Pharmacological Characterization of the Vascular Muscarinic Receptors Mediating Relaxation and Contraction in Rabbit Aorta

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ABSTRACT

Studies were performed in the rabbit aortic rings, precontracted with norepinephrine, to determine the subtype(s) of muscarinic receptors involved in endothelium-dependent relaxation and contraction in the absence of endothelium elicited by cholinergic stimuli. Acetylcholine (ACh) and arecaidine propargyl ester (APE), a M₂ and M₃ agonist, produced a close-dependent relaxation and contraction in endothelium-intact and endothelium-denuded rabbit aortic rings, respectively. Both of these responses were blocked by the muscarinic receptor antagonist atropine. M₁ selective agonist McN-A-343 [4-(3-chlorophenyl)-carbamoyloxy]-2-butylnitrimethylammonium chloride did not produce any effect on the tone of precontracted aortic rings. ACh- and APE-induced relaxation in aortic rings with intact endothelium was selectively blocked by M₃ receptor antagonists hexahydro-sila-difenidol and p-fluoro-hexahydro-sila-difenidol (pA₂ of 7.84 and 7.18) but not by M₁ antagonist pirenzepine or M₂ receptor antagonists AF-DX 116 [11-(2-[(diethylamino)methyl]-1-piperidiny]acetyl)-5,11-dihydro-6H-pyrido-[2,3-b][1,4]-benzo-diazepin-6-one] and methoctramine. ACh- and APE-induced contraction was inhibited by M₂ receptor antagonists AF-DX 116 and methoctramine (pA₂ of 7.11 and 6.71) but not by pirenzepine, hexahydro-sila-difenidol or p-fluoro-hexahydro-sila-difenidol. ACh- and APE-induced relaxation or contraction were not altered by nicotinic receptor antagonist hexamethonium or cyclooxygenase inhibitor indomethacin. These data suggest that relaxation elicited by cholinergic stimuli in endothelium-intact aortic rings is mediated via release of endothelium-derived relaxing factor consequent to activation of M₃ receptors located on endothelial cells, whereas the contraction in aortic rings denuded of their endothelium is mediated via stimulation of M₂ receptors located on smooth muscle cells.

There is a large body of evidence indicating that the various biological responses produced by cholinergic agonists in different tissues are mediated through activation of different subtypes of muscarinic receptors. The concept of heterogeneity of muscarinic receptors was first proposed on the basis of binding properties and tissue distribution of the cloned ml to m₅ receptors. This subclassification is based mainly on the affinities of different antagonists such as AF-DX 116 [11-(2-[(diethylamino)methyl]-1-piperidiny]acetyl)-5,11-dihydro-6H-pyrido-[2,3-b][1,4]-benzo-diazepin-6-one] and methoctramine. ACh- and APE-induced contraction was inhibited by M₂ receptor antagonists AF-DX 116 and methoctramine (pA₂ of 7.11 and 6.71) but not by pirenzepine, hexahydro-sila-difenidol or p-fluoro-hexahydro-sila-difenidol. ACh- and APE-induced relaxation or contraction were not altered by nicotinic receptor antagonist hexamethonium or cyclooxygenase inhibitor indomethacin. These data suggest that relaxation elicited by cholinergic stimuli in endothelium-intact aortic rings is mediated via release of endothelium-derived relaxing factor consequent to activation of M₃ receptors located on endothelial cells, whereas the contraction in aortic rings denuded of their endothelium is mediated via stimulation of M₂ receptors located on smooth muscle cells.

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ABBREVIATIONS: HHSid, hexahydro-sila-difenidol; p-F-HHSid, p-fluoro-hexahydro-sila-difenidol; ACh, acetylcholine; KHB, Krebs-Henseleit buffer; NE, norepinephrine; APE, arecaidine propargyl ester; EDRF, endothelium-derived factor; cGMP, cyclic GMP.

