic neurophysiology, including the identification of the neurotransmitter norepinephrine (von Euler, 1946, cited in Lasanga, 1988), and the concept of adrenoceptors (Ahlquist, 1948, cited in Lasanga, 1988). Because of concerns regarding episodes of stroke in individuals ingesting phenylpropanolamine, it was withdrawn as a therapeutic agent in 2000 (Kernan et al., 2000).

An early report suggested that phenylpropanolamine may possess a cardiac-specific, indirect activity to release norepinephrine from sympathetic nerves (Trendelenburg et al., 1962). However, subsequent studies demonstrated that the cardiovascular effects of phenylpropanolamine result from direct activation of adrenoceptors (e.g., Moya-Huff and Maher, 1987; Hricik and Johnson, 1996). Phenylpropanolamine can inhibit the neuronal uptake of norepinephrine, although this property plays only a minor role in its cardiovascular effects (Hricik and Johnson, 1996). Phenylpropanolamine binds to all three subtypes of \( \alpha_1 \)-adrenoceptors (\( \alpha_{1A} \), \( \alpha_{1B} \), and \( \alpha_{1D} \)) with relatively low affinity (Buckner et al., 2002) and functions as a low-efficacy agonist at these receptors (Minneman et al., 1983; Minneman and Johnson, 1984; Fox et al., 1985; Alberts et al., 1999; Nishimatsu et al., 1999; Buckner et al., 2002). It has minimal activity at \( \beta \)-adrenoceptors (Moya-Huff and Maher, 1987; Hull et al., 1993) and is therefore considered a selective \( \alpha \)-adrenergic agonist. Although often described as an \( \alpha_1 \)-adrenergic agonist, phenylpropanolamine binds to \( \alpha_2 \)-adrenoceptors with approximately 35-fold higher affinity compared with \( \alpha_1 \)-adrenoceptors and may be an efficacious agonist at these receptors (Buckner et al., 2002).

\( \alpha_1 \)- and \( \alpha_2 \)-Adrenoceptors are both expressed on vascular smooth muscle cells and initiate vasconstriction (Guimarães and Moura, 2001). Although \( \alpha_1 \)-adrenoceptors are expressed...
by most blood vessels, functional constrictor α₁-adrenoceptors have a unique distribution in the human vasculature (e.g., Guimarães and Moura, 2001). Because of their role in vascular thermoregulation, α₁-adrenoceptors are more active in cutaneous compared with deep blood vessels (Chotani et al., 2000, 2004). They also have considerable activity in blood vessels of the nasal mucosa, where their activity can predominate over α₂-adrenoceptors (Andersson and Bende, 1984; Lacroix and Lundberg, 1989; Wang and Lung, 2003). Indeed, activation of α₂-adrenoceptors may be the preferred choice for causing nasal vasconstriction and nasal decongestion (McLeod et al., 2001). The present experiments were therefore performed to evaluate the activity of phenylpropanolamine at vascular α₁- and α₂-adrenoceptors. Blood vessels were selected that should contain functional α₁- and α₂-adrenoceptors (mouse tail artery and human dermal veins) or contain only α₁-adrenoceptors (mouse mesenteric artery) (e.g., Flavahan et al., 1984; Nase and Boegehold, 1998; Chotani et al., 2000).

Materials and Methods

Blood Vessel Chamber. Male mice (C57BL6) were euthanized by CO₂ asphyxiation. Small arteries/arterioles were then rapidly and carefully isolated from the mesenteric and tail circulations and placed in cold Krebs-Ringer-bicarbonate solution: 118.3 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 2.5 mM CaCl₂, 25.0 mM NaHCO₃, and 11.1 mM glucose (control solution). Skin biopsies (6 mm, inner aspect of the upper arm) were obtained from human volunteers, following irradiation of the site with local anesthetic (Chotani et al., 2004). Small cutaneous dorsal veins were then carefully isolated from the biopsy and placed in control solution. Blood vessels were cannulated at both ends with glass micropipettes, secured using 12-0 nylon monofilament suture, and placed in a microvascular chamber (Living Systems, Burlington, VT) (Chotani et al., 2000). Blood vessels were studied in the absence of flow and maintained at a constant transmural pressure (P TMP) of 60 mm Hg (small arteries) or 7.5 mm Hg (small veins). The chamber was placed on the stage of an inverted microscope (Nikon TMS-F; Nikon, Tokyo, Japan) connected to a video camera and superfused with control solution (maintained at 37°C, gassed with 16% O₂, 5% CO₂, balance N₂; pH 7.4). The blood vessel image was projected onto a video monitor and internal diameters continuously monitored by a video dimension analyzer (Living Systems) and BIOPAC data acquisition system (Santa Barbara, CA). Animal procedures were approved by the Ohio State University Animal Care and Use Committee. Human volunteers gave informed consent, and the biopsy procedure was approved by the Ohio State University human subjects IRB Committee.

