Differences in \(\alpha\)-Adrenergic Responsiveness Between Human Internal Mammary Arteries and Saphenous Veins

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Little is known regarding specific biologic and pharmacologic differences between human internal mammary arteries and saphenous veins. To better define the role of \(\alpha\)-adrenoceptor-mediated vasoconstriction in human internal mammary arteries and saphenous veins, we obtained fresh specimens of both vessels from 32 patients undergoing coronary artery bypass surgery. Dose-response curves were generated for the relatively selective \(\alpha_1\)-receptor agonist phenylephrine, the \(\alpha_2\)-receptor agonist BHT-920, and the \(\alpha_1\) and \(\alpha_2\)-receptor agonist norepinephrine. Phenylephrine elicited similar contractile responses in internal mammary arteries and saphenous veins, with a mean EC\(_{50}\) (the effective concentration necessary to produce 50% of the maximal contraction) of 1.4\(\times\)10\(^{-6}\) M for internal mammary arteries and 1.8\(\times\)10\(^{-6}\) M for saphenous veins (\(p=\text{NS}\)). Selective stimulation of \(\alpha_2\)-receptors with BHT-920 elicited a marked contractile response only in saphenous veins. Dose-response curves for phenylephrine and BHT-920 were shifted to the right for both vessels in the presence of the \(\alpha_2\)-receptor antagonist prazosin and the \(\alpha_2\)-receptor antagonist yohimbine, respectively. Norepinephrine elicited contraction at a lower concentration in saphenous veins than in internal mammary arteries with a mean EC\(_{50}\) of 7.8\(\times\)10\(^{-8}\) M for saphenous veins and a mean EC\(_{50}\) of 3.4\(\times\)10\(^{-7}\) M for internal mammary arteries (\(p<0.05\)). The results suggest that \(\alpha\)-adrenoceptor-mediated vasoconstriction is caused primarily by \(\alpha_1\)-receptors in human internal mammary arteries and by \(\alpha_1\)- and \(\alpha_2\)-receptors in human saphenous veins. (Circulation 1989;79:1264–1270)

A marked difference in the long-term patency rates of saphenous vein and internal mammary artery grafts has been well established.\(^1\)\(^-\)\(^4\) Although the patency rates of both types of vessels are similar during the first few postoperative years, at 10 years, the patency rate of internal mammary artery grafts approaches 95% without significant stenoses, but over 60% of saphenous vein grafts are either occluded or have major atherosclerosis.\(^5\)\(^-\)\(^7\)

The reasons for these differences in patency rates are unclear. Several theories have been proposed. One of the more popular is the possibility that the “vasoactive properties” of the internal mammary artery are protective against atherosclerosis, whereas the absence of such properties in the saphenous vein cause it to be predisposed to plaque formation when the vein is exposed to systemic pressure.\(^1\)

There appears to be something unique about the internal mammary artery other than the general difference between biologic properties of arteries and veins because free radial arteries used as bypass grafts have a substantially higher rate of occlusion than do saphenous veins.\(^8\)

Furthermore, the internal mammary artery is well known to have a very low incidence of atherosclerosis, even in patients with diffuse coronary and peripheral vascular disease, and rarely does the internal mammary artery have to be studied angiographically before surgery.

Another important difference between the human internal mammary artery and saphenous vein relates to flow rates in the two vessels when used as conduits for coronary artery bypass grafts. Although the saphenous vein graft has a predictably high flow rate, which appears to be directly proportional to vessel caliber, the flow rate in the internal mammary artery conduit is highly variable with markedly different flow rates for vessels of the same...
apparent caliber. This finding has led many surgeons to avoid using the internal mammary artery as a conduit in patients who are hemodynamically unstable and who need high flow rates immediately after surgery. However, little is known regarding the differential pharmacology of the human internal mammary artery and saphenous vein. Intraoperative flow studies have failed to yield significant differences between the response of both vessels to various pharmacologic agents. However, multiple problems exist in these intraoperative studies. Foremost is the inability to control the multiple variables that may hide significant differences in the direct effects of agonists on the two vessels.

Previous animal studies in situ and in vitro have suggested that large conduit vessels often lack \( \alpha \)-adrenoceptor function and thus differ from many veins. Therefore, human internal mammary arteries and saphenous veins may differ in the subtypes of \( \alpha \)-adrenoceptors present. To our knowledge, this had not been studied in the human internal mammary arteries. Furthermore, a preliminary study on postmortem samples of human saphenous veins suggested that the tissue may have rather sparse \( \alpha \)-adrenoceptors because of the lack of responsiveness to clonidine. However, because of the partial agonist properties of clonidine, the interpretation of these results is difficult.