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contraction produced by cholinergic agents are blocked by thelum-denuded vascular preparations, cholinergic agonists in blood vessels, of distinct subtypes of muscarinic receptors is not known. Therefore, the purpose of the present study was to characterize the subtype of muscarinic receptors involved in the action of cholinergic stimuli on relaxation and contraction of endothelium-intact and endothelium-denuded rabbit aortic rings, respectively. The effect of ACh, selective M₁ agonist McN-A-343 [4-[N-(3-chlorophenyl)carbamoyloxy]-2-butyltrimethylammonium chloride] (Hammer and Giachetti, 1982; Eltze et al., 1988) and M₂ and M₃ agonist APE (Mutschler and Lambrecht, 1984; Moser et al., 1989, 1990) were investigated on relaxation and contraction of endothelium-intact and endothelium-denuded aortic rings, respectively, superfused with KHB in the absence and presence of muscarinic receptor antagonist atropine, M₁ selective antagonist pirenzepine, M₂ antagonists AF-DX 116 (Hammer et al., 1986; Giachetti et al., 1986; Dukelsky et al., 1987; Jaiswal and Malik, 1988; Jaiswal et al., 1988) and methoctramine (Melchiorre et al., 1987), as well as M₃ selective antagonists HHSiD (Mutschler et al., 1987; Lambrecht et al., 1988; Waelbroeck et al., 1989) and p-F-HHSiD (Lambrecht et al., 1988, 1989).

Methods

Male New Zealand White rabbits (1.0–2.0 kg) (Myrtle’s Rabbitry, Thompson Station, TN) were anesthetized i.v. with sodium pentobarbital, 30 mg/kg (Abbott Laboratory, North Chicago, IL), the abdomen was opened and heparin (100 U/kg) was administered into the vena cava. The aorta was removed carefully, to protect the endothelial lining, and transferred to a beaker containing gassed (95% O₂ and 5% CO₂) KHB of following composition (in millimolar): NaCl, 114; KCl, 4.7; KH₂PO₄, 1.2; NaHCO₃, 25.0; CaCl₂, 2.5; MgSO₄, 1.2; and glucose, 5.5. The aorta was cleaned free of adhering fat and connective tissues and then cut into 2.5-mm-wide transverse rings using a razor blade. Each aortic ring was mounted on the force displacement transducer at the lower nichrome wire was fixed to the bottom of the chamber with a thread for the adjustment of the resting tension. Changes in isometric tension were recorded on a Grass polygraph. Before the start of any experimental intervention, all rings were allowed to equilibrate for 60 min while superfused continuously with KHB. Endothelium was removed from some aortic rings by gently rubbing the intima with a wooden stick for 45 to 60 sec. The removal of endothelial cells were verified histologically using a silver-staining method (Abrol et al., 1984).

Experimental Protocol

After equilibration for 60 min at 37°C, rings were stretched to the previously determined optimum resting tension of 1.5 g. For relaxation studies, submaximal increase in tone was achieved by superfusing the aortic rings continuously with 10⁻⁷ M of NE, whereas for contraction studies aortic rings were superfused with 10⁻⁸ M of NE so as to maintain similar experimental conditions as in aortic rings with intact endothelium. The following experimental protocols were used for endothelium-intact and endothelium-denuded aortic rings.

Protocol 1. These series of experiments were conducted to investigate the effect of muscarinic agonists ACh (0.001–10 nmol), McN-A-343 [4-[N-(3-chlorophenyl)carbamoyloxy]-2-butyltrimethylammonium chloride] (0.001–1000 nmol) or APE (0.001–10 nmol) to decrease and increase isometric tension in endothelium-intact and in endothelium-denuded aortic rings, respectively. After an equilibration period of 30 min, muscarinic agonists were administered as a bolus into superfusing solution contained in a volume of 100 μl and the responses were recorded on a physiograph. The vehicle of these agents was also administered in an additional series of aortic rings during the same time period as muscarinic agonists.