Experimental Protocol. Blood vessels were allowed to equilibrate for 30 to 40 min before commencing experiments. Concentration-effect curves to the selective α₁-adrenergic agonist phenylephrine, the selective α₂-AR agonist UK14,304 [5-bromo-N-(4,5-dihydro-1H-imidazol-2-yl)-6-quinoxalinamine], or to phenylpropanolamine were generated by increasing the concentration of the agonists in half-log increments, once the constriction to the previous concentration had stabilized (Flavahan et al., 1984; Chotani et al., 2000). Following completion of the concentration-effect curve, the influence of the agonists was terminated by repeatedly exchanging the buffer solution and allowing the blood vessels to return to their stable baseline levels. In some experiments, concentration-effect curves to phenylpropanolamine were determined under control conditions and then in the presence of the selective α₁-adrenergic antagonist rauwolscine (10⁻⁷ M) and/or the selective α₂-adrenergic antagonist prazosin (3 × 10⁻⁷ M) (Flavahan et al., 1984). When these inhibitors were used, the blood vessels were incubated for 30 min with the drugs prior to and during exposure of the arteries to the agonist. Experiments were also performed to confirm that repeated exposure of blood vessels to phenylpropanolamine in the absence of antagonists evoked similar constrictor responses. In some experiments, endothelial cells of mesenteric and tail arteries were removed by gently placing a wire (70 μ in diameter) through the vessel lumen. This procedure abolished endothelium-dependent relaxation to acetylcholine (10⁻⁵ to 10⁻⁶ M), assessed during constriction to phenylephrine (by ~35% baseline diameter).

Drugs. Acetylcholine chloride, phenylephrine hydrochloride, phenylpropanolamine hydrochloride, prazosin hydrochloride, rauwolscine hydrochloride, and UK14,304 were obtained from Sigma-Aldrich (St. Louis, MO). Stock solutions of drugs were prepared freshly each day and stored at 4°C during the experiment. Drugs were dissolved in distilled water with the exception of UK14,304, which was dissolved in dimethyl sulfoxide (highest chamber concentration of 0.001%). Drug concentrations are described as final molar concentration (moles/liter) in the chamber superfusate.

Data Analysis. Vasoconstriction and vasodilation were expressed as a percentage of the internal diameter of the blood vessel prior to administrating the agent. Because of the phasic behavior of vasmotion in tail arteries, the signal was electronically averaged to obtain diameter measurements (Chotani et al., 2000). Data are expressed as means ± S.E.M. for n number of experiments, where n equals the number of animals or humans from which blood vessels were studied. Because intense constriction of isolated blood vessels may result in arterial injury, constrictor responses were restricted to ~50% of baseline diameter. Because of this restriction, we were unable to determine the maximal responses to α₁-adrenergic activation. Concentration-effect curves to constrictor agonists were analyzed by determining the agonist concentration causing 15% constriction (CC₁₅) (Chotani et al., 2000). Statistical evaluation of the data was performed by Student’s t test for either paired or unpaired observations. When more than two means were compared, analysis of variance was used. If a significant F value was found, Scheffe’s test for multiple comparisons was employed to identify differences among groups. Values were considered to be statistically different when P was <0.05.

Results

Constriction in Mouse Arteries. When assessed at a P TMP of 60 mm Hg, the internal diameters of tail and mesenteric arteries were similar: 124.9 ± 10.0 μ (n = 16) and 136.3 ± 6.5 μ (n = 10), respectively (P = NS). The selective α₁-adrenergic agonist phenylephrine (10⁻⁹ to 10⁻⁶ M) caused concentration-dependent constriction of both types of artery, with a 2.7-fold higher potency in tail compared with mesenteric arteries (log CC₁₅ values of −6.84 ± 0.08 and −6.41 ± 0.17, respectively; n = 10, P < 0.05) (Fig. 1A). The selective α₂-adrenergic agonist UK14,304 (10⁻⁹ to 10⁻⁷ M) caused constriction of tail arteries (log CC₁₅ of −8.72 ± 0.08, n = 9) but not mesenteric arteries (n = 10) (Fig. 1B).