Therefore, as an initial step to understand the pharmacologic responsiveness of these vessels used for coronary artery bypass grafts, we attempted to characterize the \( \alpha \)-adrenoceptor subtypes in vitro for human internal mammary arteries and saphenous veins.

Methods

Tissue Preparation

Saphenous vein and left internal mammary artery segments obtained from 32 patients undergoing coronary artery bypass graft surgery were studied. The mean age of the patients was 65 years (range, 55–75 years). Twenty-two were men and 10 were women. Twenty-nine patients were treated before surgery with nitrates, calcium channel blockers and \( \beta \)-blockers. Of the remaining three patients, two were treated with nitrates and \( \beta \)-blockers, and one was treated with nitrates and calcium channel blockers. All patients were anesthetized in a similar fashion with opioid anesthesia with oxygen and occasional low-dose inhalational agents. In 24 patients, both saphenous vein and left internal mammary artery segments were obtained; in six patients, only saphenous vein segments were obtained, and in two patients, only left internal mammary artery segments were obtained. Use of the tissue was approved by the Human Studies Committee of Beth Israel Hospital.

The saphenous veins were excised from the upper thighs and calves and measured for amount needed for bypass grafting. A 5 mm to 1 cm segment of excess tissue was cut from the distal end. The internal mammary artery was dissected on a broad pedicle from the left anterior chest wall. The distal pedicle was excised. In the operating room, both internal mammary artery and saphenous vein specimens were immediately placed in oxygenated physiologic saline solution (PSS) and carried to the laboratory where both vessels were further dissected from surrounding muscle, fascia, and adventitia while being oxygenated in PSS. After dissection, the vessels were cut longitudinally, and 1 mm x 3–10 mm circular strips were cut from the vessel according to techniques previously described. The endothelium was removed by gentle abrasion with a rubber-coated applicator in a manner that has been shown morphologically by others to result in essentially complete removal of the endothelium. We have confirmed, functionally, that this is effective in a wide variety of animal and human vessels. Maintaining an intact endothelium is very difficult, and we have found, in separate preliminary studies, that the endothelium of human tissues is generally destroyed by routine surgical handling. Therefore, for consistency, we purposely eliminated the endothelium in all of these studies. A Surgical Tevdek II 9-0 suture (Deknatel, Queens Village, New York) was tied to either end of the strips.

Muscle Tension Recording

The saphenous vein and internal mammary strips were placed into organ baths containing oxygenated PSS and were attached at one end to a muscle holder, and at the other end to a Gould UC-2 strain gauge transducer (Cleveland, Ohio). Care was taken to keep the muscle strips oxygenated continuously in the PSS. Resting length was recorded, after which the strips were stretched to 150% of resting length to approximate L\(_{max}\). The signal obtained was amplified by a Neilson transducer amplifier (Rochester, Minnesota) and recorded on a Gould thermal chart recorder. The muscle strips were allowed to equilibrate at room temperature (22° C) in the PSS for 2 hours or until a steady state was reached. Next, the organ bath was warmed to 37° C with the use of a Lauda circulating water bath (Brinkman Instruments, Westbury, New York). The muscle strips were again allowed to equilibrate for 2 hours or until a steady state was reached.

Solutions of elevated potassium concentration were obtained by equimolar replacement of sodium chloride with potassium chloride in the PSS. Baseline responses to 96 mM K\(^+\) were obtained in all muscle strips. Any muscle strips pulling less than 200 mg net force were excluded from the study. We excluded these specimens because previous studies evaluating vascular smooth muscle from various species showed that if muscle strips of similar size to our specimens pull less than this amount of force, they are likely not viable. After the response to potassium, muscle strips were washed four times with normal PSS and again allowed to equilibrate.
for 2 hours or until steady state was achieved. All muscle strips returned to baseline tension or were excluded. Phenylephrine, BHT-920, and norepinephrine were injected, in various concentrations, directly into the organ baths to reach the final concentration indicated on the dose-response curves with washout by the aforementioned technique. Drug selection was purposefully random to ensure that the order in which the drugs were used did not alter the responses. Phenylephrine was used as a prototype of a relatively selective α1-receptor agonist and BHT-920 of a relatively selective α2-receptor agonist; however, it should be kept in mind that neither of these drugs are absolutely selective and can have effects on other receptor types. In this context, it should be mentioned that phenylephrine has been reported to have some β-receptor agonist properties in some preparations,18,19 although we saw no evidence for such properties in this study.