Protocol 2. The second series of experiments were performed to investigate the effect of muscarinic receptor agonists ACh and APE in the absence and presence of selective M₁, M₂ and M₃ muscarinic receptor antagonists on the isometric tension of aortic rings preconstricted with NE. The experimental protocol consisted of two or more periods depending upon the antagonists used. The rings were equilibrated with antagonists for 30 min. ACh, APE (1–100 nmol) or their vehicle were administered during all periods. After the responses of aortic rings to ACh or APE were recorded, the rings were superfused with muscarinic receptor antagonists atrpine (10⁻⁷ to 10⁻⁴ M), M₁ receptor antagonist pirenzepine (10⁻⁷ to 10⁻⁴ M), M₂ receptor antagonists AF-DX 116 [11-[(2-[diethylamino)methyl)-1-piperidinyl]acetlyl]-5,11-dihydro-6H-pyrido-[2,3-b]-[1,4]-benzo-diazepin-6-one (10⁻⁷ to 10⁻⁴ M) or methoctramine (10⁻⁷ to 10⁻⁴ M), M₃ receptor antagonists HHSiD (10⁻⁸ to 10⁻⁴ M) or p-F-HHSiD (10⁻⁴ to 10⁻⁸ M), at a lower concentration in second period and at a higher concentration in third or subsequent periods and the responses of aortic rings to muscarinic agents were determined. The effect of muscarinic agonists in the presence of the vehicle of the above muscarinic receptor antagonists were also examined on aortic rings during these time periods.

Protocol 3. The third series of experiments were performed to determine the effect of nicotinic receptor antagonist hexamethonium (10⁻⁴ M) and cyclooxygenase inhibitor indomethacin (5.0 × 10⁻⁵ M) on the responses elicited by bolus injections of muscarinic agonists of the endothelium-intact and endothelium-denuded aortic rings preconstricted with NE, as in the second series of experiments. The effects of muscarinic receptor antagonists used in this study were also investigated on the vasodilator effect of noncholinergic agent calcium ionophore A23187 (10⁻⁷ M).

Drugs. The following drugs used in this study were purchased: ACh chloride, NE bitartrate, atropine sulfate, hexamethonium bromide and indomethacin (Sigma Chemical Co., St. Louis, MO); and methoc tramine (Research Biochemicals, Inc., Natick, MA). The following drugs were synthesized: APE, HHSiD and p-F-HHSiD (Mutschler and Hultsch, 1973; Tacke et al., 1985). The following drugs were gifts: McN-A-343 (Dr. Hammer, Instituto de Angeli, Milan, Italy); pirenzepine; and AF-DX 116 (Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT).

Data analysis. Data were expressed as the mean ± S.E.M. and comparisons between means were made using Student’s t test. Differences between means were considered significant at P < .05. The decrease (vasodilation) and increase (vasoconstriction) in tension produced by cholinergic agonists was presented as percentage of change from the tension raised by superfusing the aortic rings with NE solution. The affinity of muscarinic receptor antagonists was determined according to Arunlakshana and Schild (1959) and expressed in.
terms of $\text{pA}_2$ values. $\text{pA}_2$ values ($-\log [(\text{agonist})/(\text{dose ratio} - 1)]$) for antagonists were determined as described by MacKay (1978).

### Results

Effect of ACh, McN-A-343 and APE on isometric tension in aortic rings precontracted with NE (fig. 1). ACh produced a dose-dependent decrease in tension in NE (10$^{-7}$M)-precontracted endothelium-intact aortic rings, producing a maximal effect at 10 nmol. McN-A-343, a selective $M_1$ agonist, did not alter the tone in NE-precontracted aortic rings even up to a dose of 1000 nmol; whereas, APE, a $M_2$ and $M_3$ receptor agonist, relaxed the NE-precontracted rings in a dose-dependent fashion (fig. 1A).

