Characterization of Intrinsic Sympathomimetic Activity of Carteolol in Rat Cardiovascular Preparations

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Abstract. We evaluated in vitro, in myocardial and vascular preparations isolated from reserpine-treated rats, the intrinsic sympathomimetic activity (ISA) of carteolol, a β₁/β₂-adrenoceptor blocking agent used in cardiovascular and non-cardiovascular diseases. In spontaneously beating atria, carteolol, at low concentrations (0.01 and 0.1 μM), antagonized the positive inotropic effect of isoprenaline, whereas at higher concentrations (1 μM to 1 mM), it caused an increase in the force of contraction (EC₅₀: 4.6 ± 0.1 μM, Eₘₐₓ: 17.1 ± 1.1%, with respect to the maximum isoprenaline response) and a slight increase (7.8 ± 1.9% over basal values) in the heart rate. The positive inotropic effect of carteolol was abolished by concentrations of propranolol or timolol (10 μM) much higher than those blocking isoprenaline effects in the same preparations. A similar positive inotropic effect was also observed in electrically driven left atrium and in Langendorff perfused hearts. Functional and biochemical evidences supported the involvement of cAMP in the cardiac action of carteolol. In peripheral arteries (femoral and tail) pre-contracted with phenylephrine, carteolol exerted ISA-related relaxing effects, independent of the presence of endothelium and sensitive to high concentrations (10 μM) of conventional β-blockers. On the basis of these results, we propose to categorize carteolol as a non-conventional partial agonist of both cardiac and vascular β-adrenoceptors.

Keywords: carteolol, partial intrinsic β-agonist activity, positive inotropic effect, peripheral vasodilator activity, atypical β-adrenoceptor

Introduction

Carteolol is a β₁/β₂-adrenoceptor blocking agent (1–3), currently used in management of patients with cardiovascular and non-cardiovascular diseases. Similarly to many other β-blockers, carteolol is endowed with intrinsic sympathomimetic activity (ISA) (4); therefore, it may be categorized as a partial agonist. Many findings indicate that in addition to the conventional partial agonists, which produce effects proportional to their β-adrenoceptor occupancy, other β-blockers endowed with ISA, such as (–)-CGP12177 (5, 6), (–)-pindolol (7), bucindolol (8), cyanopindolol (5), and alprenolol (9), now classified as non-conventional partial agonists, show agonist activity at concentrations considerably greater than those antagonizing the effects of catecholamines (10). This typical behavior is currently explained by the existence of two different active sites or conformations of the cardiac β₁-adrenoceptor: one site where classic agonists (catecholamines) and β-antagonists bind, and the other one where these non-conventional partial agonists act (11, 12). Since this site is relatively resistant to blockade by concentrations of propranolol (5) that effectively antagonize the effects of catecholamines (13), it is now defined as the propranolol-resistant state of the β₁-adrenoceptor (8).

A dissociation between the concentrations causing β-adrenoceptor blockade and those causing stimulation has been reported, but not explained, for many drugs long before (14) the demonstration of the existence...
of two different active sites of the $\beta_1$-adrenoceptor. Interestingly, in 1979, in isolated dog atrium preparations, Chiba (15) observed this typical behavior also for carteolol. More recently, Takayanagi et al. (16) reported that in vivo carteolol doses able to block $\beta$-adrenoceptors were 400 – 1000 times lower than those inducing increase in heart rate. In the light of the recent finding concerning the non-conventional partial agonists and considering that evaluation of ISA-related cardiovascular effects of $\beta$-blockers continues to be an important issue (17), the aim of the present research was to reconsider the effects of carteolol, in myocardial and vascular preparations isolated from reserpine-treated rats. The use of catecholamine-depleted tissues in order to avoid the underlying sympathetic tone is very important, since ISA of $\beta$-blockers, in particular their effect on cardiac contractility, is considered rather difficult to demonstrate in vitro (18), mainly because it critically depends on the activation state of the $\beta$-adrenoceptors (19) and/or on the degree of the underlying sympathetic tone (20).

