Modulatory role of a constitutively active population of α1D-adrenoceptors in conductance arteries

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Ziani, Khalid, Regina Gisbert, Maria Antonia Noguera, Maria Dolores Ivorra, and Pilar D’Ocon. Modulatory role of a constitutively active population of α1D-adrenoceptors in conductance arteries. Am J Physiol Heart Circ Physiol 282: H475–H481, 2002. First published October 18, 2001; 10.1152/ajpheart.00411.2001.—A constitutively active population of α1D-adrenoceptors in iliac and proximal mesenteric arteries was studied. The increase in resting tone (IRT) that evidences it was observed only in iliac and proximal mesenteric and was inhibited by prazosin (pIC50 = 9.57), 5-methylurapidil (pIC50 = 7.61), and BMY 7378 (pIC50 = 8.77). Chloroethylchlonidine (100 μmol/l) did not affect IRT, but when added before the other antagonists it blocked their effect. The potency shown by BMY 7378 confirms the α1D-subtype as responsible for IRT. BMY 7378 displayed greater inhibition of adrenergic responses in iliac (pIC50 = 7.57 ± 0.11) and proximal mesenteric arteries (pIC50 = 8.05 ± 0.2) than in distal (pIC50 = 6.94 ± 0.13) or small mesenteric arteries (pIC50 = 6.30 ± 0.14), which confirms the functional role of the α1D-adrenoceptor in iliac and proximal mesenteric arteries. This subtype prevents abrupt changes in iliac and proximal mesenteric artery caliber when the agonist disappears, and this modulatory role is evidenced by the slower decay in the response to norepinephrine after removal.

Conductance vessels; resistance vessels; constitutive activity; α1D-adrenoceptors

It has been clearly shown that activation of α1-adrenoceptors mediates vasoconstriction, and considerable progress has been made toward elucidating the molecular structures and signal transduction mechanisms of these adrenoceptors. Molecular cloning studies have identified three α1-adrenoceptor cDNAs (α1A, α1B, and α1D), and their primary structure corresponds to the model of the superfamily of G protein-coupled receptors. Moreover, three distinct α1-adrenoceptor subtypes (α1A, α1B, and α1D) that correlate well with the cloned α1-adrenoceptors have been identified pharmacologically in functional and binding experiments (8, 9). These three subtypes display high, subnanomolar affinities for prazosin. Furthermore, functional studies have provided evidence of the existence of an additional α1L-adrenoceptor subtype displaying low affinity for prazosin and some other α1-adrenoceptor antagonists (3, 4, 23, 33). This α1L-adrenoceptor has no molecular correlate. However, the physiological role of each α1-adrenoceptor in the vascular smooth muscle is unclear, and the expression of an α1-adrenoceptor subtype in a vessel is not always related to a functional role of this subtype in the contractile tone of the vessel (10, 12, 16, 17, 29, 33).

In addition to the complex functionality of the different subtypes of α1-adrenoceptors, we have shown in previous studies (7, 24, 26) the existence of a population of constitutively active α1D-adrenoceptors in the rat aorta but not in the tail artery. The existence of this active conformation has been revealed mainly in artificial models such as receptor mutants or systems that show an overexpression of a certain type of receptor (1, 13, 18, 19, 21, 31). However, until now, only our studies in native tissues (7, 24, 26) and two recent papers (5, 20) on cloned adrenoceptors have given new evidence of the existence of a population of constitutively active α1D-adrenoceptors.

In our previous work, we suggested that this subtype, by remaining in an active state when the agonist is removed, could be responsible for the slower disappearance of the contractile response to the agonist in the aorta than in the tail artery, but further studies in different vessels are needed to confirm this hypothesis. The present report analyzes the constitutive activity of α1D-adrenoceptors by examining its functional role in the contractile tone of different rat vessels, including conductance and resistance arteries, to glean more information on the physiological implications of the constitutive activity of α1D-adrenoceptors in the functionality of the cardiovascular system.