In isolated aortic rings denuded of their endothelium and submaximally precontracted with 10$^{-6}$ M of NE, ACh at 0.1 to 100 nmol increased the tone in a dose-dependent manner, producing a maximal effect at 100 nmol. Further increase in the dose of ACh did not produce an additional enhancement of the contractile response (fig. 1B). McN-A-343, a $M_1$-receptor agonist, did not alter the contractile responses of aortic rings up to 1000 nmol (fig. 1B). Administration of APE at a dose of 0.1 nmol did not have any effect, but APE produced a significant increase in the contractile responses of aortic rings at 1 to 100 nmol in a dose-dependent manner. APE produced a maximal response at 100 nmol. A further increase in the dose of APE did not have any additive effect on contractile responses (fig. 1B). Administration of vehicle did not produce any changes in the tone of the aortic rings.

Comparisons were made of the relative potencies of ACh and APE to produce relaxation and contraction in the endothelium-intact and endothelium-denuded aortic rings, respectively (table 1). ACh and APE were nearly equipotent in producing contraction in endothelium-denuded aortic rings and relaxation in endothelium-intact aortic rings, but about 10- to 40-fold higher concentrations of these two agonists were necessary in denuded aortic rings to get an effect.

**Effect of muscarinic receptor antagonists on relaxation produced by ACh and APE in endothelium-intact aortic rings precontracted with NE. Effect of ACh (fig. 2–4).** The effect of ACh (1 and 10 nmol) to produce relaxation of aortic rings with intact endothelium was reduced significantly by the infusion of nonselective muscarinic receptor antagonist atropine at 10$^{-7}$ and 10$^{-6}$ M (fig. 2A). Infusion of $M_1$-receptor antagonist pirenzepine at 10$^{-7}$ M did not have any effect but at 10$^{-6}$ M reduced the ACh (10 nmol)-induced relaxation from 26 ± 5 to 11 ± 2% (fig. 2B); a 10-fold increase in the concentration of pirenzepine did not produce any further change. In the presence of $M_2$ receptor antagonist AF-DX 116 (10$^{-7}$ and 10$^{-6}$ M), the relaxation elicited by ACh was not altered (fig. 2C). A further increase in the concentration of AF-DX 116 did not produce any change. Methoctramine (10$^{-7}$ to 10$^{-6}$ M) also did not alter ACh-induced relaxation (data not shown).

Figure 3 illustrates the effects of $M_1$-receptor antagonists HHSiD and p-F-HHSiD on relaxation response produced by 10 nmol of ACh. Lower concentration of HHSiD (10$^{-6}$ M) did not alter the relaxing effect of ACh but at 10$^{-7}$ M, HHSiD inhibited the relaxation produced by ACh from 44 ± 7 to 19 ± 4% (n = 6; P < .05). Further increase in the concentration of HHSiD to 10$^{-6}$ M abolished the relaxation response to ACh (fig. 3A). p-F-HHSiD, another $M_2$-receptor antagonist, at 10$^{-8}$ M, did not alter the relaxation response to ACh but reduced it at 10$^{-7}$ and 10$^{-6}$ M; increasing the p-F-HHSiD concentration to 10$^{-6}$ M completely abolished the relaxation produced by ACh (fig. 3B).

Analysis of the data according to Arunlakshana and Schild (1959) gave a straight line for both HHSiD and p-F-HHSiD

### TABLE 1

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Endothelium-Intact $E_{D_{50}}$</th>
<th>Endothelium-Denuded $E_{D_{50}}$</th>
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<tr>
<td></td>
<td>nmol</td>
<td>nmol</td>
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<tr>
<td>ACh</td>
<td>0.25 ± 0.08</td>
<td>10 ± 2.48</td>
</tr>
<tr>
<td>APE</td>
<td>0.5 ± 0.099</td>
<td>5.0 ± 1.32</td>
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against ACh-induced relaxation in the endothelium-intact aortic rings (fig. 4). The pA₂ values for atropine, HHSiD, p-F-HHSiD and pirenzepine are given in table 2. Slopes of Schild-plots were not significantly different from unity (P < .05).