Materials and Methods

Animals

Wistar male rats (300 – 380 g), obtained from Harlan Italy (S. Pietro al Natisone, Udine, Italy), were kept in controlled environmental conditions (temperature: 23 ± 2°C, light-dark cycle: 7 a.m. to 7 p.m.). Animals had free access to a standard laboratory diet and water. All animal-use procedures described in this paper were in accordance with the ethical principles and guidelines adopted by the European Community, law 86/609/CEE. The experimental protocol was approved by the local veterinary committee.

For all experiments, in order to obtain myocardial and vascular tissues depleted in endogenous catecholamines, the animals were treated daily for 2 days with reserpine (2 mg/kg, i.p.) before sacrifice.

At the time of sacrifice, animals were anesthetized by inhalation of methoxyfluran and then killed by cervical dislocation; the heart and tail and femoral arteries were then rapidly removed.

Isolated cardiovascular preparations

Myocardial preparations: The experiments were carried out on both spontaneously beating atria and electrically-driven (1 Hz) left atrium, as previously described (21). Since myocardial preparations were isolated from reserpine-treated animals, before the beginning of the experiments, the depletion of catecholamines was verified by lack of any positive inotropc effect following addition of tyramine (1.5 $\mu$M). Moreover, in each atrial preparation, the maximum positive inotropic effect ($E_{\text{max}}$) induced by 0.1 $\mu$M isoprenaline was determined before the addition of carteolol. Non-cumulative concentration-effect curves were obtained for increasing concentrations (from 0.01 $\mu$M to 1 mM) of carteolol. Two minutes after the maximum response to each drug concentration was reached, the atria were washed before the addition of a higher concentration. Inotropic effects were defined as the difference between the force of contraction before and after drug addition and were expressed as percentages of the $E_{\text{max}}$ induced by 0.1 $\mu$M isoprenaline in the same atria. The EC$_{50}$ value for carteolol was defined as the concentration producing half the maximum effect obtainable with the drug and was calculated by means of Prism 3.03 Software (GraphPad, San Diego, CA, USA) from concentration-response data evaluated by sigmoidal curve fitting. In spontaneously beating atria, chronotropic effects were defined as the difference between the heart rate (beats /min) before and after drug addition, and they were expressed as percentages of variation over the basal value.

Isometric contraction curves were analyzed for time to peak force ($t_1$), relaxation time ($t_2$), mean velocity of force development ($S_1$), and mean velocity of relaxation ($S_2$), according to Reiter (22).

Where indicated, propranolol or timolol or 3-isobutyl-1-methylxanthine (IBMX) were added to the bath medium 15 – 20 min before the addition of carteolol.

Langendorff isolated perfused hearts: Hearts were prepared and retrogradely perfused via the aorta at a constant flow rate of 8 – 9 ml/g tissue/min, as previously described (23). After a stabilization period of 30 min, the atria were excised and the heart was driven at the frequency of 6 Hz through platinum electrodes placed on the upper part of the right ventricle. Heart contractility was measured by means of an intra-ventricular rubber balloon. The drug was added to the perfusion medium and the time course of cardiac effects was continuously recorded.

Vessel preparations: Femoral or tail artery rings were prepared as previously described (24). The contractility of vascular tissues was recorded by means of a high-sensitivity transducer connected to a chart recorder (type DYO and Unirecord System, Model 7050; Ugo Basile, Varese, Italy). Where indicated, the endothelium was removed by gently rubbing the intimal surface with a polyester string. Vessel segments were allowed to equilibrate for 60 min before viability was assessed with a standard procedure, consisting of contraction induced by 1 $\mu$M phenylephrine and relaxation by 2 $\mu$M acetylcholine, when the extent of contraction had reached the plateau. The denuded preparations did not respond to
acetylcholine, thus indicating loss of endothelial functional integrity (25). Where indicated, propranolol or timolol were added to the bath medium 15 min before the vasoconstrictor agent.

Biochemical assays

Preparation of membranes from rat ventricular tissue: Rat cardiac tissue was homogenized in 10 volumes (w/v) of ice-cold 50 mM Tris-HCl, pH 7.4. The homogenate was centrifuged at 48000 × g for 10 min. The pellet was washed in the same buffer, centrifuged again at 48000 × g for 10 min, and used for binding assay and adenylate cyclase activity measurement. Protein content was determined according to Lowry et al. (26), using bovine serum albumin as standard.