Dose-response curves were repeated for phenylephrine and BHT-920 in the presence of the α1-receptor antagonist prazosin and the α2-receptor antagonist yohimbine, respectively. In all experiments used, muscle strips were challenged with 96 mM K+ at the end of the experiment and were within 20% of force generated at the beginning.

Solutions and Drugs

The PSS used throughout this study was a Kreb’s solution that contained (mM): Na 137.4, K+ 5.9, Ca 2.5, Mg 1.2, Cl 134, bicarbonate 15.5, hydrogen phosphate 1.2, dextrose 11.5. It was bubbled with a 95% O2-5% CO2 gas mixture before and during use in any part of the experiment maintaining the pH between 7.3 and 7.4. The α-receptor agonist BHT-920 [6-allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo-(4,5-d)]azepin-dihydro-chloride; Boehringer Ingelheim Pharmaceuticals, Ridgefield, Connecticut] and phenylephrine (CIBA Pharmaceutical, Summit, New Jersey) were dissolved in distilled water. Norepinephrine (Sigma Chemical, St. Louis, Missouri) was dissolved in distilled water and a 0.2-mM concentration of ethylenediaminetetraacetic acid (EDTA) (final bath concentration). Prazosin (Pfizer Laboratories, New York, New York) was dissolved in dimethyl sulfoxide (DMSO) and distilled water. Yohimbine (Sigma) was dissolved in warm ethanol and distilled water.

Data Analysis

For normalization, all of the responses to the various agonists and antagonists were plotted as a percentage of the maximal contraction to potassium for each muscle strip. The EC50 (effective concentration of an agonist required to cause 50% of the maximal contractile response to the agonist) was determined for each individual strip to each agonist tested. Cross-sectional area was approximated from the length and weight of the tissue with tension being given in newtons per square meter of cross-sectional area.17 Statistical analysis was by the two-tailed t test.

Results

The mean dose-response curves for the effects of phenylephrine on the human left internal mammary artery and human saphenous vein strips are plotted in Figure 1. The contractile responses of 16 individual internal mammary artery strips and 12 saphenous vein strips obtained from 12 different patients are expressed as a percentage of the maximal tension generated to potassium. The curves for both vessels are similar; the mean EC50 for the internal mammary arteries was 1.5×10^-6 M, and for the saphenous veins it was 1.8×10^-6 M (p=NS). In the presence of the α1-receptor antagonist prazosin (n=5), both curves are shifted to the right (Figure 1).

Figure 2 illustrates typical recordings comparing the responses of the left internal mammary arteries and saphenous veins from the same patient to the relatively selective α2-receptor agonist BHT-920. The recordings reveal markedly different responses.
The internal mammary artery strip contracts weakly to increasing concentrations of BHT-920, whereas the saphenous vein strip shows a marked contraction beginning at a concentration of $1\times10^{-7}$ M. These differences in responsiveness were found in all strips studied. The effects of BHT-920 on 15 internal mammary artery and 12 saphenous vein strips obtained from 12 different patients are summarized in Figure 3. The mean dose-response curves for both vessels to BHT-920 are plotted as a percentage of the maximal tension to potassium. The internal mammary artery strips contract little even at relatively high doses of BHT-920, whereas the saphenous vein strips show a characteristic sigmoid-shaped dose-response curve. The mean $EC_{50}$ is $1.7\times10^{-6}$ M for the internal mammary artery strips and $1.9\times10^{-7}$ M for the saphenous vein strips ($p<0.05$). In the presence of the $\alpha_2$-receptor antagonist yohimbine ($n=6$), both curves are shifted to the right (Figure 3). The mean maximal contraction (expressed as a percentage of maximal contraction to potassium±SEM) was $14\pm3\%$ for the internal mammary arteries and $73\pm6\%$ for saphenous veins ($p<0.001$).

Figure 4 illustrates the mean dose-response curves for norepinephrine in 10 left internal mammary artery and nine saphenous vein strips from seven patients. Both vessels contracted in a dose-related fashion to norepinephrine. However, the dose-response curve is shifted to the left for the saphenous vein strips. This difference is reflected in the $EC_{50}$ for the effect of norepinephrine on both vessels (calculated for each individual experiment), which was $7.8\times10^{-9}$ M for the saphenous vein strips and $3.4\times10^{-7}$ M for the internal mammary artery strips ($p<0.001$).