In addition to vascular smooth muscle α₂-adrenoceptors, which initiate constriction, endothelial cells express α₂-adrenoceptors that can mediate dilation (Flavahan et al., 1989). The inability of α₂-adrenoceptor stimulation to initiate constriction could therefore reflect increased activity of endothelial α₂-adrenoceptors in mesenteric compared with tail arteries. In mesenteric arteries constricted by ~35% of baseline diameter with phenylephrine, UK14,304 (10⁻⁹ to 10⁻⁷ M) caused relaxation (maximal observed effect equal to 10.9 ± 2.5% of baseline diameter, n = 4, P < 0.05), which was abolished by endothelial denudation (Fig. 2A). During a similar degree of constriction with phenylephrine, acetylcholine
(10^{-6} \text{ M}) caused relaxation equal to 33.0 \pm 2.6\% of baseline diameter \((n = 4, P < 0.01)\), completely reversing the phenylephrine constriction. In tail arteries constricted by \(\sim 35\%\) with phenylephrine, UK14,304 \((10^{-9} \text{ to } 10^{-7} \text{ M})\) caused further constriction, negating analysis of endothelium-dependent relaxation (data not shown). Acetylcholine \((10^{-6} \text{ M})\) caused complete endothelium-dependent relaxation of phenylephrine-induced constriction in tail arteries (data not shown). The pattern of vasoconstriction to UK14,304 \((10^{-9} \text{ to } 10^{-7} \text{ M})\) in endothelium-containing and -denuded arteries was similar, causing constriction of tail arteries but not mesenteric arteries (Figs. 1B and 2B).

Phenylpropanolamine \((10^{-7} \text{ to } 3 \times 10^{-4} \text{ M})\) constricted both types of artery but was more potent in tail arteries compared with mesenteric arteries: 62-fold in arteries with endothelium \((\log \text{CC}_{15} \text{ values of } -6.07 \pm 0.25 \text{ and } -4.28 \pm 0.10, \text{ respectively}; \ n = 4, P < 0.001)\) and 93-fold in arteries without endothelium \((\log \text{CC}_{15} \text{ values of } -6.18 \pm 0.12 \text{ and } -4.21 \pm 0.13, \text{ respectively}; \ n = 4, P < 0.001\) (Fig. 3). In mesenteric arteries, constriction to phenylpropanolamine was not significantly affected by the selective \(\alpha_2\)-adrenergic antagonist rauwolscine \((10^{-7} \text{ M})\) but was abolished by the selective \(\alpha_1\)-adrenergic antagonist prazosin \((3 \times 10^{-7} \text{ M})\) (Fig. 4). In contrast, constriction of tail arteries to phenylpropanolamine was significantly inhibited by rauwolscine \((3 \times 10^{-7} \text{ M})\) \((\log \text{ shift of } 1.75 \pm 0.16 \text{ in the agonist concentration-effect curve}, \ n = 6, \ 56\text{-fold shift})\) (Fig. 5) but not significantly affected by prazosin \((3 \times 10^{-7} \text{ M})\), either in the absence or presence of rauwolscine (Fig. 5). In both types of artery, repeated administration of phenylpropanolamine \((10^{-7} \text{ to } 3 \times 10^{-4} \text{ M})\), in the absence of antagonists, caused reproducible constrictor responses (Figs. 4 and 5).

**Constriction in Human Dermal Veins.** At a \(P_{TM}\) of 7.5 mm Hg, the internal diameter of human dermal veins was...
Selective 1-adrenergic agonist phenylephrine and the selective 2-adrenergic agonist UK14,304 each caused constriction of human veins (log CC15 values of 6.43 ± 0.18 and 8.05 ± 0.13, respectively; n = 8) (Fig. 6A). Phenylpropanolamine caused constriction of human veins (log CC15 value of 5.30 ± 0.03, n = 8) that was significantly inhibited by the selective 2-adrenergic antagonist, rauwolscine (10⁻⁷ M) (log shift of 1.46 ± 0.14, n = 4, P < 0.005, 29-fold shift) but was not affected by the selective 1-adrenergic antagonist prazosin (3 × 10⁻⁷ M) (Fig. 6B). As with mouse blood vessels, repeated administration of phenylpropanolamine in the absence of antagonists caused reproducible constriction in human veins (data not shown).