β-Adrenoceptor binding assay: Binding studies were performed essentially according to Cerbai et al. (27). The affinity of the drugs for β/β-adrenoceptors was determined by measuring the displacement of 2 nM [3H]CGP12177 by increasing concentrations (0.1 nM – 10 μM) of the tested compounds. Non-specific binding was determined in the presence of 10 μM (+)-propranolol and was about 30% of total binding. After 120 min of incubation at 25°C in a medium (0.25 ml final volume) containing 50 mM Tris-HCl (pH 7.4), 10 mM MgCl₂, and membrane suspensions (100 – 120 μg of proteins), bound and free radioactivity were separated by filtering the assay mixture through Whatman GF/C glass-fiber filters using a Brandel cell harvester. Filters, washed three times with 50 mM Tris-HCl (pH 7.4) at 4°C, were then placed in liquid scintillation vials containing 5 ml of Aquassure (Packard). The radioactivity retained on filters was determined in a Beckman Liquid Scintillation Spectrometer at 55% efficiency. Ki values were calculated from the Cheng-Prusoff equation (28). Binding data were analyzed using the non-linear-regression curve-fitted computer program Ligand (29).

Assay of adenylate cyclase activity: Membrane preparations (100 μg/100 μl) obtained from rat heart were pre-incubated for 10 min in a shaking bath at 37°C in a medium (0.5 ml) containing 50 mM Tris-HCl (pH 7.4), 10 mM MgCl₂, 1 mM EDTA (pH 7.4), 5 μM GTP, and 0.5 mM IBMX, as a non-specific phosphodiesterase inhibitor. At the end of pre-incubation, 0.5 mM ATP and increasing concentrations (1 nM to 10 μM) of carteolol were added. After 10 more min of incubation, the reaction was stopped by transferring the tubes to a boiling water bath for 2 min. The samples were then cooled to room temperature, centrifuged at 2000 × g for 10 min at 4°C, and the supernatant was used to determine cAMP, essentially according to the method of Varani et al. (30). Under our experimental conditions, the basal value of adenylate cyclase activity was 12.3 ± 3 pmol cAMP/10 min per 100 μg of proteins. EC₅₀ values in the cAMP assay were calculated with the non-linear least-squares curve fitting program Prism 3.03 (GraphPad).

Drugs

L-Phenylephrine hydrochloride, acetylcholine chloride, (+)-propranolol hydrochloride, reserpine, timolol maleate salt, tyramine hydrochloride, (+)-isoprenaline hydrochloride, carbamylcholine (carbachol) chloride, prazosin hydrochloride, IBMX, and ATP were from Sigma Chemical Co. (St. Louis, MO, USA). Carteolol hydrochloride was provided by SIFI S.p.A. (Catania, Italy). [3H]CGP12177 ((–)–4-(3-t-hydroxypropoxy-5,7-3H]benzimidazol-2-one) and [3H]cAMP were obtained from Amersham Italia (Milan, Italy). All other reagents were of analytical grade and obtained from commercial sources. Carteolol was prepared in DMSO; the final concentration of which never exceeded 0.3% (v/v) in the organ bath; in these conditions, the solvent did not modify the reactivity of cardiovascular tissues. All other agents were dissolved in saline.

Statistical analyses

Data are expressed as arithmetic means ± S.E.M. Statistical analyses were performed using paired t-tests or one-way analysis of variance (ANOVA). A P value of less than 0.05 was considered statistically significant.

Results

Effect of carteolol in isolated rat heart preparations

In rats spontaneously beating atria, carteolol, at low concentrations (0.01 – 0.1 μM), antagonized the effects of increasing concentrations of the full β-adrenoceptor agonist isoprenaline. As clearly shown in Fig. 1A, the concentration-response curve for the positive inotropic effect caused by isoprenaline was parallel rightward-shifted in the presence of carteolol. This shift was particularly evident in the presence of 0.1 μM carteolol. Moreover, 0.1 μM carteolol significantly reduced the positive chronotropic effect of isoprenaline (Fig. 1B).