The left internal mammary artery strips were more responsive to potassium than were the saphenous vein strips. The mean $EC_{50}$ for potassium was $17$ mM for the internal mammary artery strips and $23$ mM for the saphenous vein strips ($p<0.01$). However, the mean maximal force per unit area ($\pm$SEM, $n$) generated to potassium was $2.8\times10^{4}$ N/m$^2$ ($\pm0.6$ N/m$^2$, $n=11$) for internal mammary artery strips and $5.0\times10^{4}$ N/m$^2$ ($\pm0.9$ N/m$^2$, $n=10$) for saphenous vein strips ($p=NS$).

The findings of similar contractile responses to phenylephrine by both saphenous vein and left internal mammary artery strips, marked contractile responses of saphenous vein strips to BHT-920 with little or no response in the internal mammary artery strips, and similar contractile responses to norepinephrine with the saphenous vein strips contracting at lower concentrations occurred regardless of order.

**Figure 2.** Tracings of the response of single left internal mammary artery (LIMA) and saphenous vein (SV) strips from the same patient compared with increasing doses of BHT-920.

**Figure 3.** Cumulative dose-response curves (expressed as percent of maximal tension to potassium) of the human isolated left internal mammary artery (LIMA) and saphenous vein (SV) to BHT-920 in the presence of ($n=5$) and absence ($n=14$) of yohimbine ($\pm$SEM). Lines were fitted to the data points by eye.
of agonists used. Difference in age, sex, or preoperative medications used by the patients made no demonstrable difference in the contractile responses of the saphenous vein and internal mammary artery strips. The mean EC$_{50}$ for each agonist tested is summarized in Table 1.

**Discussion**

Because of its excellent long-term patency rate, the internal mammary artery is increasingly used as a coronary artery bypass conduit.$^1$ However, because there are only right and left internal mammary arteries of finite length for any given patient, rarely can the arteries be used as the sole bypass graft.$^8$ For this reason, as well as the occasional low initial flow rates present in internal mammary artery conduits, saphenous veins are used in addition to internal mammary arteries in most patients undergoing coronary artery bypass graft surgery.$^1$ With the marked differences in long-term patency and short-term flow rates between internal mammary arteries and saphenous veins, a better understanding of the pharmacologic and biologic properties of these two vessels is warranted. Our study focused on the differences in α-receptor characteristics between both vessels.

α-Receptors can be subdivided into α$_1$- and α$_2$-subtypes based on their responses to different types of agonists.$^{20-25}$ α$_1$-Receptors were originally thought to be located exclusively on postsynaptic sites and that α$_2$-receptors were located presynaptically on adrenergic terminal axons.$^{26}$ The α$_1$-receptors were believed to be excitatory, leading to vascular smooth muscle contraction, whereas the α$_2$-receptors were believed to be only inhibitory, leading to vascular smooth muscle relaxation. In the mid 1970s, α$_2$-receptors were found on postsynaptic sites and in vascular smooth muscle from various species of animals.$^{21,22,27-33}$ Rather than having an inhibitory action such as that caused by presynaptic α$_2$-receptor stimulation, stimulation of postsynaptic α$_2$-receptors was excitatory, causing vascular smooth muscle contraction. During the past several years, evidence has been well established for the existence of postsynaptic α$_1$- and α$_2$-receptors that mediate vasoconstriction and maintenance of basal tone in arteries and veins in humans.$^{34-37}$

Our initial observation that the relatively selective α$_1$-receptor agonist phenylephrine caused contraction in both the human internal mammary artery and saphenous vein suggested that α$_1$-receptors are present in both vessels. We compared these and subsequent responses to the maximum contractile response elicited by potassium. Potassium served as a control because no significant difference was found between the maximum force generated per unit area by potassium in both vessels. Compared in this fashion, the cumulative dose-response curves for both types of vessels were nearly superimposable, and the mean EC$_{50}$ for phenylephrine for both types of vessels was not significantly different. This suggests that the activity of α$_1$-adrenoceptors in both vessels is similar. In contrast, the relatively selective α$_2$-receptor agonist BHT-920 caused little contractile response in the internal mammary artery but a marked response in the saphenous vein, suggesting that α$_2$-receptors are present in the human saphenous vein but not in the internal mammary artery. These findings are consistent with studies on canine saphenous veins, and they also help to clarify the confusing results previously obtained with the partial α$_2$-receptor agonist clonidine in saphenous veins from human cadavers.$^{13}$ Also, these are the first findings to suggest the absence of α$_2$-receptors in the human internal mammary artery.