METHODS

Rings of the aorta, iliac artery, and proximal or distal (with respect to the aorta) mesenteric arteries (~3–5 mm in length) of female Wistar rats (200–220 g) were denuded of the endothelium by gentle rubbing and suspended in a 10-ml organ bath containing physiological solution maintained at...
37°C and gassed with 95% O₂-5% CO₂. An initial load of 1 g was applied to each preparation and maintained throughout a 75- to 90-min equilibration period. After this time, contractile responses to agonists were elicited according to the experimental procedures described in Experimental Design. The pretension of 1 g was kept constant, but there was a loss of tension (<10–15%) when the preparations were placed in Ca²⁺-free medium. Tension was recorded isometrically by Grass FTO3 force-displacement transducers, and data were recorded on a disc (MacLab).

Mesenteric arterial trees were dissected and cleared of surrounding adipose tissue. As described previously (22), a ring segment (2 mm in length) from the second branch of the arterial tree was mounted in a myograph (J. P. Trading; Aarhus, Denmark) with 6-ml organ baths containing physiological solution at 37°C and was gassed with 95% O₂-5% CO₂. After a 30-min stabilization period, the internal diameter of each vessel was set to a tension equivalent to 0.9 times the estimated diameter at 100 mmHg of effective transmural pressure (l_{100} = 90–180 μm) according to the standard procedure of Ref. 22. Tension was recorded isometrically, and data were recorded on a disk (MacLab).

The absence of relaxant response (>10%) after acetylcholine (10 μmol/l) addition to preparations precontracted with noradrenaline (1 μmol/l) indicated the absence of a functional endothelium in all the rings.

**Experimental Designs**

Maximal response to α₁-adrenoceptor agonists. A single agonist curve was obtained by cumulative addition of several concentrations of phenylephrine (1 nmol/l–100 μmol/l) or norepinephrine (1 nmol/l–100 μmol/l) to determine the concentration of each agonist needed to obtain the sustained maximal contractile response in each tissue. This concentration was 10 μmol/l in iliac and proximal and distal mesenteric arteries and 30 μmol/l in small mesenteric arteries.

Experimental procedure that evidences the constitutive activity of α₁D-adrenoceptors. Figure 1 shows the experimental procedure designed to study the depletion of intracellular Ca²⁺ stores sensitive to norepinephrine in Ca²⁺-free medium and the increase in resting tone (IRT) obtained in iliac and PMA by subsequent exposure to Ca²⁺-containing solution during the refilling of the NE-sensitive Ca²⁺ stores. Agonist was added in Ca²⁺-containing solution (Ca²⁺), and, after the tissues were washed (W) and the basal tone recovered, the tissue was incubated for 20 min in Ca²⁺-free EDTA-containing solution (Ca²⁺-free). After this time, the agonist was applied (NE1 and NE2), and the tissue was washed until no contraction was induced, indicating complete depletion of internal Ca²⁺ stores sensitive to NE. The tissue was then incubated for 20 min in Krebs solution, and a spontaneous IRT (IRT1) of the iliac and PMA (but not the DMA or SMA) was observed. After the tissues were washed and loaded for 20 min in Ca²⁺-free solution, NE (NE3 and NE4) was added again. In the experiments designed to assess the effects of different α₁-adrenoceptor antagonists on IRT, the arteries were pretreated with different concentrations of these agents 10 min before and during (20 min) the second IRT (IRT2) was induced with a new loading in Krebs solution.