Discussion

Although 1-adrenoceptors mediate constriction of most blood vessels, 2-adrenoceptors have a more restricted distribution in the vasculature. Within the arterial system, smooth muscle 2-adrenoceptors are generally not functional in large arteries, with their activity increasing in distal vessels (Flavahan et al., 1987; Nielsen et al., 1990; Chotani et al., 2000). This reflects variation in 2-adrenoceptor expression by smooth muscle cells, resulting from differential transcrip-
tional activation of $\alpha_2$-adrenoceptor genes (Chotani et al., 2004). In most vascular beds, the activity of $\alpha_2$-adrenoceptors in distal arteries and arterioles remains relatively weak (Steen et al., 1984b; Nielsen et al., 1990; Nase and Boegehold, 1998), whereas in certain systems, notably the cutaneous circulation, $\alpha_2$-adrenergic constrictor activity is greatly increased (Flavahan et al., 1987). Indeed, in the present study, the $\alpha_2$-adrenergic agonist phenylephrine constricted mesenteric and cutaneous tail arteries, whereas $\alpha_2$-adrenoceptor

**Fig. 5.** Effect of $\alpha$-adrenergic antagonists on vasoconstrictor responses to phenylpropanolamine (10$^{-9}$ to 10$^{-6}$ M) in mouse tail arteries. Top panel, responses to phenylpropanolamine were obtained in the absence (□), then in the presence of the selective $\alpha_2$-adrenergic antagonist rauwolscine (10$^{-7}$ M) (●), followed by rauwolscine (10$^{-7}$ M) plus the selective $\alpha_1$-adrenergic antagonist prazosin (3 $\times$ 10$^{-7}$ M) (◆). Middle panel, responses to phenylpropanolamine were obtained in the absence (□) then in the presence of the selective $\alpha_1$-adrenergic antagonist prazosin (3 $\times$ 10$^{-7}$ M) (◆). Bottom panel, in time control experiments, three consecutive concentration-effect curves were obtained to phenylpropanolamine in the absence of antagonists (first curve, □; second curve, □; third curve, ◆). In all experiments, vasoconstriction was assessed as changes in internal diameter of the blood vessels and is expressed as a percentage of the stable baseline diameter. Data are presented as means ± S.E.M. for $n = 3$ to 7.

**Fig. 6.** Vasoconstrictor responses in human isolated dermal veins. A, vasoconstrictor effects of the selective $\alpha_1$-adrenergic agonist, phenylephrine (10$^{-9}$ to 10$^{-6}$ M, ○) or the selective $\alpha_2$-adrenergic agonist, UK14,304 (10$^{-9}$ to 3 $\times$ 10$^{-8}$ M; ●). B, vasoconstrictor effects of phenylpropanolamine (10$^{-7}$ to 3 $\times$ 10$^{-6}$ M) in absence (□) and presence of the selective $\alpha_2$-adrenergic antagonist rauwolscine (10$^{-7}$ M) (●) or the selective $\alpha_1$-adrenergic antagonist, prazosin (3 $\times$ 10$^{-7}$ M) (◆). Vasoconstriction was assessed as changes in internal diameter of the blood vessels and is expressed as a percentage of the stable baseline diameter. Data are presented as means ± S.E.M. for $n = 8$ (A) or 4 (B).
activation with UK14,304 constricted only the tail arteries. This latter effect is mediated by α₂A-αdrenoceptors (Chotani et al., 2000). The failure of α₁-αdrenoceptors to mediate constriction of mesenteric arteries did not reflect activity of endothelial α₂A-αdrenoceptors. Although UK14,304 caused a small endothelium-dependent relaxation in mesenteric arteries, endothelium denudation did not uncover constriction to the α₂-αdrenoergic agonist.