**TABLE 1.** Mean EC$_{50}$ of α-Receptor Agonists and Potassium in Human Internal Mammary Artery and Saphenous Vein Muscle Strips

<table>
<thead>
<tr>
<th>Vessel</th>
<th>K$^+$ (mM)</th>
<th>Phenylephrine ($\times 10^{-6}$ M)</th>
<th>BHT-920 ($\times 10^{-7}$ M)</th>
<th>Norepinephrine ($\times 10^{-7}$ M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left internal mammary artery</td>
<td>17±1.4</td>
<td>1.4±0.2</td>
<td>17±6.0</td>
<td>3.4±0.6</td>
</tr>
<tr>
<td>Saphenous vein</td>
<td>23±1.6</td>
<td>1.8±0.2</td>
<td>1.9±0.7</td>
<td>0.78±0.03</td>
</tr>
<tr>
<td>Difference</td>
<td>p&lt;0.01</td>
<td>NS</td>
<td>p&lt;0.05</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

All values are mean±SEM.
The rightward shift in the dose-response curves of both vessels to phenylephrine and BHT-920 in the presence of the \(\alpha\)-receptor antagonists prazosin and yohimbine, respectively, indicates that the differences noted in \(\alpha\)-adrenoceptor responsiveness between both vessels are indeed secondary to differences in \(\alpha\)-adrenoceptor activity.

The question arises whether the observed differences in \(\alpha\)-adrenoceptor function could result from differences in presynaptic \(\alpha\)-adrenoceptors on nerve terminals that may be present in these in vitro strips. The isolation of single smooth muscle cells would be required to completely answer this question. However, presynaptic \(\alpha\)-adrenoceptors are generally thought to be inhibitory with respect to transmitter release, and Rorie et al have shown that there is little basal release of norepinephrine from human saphenous veins. Also, because these are inhibitory actions, it would be difficult to explain the differences in contractile amplitude in response to the application of the relatively selective \(\alpha\)-adrenoceptor subtype agonists.

The hypothesis that human internal mammary arteries possess only \(\alpha_1\)-receptors and human saphenous veins possess both \(\alpha_1\) and \(\alpha_2\)-receptors was tested further when we exposed both vessels to norepinephrine. Norepinephrine, having both \(\alpha_1\)- and \(\alpha_2\)-receptor agonist properties, may be expected to cause a larger response at lower concentrations in a vessel with both types of receptors present. During each experiment, the saphenous vein strips contracted at a lower concentration than did the internal mammary artery strips. The cumulative dose-response curve for the effect of norepinephrine on the saphenous veins was shifted to the left when compared with that of the internal mammary arteries until a maximum tension was reached. In addition, the mean \(EC_{50}\) for norepinephrine for the saphenous vein strips was significantly lower than that for the internal mammary artery strips. These findings suggest the recruitment of both \(\alpha_1\)- and \(\alpha_2\)-receptors in human saphenous veins and only \(\alpha_1\)-receptors in internal mammary arteries. This also confirms what was seen with the individual \(\alpha_1\)- and \(\alpha_2\)-receptor agonists. Therefore, our findings strongly support the presence of \(\alpha_1\)- and \(\alpha_2\)-receptors in human saphenous veins and \(\alpha_1\)-receptors alone in human internal mammary arteries.

These differences in receptor properties may have therapeutic implications; however, further information is necessary before predictions could be made on the overall effect of selective agonists or antagonists of \(\alpha\)-adrenoceptor subtypes on the intact coronary circulation in disease states. In particular, animal studies have not only indicated that \(\alpha_2\)-receptors can be sparse in large conduit arteries, as we have found in the human internal mammary artery, but have also indicated that \(\alpha_2\)-receptors may predominate in resistance vessels and small coronary arteries.10,11 Thus, it would be difficult to predict the effect of an \(\alpha_2\)-receptor vasopressor on the intact human coronary circulation. Because only \(\alpha_1\)-receptors appear to be present in internal mammary arteries, however, the topical application of an \(\alpha_1\)-receptor antagonist may prove prudent.

It is well known that the presence of an intact endothelium may alter responses of vascular smooth muscle to a variety of agonists.39 Because of the difficulty in maintaining an intact endothelium for the prolonged periods necessary for our protocol, we purposefully removed the endothelium at the beginning of each experiment. Recently, differences also were reported in the endothelium-dependent responses of human internal mammary artery and saphenous vein grafts.40 In addition, further studies, including studies of the effect of \(\beta\)-receptor agonists and studies with long-term denervated specimens, need to be performed. However, our study is the first to document specific biologic differences in receptor properties between the smooth muscle of internal mammary arteries and saphenous veins. These findings may have important implications for differences in long-term patency rates.

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