Fig. 1. Experimental procedure designed to study the depletion of intracellular Ca²⁺ stores sensitive to norepinephrine (NE) in the iliac (A) and proximal (PMA; B), distal (DMA; C), and small mesenteric arteries (SMA; D) in Ca²⁺-free medium and the increase in resting tone (IRT) obtained in the iliac and PMA by subsequent exposure to Ca²⁺-containing solution during the refilling of the NE-sensitive Ca²⁺ stores. Agonist was added in Ca²⁺-containing solution (Ca²⁺), and, after the tissues were washed (W) and the basal tone recovered, the tissue was incubated for 20 min in Ca²⁺-free EDTA-containing solution (Ca²⁺-free). After this time, the agonist was applied (NE1 and NE2), and the tissue was washed until no contraction was induced, indicating complete depletion of internal Ca²⁺ stores sensitive to NE. The tissue was then incubated for 20 min in Krebs solution, and a spontaneous IRT (IRT1) of the iliac and PMA (but not the DMA or SMA) was observed. After the tissues were washed and loaded for 20 min in Ca²⁺-free solution, NE (NE3 and NE4) was added again. In the experiments designed to assess the effects of different α₁-adrenoceptor antagonists on IRT, the arteries were pretreated with different concentrations of these agents 10 min before and during (20 min) the second IRT (IRT2) was induced with a new loading in Krebs solution.
enteric arteries by subsequent exposure to Ca\(^{2+}\)-containing solution during the refilling of these stores.

Concentration-response curves of inhibition of the IRT to selective \(\alpha_1\)-adrenoceptor antagonists. In a separate series of experiments, the effects of prazosin (0.001 nmol/l–1 \(\mu\)mol/l), 5-methylurapidil (0.001 nmol/l–1 \(\mu\)mol/l), 8-[2-[4-(2-methoxyphenyl)-1-piperazynil]-8-azaspiro[4,5]decane-7,9-dione dihydrochloride (BMY 7378; 0.001 nmol/l–1 \(\mu\)mol/l), and chlorothelyclidone (100 \(\mu\)mol/l) were assessed on IRT in the aorta, iliac, and proximal mesenteric arteries. In this case, the experimental procedure was similar to that shown in Fig. 1, but 10 min before and during the second loading period in Ca\(^{2+}\)-containing solution for the refilling of internal Ca\(^{2+}\) stores previously depleted by norepinephrine, a given concentration of each antagonist was added. The magnitude of the second IRT (IRT2) in the presence of each concentration of each compound was expressed as a percentage of the reference IRT (IRT1) obtained in the absence of any agent (see Fig. 1).

Taking into account the fact that chlorothelyclidone behaves as a neutral antagonist (7), in the aorta and iliac artery, where chlorothelyclidone (100 \(\mu\)mol/l) did not elicit any contractile response, we tested the effect of 30 min of preincubation with this alkylating agent on the inhibitory effect that prazosin and BMY 7378 have on the IRT. The experimental procedure was similar to that shown in Fig. 1, but 30 min before and during the second loading period in Ca\(^{2+}\)-containing solution for the refilling of internal stores, chlorothelyclidone (100 \(\mu\)mol/l) was added, and 10 min before and during this loading period, prazosin (10 \(\mu\)mol/l) or BMY 7378 (10 \(\mu\)mol/l) was also added; the magnitude of the IRT2 in presence of these compounds was expressed as a percentage of IRT1 obtained in the absence of any agent.

Concentration-response curves of relaxation to selective \(\alpha_1\)-adrenoceptor antagonists. Concentration-response curves were performed by addition of cumulative concentrations of prazosin (0.01 nmol/l–1 \(\mu\)mol/l), 5-methylurapidil (0.001 nmol/l–10 \(\mu\)mol/l), cyclazosin (0.001 nmol/l–1 \(\mu\)mol/l), and BMY 7378 (0.001 nmol/l–1 \(\mu\)mol/l) to tissues in which sustained contractions had been induced by a maximal concentration of phenylephrine (10 \(\mu\)mol/l) or norepinephrine (30 \(\mu\)mol/l). Relaxations were expressed as a percentage of the maximum increment in tension obtained by agonist addition.

Analysis of Results

Contractions were expressed in millinewtons of developed tension or as a percentage of the agonist-induced contractions obtained in normal physiological solution. Increases in resting tone were also expressed as a percentage of the agonist-induced contraction in normal physiological solution.