Phenypropanolamine is a low-efficacy agonist at α₁-αdrenoceptors, interacting with similar low affinity at α₁A, α₁B, and α₁D-αdrenoceptors (Kᵣ values of ~9 × 10⁻⁶ M (Minneman et al., 1983; Buckner et al., 2002). In smooth muscle preparations replete with α₁A-αdrenoceptors, phenylpropanolamine was slightly more effective (maximum, 67%; ED₅₀ value, 6 × 10⁻⁵ M) (Buckner et al., 2002). Other α-adrenergic agonists also had increased activity in rat aorta (Buckner et al., 2002), likely reflecting the large receptor reserve (Bognar and Enero, 1988), rather than preferential activity of phenylpropanolamine for α₁B-αdrenoceptors. In the present study, phenylpropanolamine caused constriction of mesenteric arteries, which was abolished by the α₁B-αdrennergic antagonist prazosin but not affected by the α₂A-αdrenoergic antagonist rauwolscine. This α₂-αdrenoergic response to phenylpropanolamine occurred at concentrations similar to those reported previously. All three subtypes of α₁-αdrenoceptors may contribute to constriction of mouse mesenteric arteries (Yamamoto and Koike, 2001; Daly et al., 2002).

Phenypropanolamine is often described as a selective α₂-αdrenoergic agonist. However, previous analyses of smooth muscle contraction were generally restricted to preparations that lacked functional α₂-αdrenoceptors (human, pig, or rabbit urethra; rat vas deferens; rat spleen; rabbit aorta), phenylpropanolamine caused constriction with maximums of 13 to 60% (compared with high-efficacy α₁A-αdrenoceptors, interacting with similar low affinity at α₁A-αdrenoceptors (Kᵣ values of ~9 × 10⁻⁶ M) (Minneman et al., 1983; Buckner et al., 2002). In smooth muscle preparations replete with α₁A-αdrenoceptors, phenylpropanolamine was slightly more effective (maximum, 67%; ED₅₀ value, 6 × 10⁻⁵ M) (Buckner et al., 2002). Other α-adrenergic agonists also had increased activity in rat aorta (Buckner et al., 2002), likely reflecting the large receptor reserve (Bognar and Enero, 1988), rather than preferential activity of phenylpropanolamine for α₁B-αdrenoceptors. In the present study, phenylpropanolamine caused constriction of mesenteric arteries, which was abolished by the α₁B-αdrennergic antagonist prazosin but not affected by the α₂A-αdrenoergic antagonist rauwolscine. This α₂-αdrenoergic response to phenylpropanolamine occurred at concentrations similar to those reported previously. All three subtypes of α₁-αdrenoceptors may contribute to constriction of mouse mesenteric arteries (Yamamoto and Koike, 2001; Daly et al., 2002).

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Phenypropanolamine binds directly to human α₂A-αdrenoceptors with a Kᵣ of 3 × 10⁻⁷ M, demonstrating an approximate 35-fold higher affinity for these receptors compared with α₁-ARs (Minneman et al., 1983; Buckner et al., 2002). Indeed, phenylpropanolamine inhibits sympathetic neuro-transmission and the release of norepinephrine (Davies et al., 1993; Buckner et al., 2002), consistent with activation of prejunctional α₁-αdrenoceptors. The results of the present study demonstrate that phenylpropanolamine functions as a preferential α₂-αdrenoergic agonist in the vasculature. In the tail artery, which unlike the mesenteric artery expresses functional α₂A-αdrenoceptors (Chotani et al., 2000), phenylpropanolamine caused constriction at 62-fold lower concentrations than those needed to activate α₁-αdrenoceptors in mesenteric arteries. Indeed, constriction to phenylpropanolamine in the tail artery was not affected by the α₁-αdrenoergic antagonist prazosin (3 × 10⁻⁷ M) but was profoundly inhibited by the α₂A-αdrenoergic antagonist, rauwolscine. Rauwolscine caused a 55-fold shift in the concentration-effect curve, consistent with a Kᵣ of 2 × 10⁻⁹ M and α₂A-αdrenoceptor antagonism (Flavahan et al., 1984; Guimaraes and Moura, 2001). Indeed, after rauwolscine, phenylpropanolamine had similar sensitivity in tail and mesenteric arteries. However, after α₂A-αdrenoergic blockade in tail arteries, the residual response to phenylpropanolamine was still resistant to inhibition by prazosin, suggesting that phenylpropanolamine had still not reached threshold for activating α₁-αdrenoceptors. Based on sensitivity to agonists and antagonists, the α₁-αdrenoceptors mediating constriction of these blood vessels are similar (Daly et al., 2002). Therefore, these results are consistent with the radioligand binding studies (Minneman et al., 1983; Buckner et al., 2002) and demonstrate that phenylpropanolamine is a preferential α₂-αdrenoergic agonist, activating α₂-αdrenoceptors at lower concentrations (>60-fold) than those required to stimulate α₁-αdrenoceptors. The increased functional selectivity of phenylpropanolamine, relative to its binding activity at these receptors, may reflect a lower efficacy at α₁-αdrenoceptors compared with α₂-αdrenoceptors (e.g., Buckner et al., 2002). The radioligand binding analysis was performed using human α₂A-αdrenoceptors (Buckner et al., 2002), whereas constriction of tail arteries is dependent on the rodent homolog of this receptor (α₂A/D-αdrenoceptor) (Chotani et al., 2000). Therefore, the results suggest that phenylpropanolamine does not discriminate between these receptors.