Effect of APE (fig. 5). The decrease in tension produced by 10 nmol of APE was inhibited by atropine (10⁻⁷ M) (fig. 5A), whereas infusion of the M₁-receptor antagonist pirenzepine at 10⁻⁶ but not at 10⁻⁷ M reduced the relaxation produced by APE (fig. 5B). The M₂-receptor antagonist AF-DX 116 at 10⁻⁶ or 10⁻⁵ M did not significantly alter the relaxation produced by APE (fig. 5C). HHSiD at 10⁻⁸ M did not alter the relaxing action of APE. A 10-fold increase in the HHSiD concentration (10⁻⁷ M) produced a significant decrease in relaxation caused by APE (from 33 ± 7 to 16 ± 2%, n = 4; P < .05); a 5-fold increase in the concentration of HHSiD abolished the relaxation response to APE (fig. 5D). p-F-HHSiD also inhibited the relaxation produced by APE in a concentration-dependent pattern (data not shown).

Effect of muscarinic receptor antagonists on the contractile response produced by ACh and APE in endothelium-denuded aortic rings precontracted with NE (10⁻⁸ M). An increase in contractile response to NE-precontracted endothelium-denuded aortic rings elicited by ACh (10 and 100 nmol) was abolished by 10⁻⁷ (fig. 6A) and 10⁻⁸ M of atropine (data not shown). Pirenzepine (M₁ receptor antagonist) at 10⁻⁶ and 10⁻⁵ M did not alter it but reduced it at 10⁻⁴ M (fig. 6B). A 10-fold increase in the concentration of pirenzepine did not
reduce further the response (data not shown). M₂ receptor antagonist AF-DX 116 at 10^{-7} M did not alter the effect of ACh; an increase in the concentration of AF-DX 116 from 10^{-4} to 5 \times 10^{-5} M reduced the contractile response elicited by ACh in a concentration-dependent manner (fig. 7A). Methoctramine, another M₂ receptor antagonist, significantly reduced ACh-induced contractile response at a concentration of 10^{-6} to 10^{-5} M (fig. 7B).

HHSiD at 10^{-7} to 10^{-4} M did not alter the contractile response produced by ACh (fig. 7C). p-F-HHSiD (10^{-4} M), another M₃ receptor antagonist used in this study, was also ineffective in inhibiting the contractile response of ACh or APE (data not shown). Figure 8 illustrates the Schild’s plots of the antagonistic action of AF-DX 116 and methoctramine on the contractile response to ACh produced in endothelium-denuded aortic rings. Table 3 shows the comparison of the pA₂ values of these antagonists for the endothelium-denuded aortic rings from Schild’s plot regression analysis. Slopes of Schild plots were not significantly different from unity (P < .05).

The APE-induced contractile response of endothelium-denuded aortic ring was also significantly reduced by atropine (10^{-6} M), AF-DX 116 (10^{-5} M) and methoctramine (10^{-5} M) but not by pirenzepine (10^{-4} M), HHSiD (10^{-4} M) or p-F-HHSiD (10^{-4} M) (data not shown).

**Effect of hexamethonium and indomethacin on the actions of ACh and APE on relaxation and contraction of endothelium-intact and endothelium-denuded aortic rings.** Hexamethonium (10^{-6} M), a nicotinic receptor blocker, and indomethacin (5 \times 10^{-6} M), a cyclooxygenase inhibitor, did not alter the relaxation or contraction produced by ACh or APE (10 nmol) in endothelium-intact or endothelium-denuded aortic rings, respectively (data not shown). The relaxation elicited by 10^{-7} M of A 23187 (53 ± 9% of NE-induced tone) was not altered by any of the muscarinic receptor antagonist used in this study.