The concentration (−log [mol/l]) needed to produce 50% relaxation or inhibition (pIC\(_{50}\)) and the Hill slope of the curve (nH) were obtained from a nonlinear regression plot (GraphPad Software; San Diego, CA) from at least seven concentrations. The results are presented as means ± SE; n is the number of determinations obtained from different animals.

Chemicals

The following drugs were obtained from Sigma (St. Louis, MO): phenylephrine, \(l\)-norepinephrine, prazosin, and cyclazosin, and the following drugs were obtained from Research Biochemicals International (Natick, MA): BMY 7378, chlorothelyclidone and 5-methylurapidil. Other reagents were of analytic grade. All compounds were dissolved in distilled water.

The composition of the physiological Ca\(^{2+}\)-containing solution was (in mmol/l) 118 NaCl, 4.75 KCl, 1.8 CaCl\(_2\), 1.2 MgCl\(_2\), 1.2 KH\(_2\)PO\(_4\), 25 NaHCO\(_3\), and 11 glucose. Ca\(^{2+}\)-free solution had the same composition except that CaCl\(_2\) was omitted and EDTA (0.1 mmol/l) was added.

RESULTS

Figure 1 shows the experimental procedure designed to study the depletion of intracellular Ca\(^{2+}\) stores sensitive to norepinephrine and the IRT obtained by subsequent exposure to Ca\(^{2+}\)-containing physiological solution during the refilling of these stores. Norepinephrine (10 \(\mu\)mol/l in iliac and proximal or distal mesenteric arteries or 30 \(\mu\)mol/l in small mesenteric arteries) evoked a sustained contraction, which was used to monitor the maximal response obtained with this agonist in each preparation. The magnitude of the maximal responses obtained in each tissue are summarized in Table 1. After the arteries were carefully washed, the return to the baseline was slower in the iliac and proximal mesenteric arteries than in the distal or small mesenteric arteries (Fig. 2). Basal tone recovery in the iliac artery took 843 ± 79 \(s\) (n = 11) and in the proximal mesenteric artery 545 ± 27 \(s\) (n = 5), whereas in the distal mesenteric artery, basal tone was recuperated in only 279 ± 66 \(s\) (n = 5) and in the small mesenteric arteries in 103 ± 16 \(s\) (n = 10).

We then changed to a Ca\(^{2+}\)-free solution, and, after 20 min in this medium, the addition of norepinephrine (1, 10, or 30 \(\mu\)mol/l) also induced a small contraction (NE1 in Fig. 1), which was used as an index of the content of agonist-sensitive intracellular stores. No contraction or an insignificant contraction was evoked upon a second application of the agonist (NE2 in Fig. 1) in the same solution, which indicates depletion of internal Ca\(^{2+}\) stores sensitive to norepinephrine. After being carefully washed, each tissue was incubated for 20 min in Ca\(^{2+}\)-containing solution to refill the intracellular Ca\(^{2+}\) stores, and a spontaneous IRT1 (see Fig. 1) was observed in the iliac and proximal mesenteric arteries but not in the distal or small mesenteric arteries (Table 1). The IRT observed was not sustained, and it decreased as slowly as the control response to