Activation of vascular α₂-αdrenoceptors generally produces a lower maximal response compared with α₁-αdrenoceptors (Flavahan and McGrath, 1984; Flavahan et al., 1984). In tail arteries, α₂-αdrenoergic stimulation generates a maximum of approximately 30% constriction, whereas α₁-αdrenoergic constriction is capable of almost complete closure of tail and mesenteric arteries. From Fig. 3, the selectivity ratio of phenylpropanolamine between tail and mesenteric arteries (or between α₂- and α₁-αdrenoceptors) is greatest at a low level of response and decreases at higher levels of constriction. This reflects the distinct profile of α₁- and α₂-αdrenoergic vascular responses and the low maximal effect of α₂-αdrenoceptors. Indeed, the increased potency of UK14,304 compared with phenylephrine (Fig. 1) also decreases at higher levels of constriction. In the most extreme case, when responses exceed the maximum response attainable by α₂-αdrenoceptors, then only α₁-αdrenoceptor activity is observed (Flavahan et al., 1984). Concentration-effect curves were assessed at agonist concentrations causing 15% constriction of baseline diameter. Although this may be a relatively low level of constriction for α₁-αdrenoceptors, it represents ~50% of the α₂-αdrenoergic maximum and is the most appropriate level for comparing...
responses (Flavahan et al., 1984; Chotani et al., 2000). Furthermore, because vascular resistance is inversely related to the 4th power of the vessel radius, a 15% decrease in diameter generates a stimulus to decrease blood flow by ~50%. This level of response is therefore of pharmacological and physiological relevance.

In contrast to their selective distribution in the arterial circulation, α-adrenoceptors are widely expressed and functional within the venous system (Flavahan et al., 1984; Steen et al., 1984a,b; Tornebrandt et al., 1985; Sjoberg et al., 1987). In the present study, human small dermal veins responded with constriction to phenylephrine or UK14,304 consistent with the presence of α1 and α2-adrenoceptors. The identity of the α1 and α2-adrenoceptor subtypes was not further characterized. As with mouse arteries, constriction of human veins by phenylephrine was not affected by prazosin but was markedly inhibited by rauwolscine. Rauwolscine caused a 29-fold increase in sensitivity to phenylephrine in mouse veins (Bognar IT and Enero MA, 1988). Therefore, in mouse and human blood vessels, phenylephrine acts as a preferential α2-adrenergic agonist, demonstrating considerable selectivity for this receptor subtype. The potency of phenylephrine was slightly reduced in human veins compared with tail arteries. This was paralleled by a similar decrease in sensitivity to UK14,304, suggesting reduced activity of α2-adrenoceptors rather than any difference in activity of phenylephrine in human small veins compared with large cutaneous veins (Guimaraes and Moura, 2001).

Regulation of nasal venous systems plays a prominent role in controlling mucosal congestion and patency of the nasal cavity (Wang and Lung, 2003). Sympathetic nasal decongestants act by constricting the vasculature of the nasal mucosa, mediated by activation of α1- and/or α2-adrenoceptors. Interestingly, α2-adrenoceptors predominate over α1-adrenoceptors in regulating blood flow and constricting of the collecting veins of the nasal mucosa (Andersson and Bende, 1984; Lacroix and Lundberg, 1989; Wang and Lung, 2003). Indeed, α2-adrenoergic activation has been proposed as a preferred mechanism for nasal decongestion (McLeod et al., 2001). When used as a nasal decongestant, therapeutic administration of phenylephrine (25 mg immediate release) generated peak circulating levels of approximately 90 ng/ml (6 × 10^-7 M) (Saltzman MB, Dolan MM, and Doyne N, 1983) and with its activity in the tail artery. This level of response is therefore of pharmacological and physiological relevance.

References


Stockley CS, Wing LM, Tenkin AL, and Miners JO (1994) Dispositional factors do not...


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