**Discussion**

Our recent studies in the isolated rabbit heart indicate that muscarinic receptor agonists ACh and APE produce coronary vasodilation followed by vasoconstriction and that the vasodilator component of the coronary response is mediated through activation of the M₃ (M₃a) subtype of muscarinic receptors, whereas coronary vasoconstriction is mediated through activation of the M₂ (M₂) subtype of muscarinic receptors (Jaiswal et al., 1988). The present study, which was undertaken to determine the subtype of muscarinic receptors involved in the action of cholinergic agonists to produce relaxation and contraction in endothelium-intact and endothelium-denuded rabbit aortic rings, respectively, suggests that: 1) both relaxation and contraction produced by cholinergic stimuli in NE-precontracted rabbit aortic rings is not due to activation of M₁ muscarinic receptor subtype; 2) relaxation produced by cholinergic agents is due to release of EDRF, consequent to activation of M₃ muscarinic receptors located on endothelium; and 3) contractile response produced by cholinergic agents in rabbit aortic endothelium-denuded rings is due to activation of M₂ receptors located in smooth muscle. The conclusion that relaxation as well as contraction produced by cholinergic stimuli in the NE-precontracted rabbit aortic ring is not due to activation of M₁ subtypes of muscarinic receptors is based on our demonstration that ACh and APE, a selective M₂ muscarinic receptor agonist, produced a concentration-dependent relaxation in endothelium-intact and contraction in endothelium-denuded aortic rings, whereas McN-A-343, a selective M₁ agonist, did not alter the tone of aortic rings with and without endothelium even up to 1000-nmol concentration. McN-A-343 also failed to influence vascular tone in the rabbit ear artery (Duckles, 1988).

Muscarinic receptors involved in the action of ACh or APE to produce relaxation and contraction in aortic rings with intact and denuded endothelium, respectively, are not of M₁.
The affinity of HHSiD for muscarinic receptors was very similar to that obtained in other vascular preparations (Duckles and Garcia-Villalon, 1990; Duckles et al., 1990). However, the pA₂ (7.18; table 2) found for p-F-HHSiD was at the lower end of the range reported for its affinity to its M₃ receptors in smooth muscles (Lambrecht et al., 1989; Eglen et al., 1990; Duckles, 1990). HHSiD and p-F-HHSiD have also been reported to have greater affinity for muscarinic receptors in the ileum (M₃) than the atrium (M₁) (Lambrecht et al., 1988, 1989). Muscarinic receptors mediating relaxation of rat and rabbit aorta to ACh exhibited lower affinity for methoctramine and pirenzepine, respectively, suggesting further that muscarinic receptors mediating relaxation in aorta are not of the M₁ or M₂ subtypes (Eglen et al., 1988; Choo et al., 1986). Although pirenzepine reduced the ACh-induced relaxation in rabbit ear artery are not of the M₁ subtype of receptors because of their low affinity toward pirenzepine (pA₂ = 6.5 ± 0.1). In the rabbit and dog aorta (Yamanaka et al., 1986) and bovine basilar artery (Vanderheyden et al., 1986), pKi values of pirenzepine for muscarinic receptors were 6.8, 6.97 and 6.4, respectively. However, Eglen and Whiting (1985) found somewhat higher pA₂ values for pirenzepine in the endothelium of rabbit aorta (7.6–7.9) and dog femoral artery (7.6), which are close to that reported for M₁ receptors in the cervical ganglia (8.36) (Brown et al., 1980; Eltze et al., 1988). Because these affinities of pirenzepine are not consistent with either M₁ or M₂ subtypes of muscarinic receptors, Eglen and Whiting (1985) have suggested that the muscarinic receptors that mediate vascular smooth muscle relaxation differs from all previously identified subtypes of muscarinic receptors.