<table>
<thead>
<tr>
<th>Arteries</th>
<th>NE, mN</th>
<th>IRT, %NE</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta</td>
<td>7.05 ± 0.32</td>
<td>71.84 ± 3.02</td>
<td>10</td>
</tr>
<tr>
<td>Iliac</td>
<td>3.99 ± 0.27</td>
<td>79.54 ± 5.92</td>
<td>22</td>
</tr>
<tr>
<td>Proximal mesenteric</td>
<td>6.65 ± 0.63</td>
<td>52.89 ± 2.95</td>
<td>8</td>
</tr>
<tr>
<td>Distal mesenteric</td>
<td>5.13 ± 0.54</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Small mesenteric</td>
<td>6.22 ± 0.83</td>
<td></td>
<td>10</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE; n, number of experiments. Increase in resting tone (IRT) is expressed as a percentage of the maximal response to norepinephrine (NE) in Krebs solution. Contractile responses to maximal concentrations of NE in the aorta, iliac, and proximal, distal, and small mesenteric arteries of the rat are shown. The magnitude of the IRT elicited after depletion of intracellular calcium stores by NA and posterior loading in Ca\(^{2+}\)-containing solution (see experimental procedure in Fig. 1) is also shown.
norepinephrine in Ca2+-containing solution disappeared after washing, as Fig. 2 shows. Returning the tissues to a Ca2+-free solution and further application of norepinephrine (NE3) 20 min later reproduced the contractile response elicited first in Ca2+-free solution, which indicates a complete refilling of internal stores (Fig. 1). A new loading in Ca2+-containing solution gave a new IRT (IRT2) similar to the first one in the iliac and proximal mesenteric arteries (Fig. 1).

Concentration-response curves of inhibition to prazosin (0.001 nmol/l–1 µmol/l), BMY 7378 (0.001 nmol/l–1 µmol/l), and 5-methylurapidil (0.001 nmol/l–1 µmol/l) were obtained (Fig. 3) by adding concentrations of antagonist 10 min before and during the second loading period in Ca2+-containing solution that permits the refilling of internal Ca2+ stores previously depleted by norepinephrine (IRT2). The magnitude of the IRT2 observed in the iliac artery during this period in the presence of each concentration of antagonist was expressed as a percentage of IRT1 obtained in the absence of any agent, and the calculated pIC50 and confidence limits were 9.93 (11.03–8.84) for prazosin (n = 5–7), 8.77 (9.36–7.64) for BMY 7378 (n = 6–7), and 7.61 (9.00–6.27) for 5-methylurapidil (n = 5–7). The potency shown by BMY 7378 against the IRT relates this contraction observed in the iliac artery to a population of constitutively active α1D-adrenoceptors, as has been shown in the rat aorta (7, 26). When the concentration of BMY 7378 or prazosin (0.1 µmol/l) that completely inhibits IRT2 in the aorta (7) and iliac artery was assayed in the proximal mesenteric artery, the IRT2 was also completely inhibited, confirming that this response could also be related to a constitutively active population of α1D-adrenoceptors.

To confirm this hypothesis, chloroethylclonidine (100 µmol/l), which failed to inhibit the IRT in the rat aorta (7), was used as a neutral antagonist to see whether it could block the action of the prazosin (1 µmol/l) and BMY 7378 (1 µmol/l) in the aorta and iliac artery. As shown in Fig. 4, the previous addition for 30 min of chloroethylclonidine (100 µmol/l), which failed to inhibit the IRT observed in these tissues, clearly blocked the inhibitory effect of the other agents on IRT in the three vessels. This concentration of chloroethylclonidine completely inhibits norepinephrine-induced contraction in the aorta and iliac artery (results not shown). In the proximal mesenteric artery we did not do this, because chloroethylclonidine (100 µmol/l) itself induces a contractile response that masks the IRT.

To corroborate the functional role of α1D-adrenoceptors in the iliac and proximal mesenteric arteries and exclude their participation in the distal and small mesenteric arteries, concentration-response curves of relaxation to prazosin (0.01 nmol/l–1 µmol/l), BMY 7378 (0.01 nmol/l–1 µmol/l), cycloproprazosin (0.01 nmol/l–1 µmol/l), and 5-methylurapidil (0.001 nmol/l–10 µmol/l) were obtained by adding cumulative concentrations of the compounds to tissues in which sustained contractions had been induced by a maximal concentration of agonist. In the iliac and mesenteric arteries, where α2-adrenoceptors could have a functional role (2, 29), the α1-adrenoceptor-selective agonist phenylephrine (10
\( \alpha_{1D}\)-ADRENOCEPTORS

Table 2. Comparison of \( \text{pIC}_{50} \) of the \( \alpha_1 \)-adrenoceptor antagonists tested on different vessels

<table>
<thead>
<tr>
<th>Arteries</th>
<th>Prazosin</th>
<th>BMY 7378</th>
<th>Cyclazosin</th>
<th>5-Methylurapidil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pIC(_{50})</td>
<td>n(_{H})</td>
<td>pIC(_{50})</td>
<td>n(_{H})</td>
</tr>
<tr>
<td>Aorta</td>
<td>9.70 ± 0.09</td>
<td>8.11 ± 0.07</td>
<td>8.65 ± 0.13</td>
<td>0.88 ± 0.06</td>
</tr>
<tr>
<td>Iliac</td>
<td>9.21 ± 0.14</td>
<td>0.85 ± 0.06</td>
<td>7.57 ± 0.11</td>
<td>0.94 ± 0.05</td>
</tr>
<tr>
<td>Proximal mesenteric</td>
<td>10.23 ± 0.08</td>
<td>0.97 ± 0.09</td>
<td>8.05 ± 0.22</td>
<td>0.63 ± 0.04</td>
</tr>
<tr>
<td>Distal mesenteric</td>
<td>9.41 ± 0.12</td>
<td>0.85 ± 0.05</td>
<td>6.94 ± 0.13</td>
<td>0.99 ± 0.06</td>
</tr>
<tr>
<td>Small mesenteric</td>
<td>8.48 ± 0.28</td>
<td>0.80 ± 0.11</td>
<td>6.30 ± 0.14</td>
<td>1.42 ± 0.47</td>
</tr>
<tr>
<td>Tail</td>
<td>9.53 ± 0.07</td>
<td>6.43 ± 0.11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE; \( n = 4\)–9 experiments. \( n_{H} \), Hill slope coefficient. Data from the aorta and tail artery are from Gisbert et al. (7).

\( \mu \text{mol/l} \) was employed. Norepinephrine (30 \( \mu \text{mol/l} \)) was used in the small mesenteric artery, where the contractile role of \( \alpha_3 \)-adrenoceptors is excluded (28). Relaxations were expressed as a percentage of the maximum increment in tension obtained by agonist addition, and the potency (pIC\(_{50}\)) and \( n_{H} \) of the fitted curves of relaxation obtained for each antagonist in the different vessels are summarized in Table 2.

In the iliac and proximal mesenteric arteries, the pIC\(_{50}\) of BMY 7378 as a relaxant of phenylephrine-induced contraction suggests the participation of \( \alpha_{1D} \)-adrenoceptors together with other subtypes (Table 2). The pIC\(_{50}\) shown by cyclazosin does not exclude the participation of the \( \alpha_{1D} \)-subtype, and the pIC\(_{50}\) and \( n_{H} \) for 5-methylurapidil suggest a mixed population of \( \alpha_1 \)-adrenoceptor subtypes. The results obtained in distal and small mesenteric arteries are summarized in Table 2, but the lack of potency of BMY 7378 excludes the functional role of the \( \alpha_{1D} \)-subtype in these tissues.

In small mesenteric arteries, the pIC\(_{50}\) obtained with prazosin (8.48 ± 0.28; Table 2) was consistently lower than its affinity for the \( \alpha_1 \)-adrenoceptors (7, 24, 26), the \( \alpha_{1A} \)-, \( \alpha_{1B} \)-, and \( \alpha_{1D} \)-subtypes (p\( K_i \) = 9.9–10.4) (16, 32) and also lower than the pIC\(_{50}\) obtained for prazosin in the iliac or proximal or distal mesenteric arteries.