Our demonstration that HHSiD and p-F-HHSiD, which have high affinity for M₃ receptors but not AF-DX116 and methoctramine (10⁻⁵ M), M₁ receptor antagonists, inhibited ACh- or APE-induced relaxation in aortic rings with intact endothelium, suggests that vascular smooth muscle relaxation caused by muscarinic receptor agonists is due to activation of M₃ receptors. The affinity of HHSiD for muscarinic receptors mediating relaxation in rabbit aorta (this study; pA₂ = 7.84, table 2) was very similar to that obtained in other vascular preparations (Duckles et al., 1990). However, the pA₂ (7.18; table 2) found for p-F-HHSiD was at the lower end of the range reported for its affinity to its M₃ receptors in smooth muscles (Lambrecht et al., 1989; Eglen et al., 1990; Duckles, 1990). HHSiD and p-F-HHSiD have also been reported to have greater affinity for muscarinic receptors in the ileum (M₃) than the atrium (M₁) (Lambrecht et al., 1988, 1989). Muscarinic receptors mediating relaxation of rat and rabbit aorta to ACh exhibited lower affinity for methoctramine and pirenzepine, respectively, suggesting further that muscarinic receptors mediating relaxation in aorta are not of M₁ or M₂ subtypes (Eglen et al., 1988; Choo et al., 1986). Although pirenzepine reduced the ACh-induced relaxation...
relaxation in endothelium-intact tissue, the pA₂ value (6.75 ± 0.56) was comparable to that found by several investigators (ranged from 6.5–6.8) in other tissues (Choo et al., 1986; Duckles and Garcia-Villalon, 1990; Hynes et al., 1986) demonstrating the low affinity of pirenzepine for muscarinic receptor-mediating relaxation. Recently, it was demonstrated that, in rat pulmonary artery muscarinic receptor, which mediates relaxation, it is also of M₃ subtype (McCormack et al., 1988).

In contrast to ACh- or APE-induced relaxation in aortic rings with intact-endothelium, the contraction produced by these agents in aortic rings denuded of their endothelia was inhibited by AF-DX 116 and methoctramine but not by HHSiD and p-F-HHSiD. These observations suggest that the contraction of vascular smooth muscle produced by ACh or APE in aortic rings denuded of their endothelium is mediated primarily via activation of M₂ receptors. Muscarinic receptor-stimulated contractile response to cholinergic agonists, which is independent of endothelium, has also been observed in some other blood vessels, such as the bovine coronary artery (Duckles, 1988), canine saphenous vein (O'Rourke and Vanhoutte, 1987) and pig coronary artery (Beny et al., 1986; Graser et al., 1986, 1987).

In these blood vessels, calcium ionophore A23187 but not cholinergic agonists produced endothelium-dependent relaxation. Whether this is due to lack of muscarinic receptors on the endothelial cells, no release of EDRF (Furchgott and Zawadsky, 1983) and p-F-HHSiD. These observations suggest that the calcium ionophore stimulates soluble guanylate cyclase and increases production of cGMP in vascular smooth muscle (Katsuki et al., 1977), it is possible that cGMP by decreasing cellular levels of calcium (Collins et al., 1986; Cornwell and Lincoln, 1989) attenuates M₂ receptor-stimulated contraction of vascular smooth muscle by cholinergic agonists. Removal of endothelium causes loss of M₂ receptor and associated generation of EDRF and thereby allow expression of M₂ receptor-mediated contraction in response to cholinergic agonists. Although there is overwhelming evidence of the release of EDRF from several blood vessels with intact-endothelium (Furchgott, 1983), studies using [³H] or [¹²⁵I] quinuclidinyl benzilate as a selective probe for muscarinic receptors have failed to provide evidence for the presence of these receptors on endothelial cells in a variety of blood vessels from several different species (Summers et al., 1987; Stephenson and Summer, 1987; Stephenson et al., 1988); muscarinic receptor ligand binding was observed in smooth muscle cells (Stephenson et al., 1988).