**DISCUSSION**

Previous results obtained in the rat aorta (7, 24, 26) show that, by activating \( \alpha_1 \)-adrenoceptors, norepinephrine releases Ca\(^{2+} \) from internal stores. When emptied, the stores can be rapidly replenished by Ca\(^{2+} \) influx during the incubation in Ca\(^{2+} \)-containing solution in the absence of the agonist, and this process manifests itself not only in the recovery of the response to norepinephrine in Ca\(^{2+} \)-free medium but also in the increased resting tone (IRT in Fig. 1) and inositol trisphosphate accumulation observed (7). As has been analyzed and discussed in previous papers (7, 24, 26), endogenous or exogenous agonists are not present; the fact that the IRT and the inositol trisphosphate accumulation related to it were inhibited by prazosin and BMY 7378, a selective antagonist of the \( \alpha_{1D} \)-subtype, suggests the existence of a population of \( \alpha_{1D} \)-adrenoceptors that remains in a constitutively active state, as we have previously proposed (7, 24–27). Moreover, the fact that this IRT was not observed in the tail artery (7), where a population of \( \alpha_{1A} \)-adrenoceptors has been described (17), suggests that this subtype does not show constitutive activity. Two questions are brought up by these results: 1) whether this is a general model for the \( \alpha_{1D} \)-subtype or depends on the vascular smooth muscle in which the adrenoceptor is expressed, and 2) what the role of this process is in the functionality of a given vessel.

To answer the first question, we analyzed this model in different rat vascular tissues: iliac and proximal, distal, and small mesenteric arteries. The experimental procedure was the same: after depletion of internal Ca\(^{2+} \) stores sensitive to norepinephrine, an IRT was observed (see Fig. 1) in the iliac and proximal mesenteric arteries but not in the distal or small mesenteric arteries. The fact that the IRT observed can be selectively inhibited by BMY 7378 suggests the existence of a population of \( \alpha_{1D} \)-adrenoceptors with constitutive activity in these vessels, as we have previously shown in the aorta (7). This affirmation is supported by the fact that chloroethylocyclidine, which acts as a neutral antagonist in the rat aorta (7) and iliac artery (present results), can block the action of the inverse agonists prazosin and BMY 7378 (see Fig. 4) as well as the effect of the agonist norepinephrine.

These results suggest that, as occurs in the rat aorta (7, 26), the \( \alpha_{1D} \)-subtype shows constitutive activity in the vessels in which it has a functional role. To confirm this role in the iliac and proximal mesenteric arteries and exclude its involvement in the functionality of the distal and small mesenteric arteries, we assayed in these tissues the activity of different selective antagonists: BMY 7378 and 5-methylurapidil, which show selective affinity for the \( \alpha_{1D} \)- and \( \alpha_{1A} \)-subtypes, respectively, and cyclazosin, which shows selective affinity for the \( \alpha_{1B} \)-subtype (6, 9, 32). The potency obtained by each selective antagonist (pIC\(_{50}\)) was interpreted as an indicator of the functionality of a subtype in a given vessel.

The results obtained confirm that the population of \( \alpha_{1D} \)-adrenoceptors that intervene in the functional response of the iliac and proximal mesenteric artery to adrenergic agonists belongs, at least in part, to the \( \alpha_{1D} \)-subtype. A similar analysis in the distal mesenteric artery was performed, but the potency of BMY 7378 was too low to account for \( \alpha_{1D} \)-adrenoceptor involvement in the response of this vessel to adrenergic stimulus.
The present results are consistent with previous studies in the iliac (29) and mesenteric arteries (12, 35) that describe a role for the α1D-subtype in the functional response of these vessels. However, the lack of a functional role for the α1D-adrenoceptor in the distal mesenteric artery has not been described previously and suggests that anatomic differences related to proximity or distance with respect to the aorta could be invoked to explain this discrepancy. According to the present results, in the part of the mesenteric artery that is close to the aorta (the proximal mesenteric artery), there remains a population of functionally active α1D-adrenoceptors, which, however, disappear little by little as the artery moves away from the aorta and become almost imperceptible in the section of the artery contiguous with the first branch of the mesenteric arterial tree (the distal mesenteric artery).

In the second branch of the small mesenteric arteries, the functionally active α1- adrenoceptor subtypes display a peculiar pharmacology. The low pIC50 obtained for prazosin was more consistent with the profile of the pharmacologically defined α1L-subtype (3, 4, 33, 34). However, other reports propose that α1B-adrenoceptors mediate contraction of small mesenteric arteries (30). Clearly, then, in this tissue the exact role of the α1-adrenoceptor subtypes is still controversial. In any case, the low potency of BMY 7378 excludes α1D-adrenoceptor participation in the functionality of small mesenteric arteries, and, as occurs in the tail (7) or distal mesenteric arteries, the IRT was not observed either.

In conclusion, the functional role of the α1D-adrenoceptors, only revealed in the iliac and proximal mesenteric arteries in the present study, implies the existence, during a certain period of time, of a functional response in the absence of the agonist that can be interpreted as the constitutive activity of these receptors. This coincides with that occurring in the rat aorta (7), a tissue in which the functionality of α1D-adrenoceptors is well defined (7, 12, 16, 23). In tissues such as the tail (7, 17), distal mesenteric, or small mesenteric arteries, where α1D-adrenoceptors do not play an functional role, the constitutive activity of a population of α1-adrenoceptors is not observed. Therefore, this constitutive activity is only shown by the α1D-adrenoceptor subtype and is observed in vessels where this subtype has a functional role.

Interestingly, the same observation about the constitutive activity of α1D-adrenoceptors was recently reported by two different groups of researchers working with cloned α1D-adrenoceptors expressed in rat-1 fibroblasts (5, 20).

The conclusion that only α1D-adrenoceptors exhibit constitutive activity and that this constitutive activity can be evidenced in different vessels when this subtype plays a functional role answers the first question referred to above. But the data gathered in the present study also clarify our second and more interesting question about the physiological role of this constitutive activity of α1D-adrenoceptors. Figures 1 and 2 show that if we compare the norepinephrine-induced contractile responses in the iliac artery and the different segments of mesenteric arteries in Ca2+-containing solution, we can observe that, after the agonist is removed, contraction disappears in the iliac and proximal mesenteric arteries as slowly as IRT decreases, but that in distal and small mesenteric arteries, the decay of the response to norepinephrine is faster. The same has been shown in the aorta, where α1D-adrenoceptors are present, compared with the tail artery, where the functionality of this subtype is excluded (7).

From these results, we can extrapolate that in physiological conditions, after norepinephrine activity and removal, a population of α1D-adrenoceptors could remain in a constitutively active state, temporarily coupled to G protein, and could be responsible for the slow disappearance of the contractile response to the agonist in a given vessel. This mechanism is not observed in vessels where α1D-adrenoceptors do not seem to play a functional role. Therefore, the presence of a population of α1D-adrenoceptors in a vessel signifies that the contractile responses of this tissue can be sustained even when the agonist is removed, and this would in turn modulate the contractile activity in this vessel, thus preventing abrupt changes in caliber when the agonist disappears. According to our findings, in conductance vessels like the aorta, proximal mesenteric, and iliac arteries, α1D-adrenoceptors play a modulatory role in the contractile tone and prevent sudden changes in blood flow. In contrast, in the portion of distal or small mesenteric arteries that are farther from the aorta, the lack of a functional role for α1D-adrenoceptors guarantees a fine, quick adjustment of contractile tone and blood flow to the adrenergic stimulus.

We can extrapolate that an imbalance in this modulating mechanism could give rise to pathologies such as hypertension or diabetes-or age-related vascular diseases, in the pathogenesis and/or maintenance of which α1D-adrenoceptors could play a role, as has been postulated by different authors (11, 14, 15, 35–37). We are currently investigating this hypothesis, but the observations reported here are the first to explain that the presence of different α1-adrenoceptor subtypes in a given vessel is due to the fact that they are involved in different tissue functions.

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