However, a recent study by Sim and Manjeet (1989) has demonstrated with [³H]ACh binding the existence of muscarinic receptors on endothelium as well as on smooth muscle in rabbit aorta. These observations suggest that endothelium-dependent relaxation caused by cholinergic agonists results from an indirect mechanism involving smooth muscle muscarinic receptors, with the endothelium playing a permissive role, possibly involving a mediator communicating from the smooth muscle to the endothelial cells to initiate the release of EDRF which, in turn, initiates smooth muscle relaxation. The possibility that the M₃ receptors in the endothelium have low affinity for quinuclidinyl benzilate cannot be excluded. Our finding that the relaxation of aortic rings elicited by ACh or APE was selectively blocked by M₂ but not by M₃ receptor antagonists and by the removal of endothelium strongly suggests that muscarinic receptors involved in ACh- or APE-induced relaxation are localized on the endothelium. Supporting this view are our recent observations that ACh enhanced prostaglandin synthesis via activation of M₂ and M₃ muscarinic receptors in the rabbit aortic rings with intact endothelia or in endothelial cells but not in endothelium-denuded aortic rings or aortic smooth muscle cells (Jaiswal and Malik, 1990; Jaiswal et al., 1991).

It should be noted that the pharmacology of the muscarinic receptors mediating relaxation and contraction, respectively, in rabbit aorta identified in the present study differs substantially from that of the M₄ receptors in the rat striatum (Waeltbroeck et al., 1990), rabbit lung and NG 108-15 cells (Lazareno et al., 1990), as well as from that of cloned m₁ and m₃ receptors (Buckley et al., 1989; Dorje et al., 1991). The rabbit aorta muscarinic receptors did not appear to correspond to the m₄ (M₄) and m₃ receptor gene products because pirenzepine is able to recognize m1, (M₁) and m₃ receptors with relatively high affinity (pKi = 7.1–7.5) (Buckley et al., 1989; Lazareno et al., 1990; Waeltbroeck et al., 1990; Wess et al., 1990), but reduced ACh- and APE-induced effects only at high concentration in...
our study in rabbit aorta. The affinities of AF-DX 116 (pKi = 6.5–6.8; Dorje et al., 1990; Waelbroeck et al., 1990) and methoctramine (7.5–8.1; Dorje et al., 1990; Lazareno et al., 1990; Waelbroeck et al., 1990) for M4 (M4) receptors were found to be high, but these antagonists did not inhibit muscarinic receptor-mediated relaxation in rabbit aorta, excluding again the contribution of M4 receptors in this effect. The binding affinities for M4 (M4) and M3 receptors of HHSSID [m4 (M4); pKi = 7.4–8.0; Buckley et al., 1989; Lazareno et al., 1990; Waelbroeck et al., 1990; m3; pKi = 7.2; Buckley et al., 1989] and p-F-HHSSID (m4; pKi = 7.5; m3 pKi = 7.0; Dorje et al., 1990) were found to be high, but in the present study, these antagonists did not inhibit ACh- and APE-induced contraction in rabbit aorta. Thus, the muscarinic receptor mediating contraction in rabbit aorta probably does not correspond to the m5 gene product. In addition, AF-DX 116 (pKi = 5.5; Dorje et al., 1990) displayed low affinity for cloned m3 receptors, but potently inhibited ACh- and APE-induced contraction of rabbit aorta. In light of the above, it is highly improbable that muscarinic m4 (M4) or m3 receptors are involved in muscarinic relaxation and contraction, respectively, of rabbit aorta.

In conclusion, the present study demonstrates that ACh- and APE-induced relaxation in rabbit aortic rings with intact endothelium is due to activation of M2 (M2) receptors located on the endothelium, whereas the contractile response elicited by cholinergic agonists in aortic rings denuded of their endothelium appears to be due to activation of the M2 (M2) subtype of muscarinic receptors located in the smooth muscle. The M2 receptor-mediated contraction is probably masked by M3 receptor-mediated relaxation caused by the release of EDRF. It is clear now that not all vascular muscarinic receptors belong to the same subclass, as also demonstrated for the muscarinic receptors on smooth muscle of the coronary (M3) and basilar artery (M3) of the pig (Van Chardhrop and Van Zwielen, 1989; Entseroth et al., 1990).

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References


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