In contrast, at higher concentrations (from 1 μM to 1 mM), carteolol caused a concentration-dependent increase in the force of contraction and a slight modification of heart rate. (Fig. 2). The effects of carteolol peaked at 0.1 mM and tended to decrease at the highest (1 mM) concentration tested. The increase in force of contraction was very rapid in onset and reached its peak within 1.5 min after drug addition to the bathing medium. When the inotropic effect of carteolol was expressed as a percentage of the maximum positive inotropic response induced by 0.1 μM isoprenaline in the same preparations (Fig. 2A), the maximum effect of
carteolol was 17.2 ± 1.1% of $E_{\text{max}}$. The intrinsic activity ($\alpha$) of carteolol, assessed as a fraction of maximum response to isoprenaline, was 0.17 ± 0.1. From the reported concentration-effect curve, an EC$_{50}$ value of 4.6 ± 0.1 µM was calculated. The positive inotropic effect of carteolol was associated with a very low modification in heart frequency (Fig. 2B). Basal heart rate, which ranged from 160 to 200 beat/min, was slightly increased by carteolol concentrations from 1 µM to 0.1 mM, the maximum increase being 7.8 ± 1.9% ($P<0.01$, with respect to control values) at 0.1 mM carteolol.

The isometric contraction curves obtained in the presence of increasing concentrations of carteolol were analyzed for $t_1$, $t_2$, $S_1$, and $S_2$ (Table 1). Results indicate that $t_1$ values were not significantly affected by carteolol, whereas a significant shortening of $t_2$ occurred at high concentrations (0.01 and 0.1 mM). At the same concentrations, $S_1$ and $S_2$ values were significantly increased, indicating increased velocities of force development and relaxation, respectively. For comparison, Table 1 also reports the values of the same parameters, calculated in the same experimental conditions, in the presence of isoprenaline. As expected, a significant shortening of $t_1$ and $t_2$ values and a marked increase in $S_1$ and $S_2$ were observed.
The positive inotropic effect of carteolol was still present in electrically driven left atrium and in Langendorff heart preparations (data not shown). In left atrium, the effect of carteolol peaked at 10 μM (21 ± 2.3% of E\text{max} induced by 0.1 μM isoprenaline in the same preparations) and then progressively decreased at the highest concentrations tested. In Langendorff heart, the positive inotropic effect of carteolol was still evident, although lower (16 ± 2% variation over the basal value, at 0.1 mM) than that observed in isolated atria.

To ascertain the involvement of β-adrenoceptor activation in the cardiac action of carteolol, we studied the effect of carteolol in rat spontaneously beating atria in the presence of the conventional ISA-deprived β-blockers, propranolol and timolol. Surprisingly, the effect of carteolol was not affected by the presence of propranolol at a concentration (0.1 μM) that abolished the cardiac effect of isoprenaline in the same preparations (data not shown). Only higher concentrations (1 – 10 μM) of propranolol or timolol were able to significantly modify the positive inotropic effect of carteolol (Fig. 3). Propranolol at 1 μM caused a rightward shift of the concentration-response curve for carteolol (EC\textsubscript{50}: 14.3 ± 0.1 μM). At a higher concentration (10 μM), both propranolol and timolol drastically inhibited the heart response to carteolol.

The positive inotropic effect of carteolol was not modified at all by the presence in the bathing fluid of the α\textsubscript{1}-antagonist prazosin (50 nM) (31), excluding any action of carteolol on α\textsubscript{1}-adrenoceptors (data not shown).

In a second set of experiments, the positive inotropic effect of carteolol was tested in the presence of carbachol (CCH) or IBMX, two drugs which, although in opposite ways, modify the cAMP-mediated responses. The results (data not shown) indicate that the effect of carteolol was completely prevented by CCH at a concentration (50 nM) which slightly inhibited basal contractility (5 – 10% decrease of developed force of contraction compared to basal value, P≥0.05), but abolished the cAMP-dependent positive inotropic response of the same atria to 0.1 μM isoprenaline. In contrast, the maximum positive inotropic effect of carteolol was increased from 17.2 ± 1.1% to 42.5 ± 3.9% (P<0.01, n = 4) by pre-treatment of atria with a concentration of IBMX (1 μM) that did not increase by itself the cardiac inotropism. In the presence of 1 μM IBMX, also the maximum chronotropic effect of carteolol was significantly (P<0.05, n = 4) enhanced, reaching a 17.5 ± 4.32% increase over the basal values, at 0.1 mM.

### Table 1. Effect of increasing concentrations of carteolol on isometric contraction parameters of rat spontaneously beating atria: comparison with effect of isoprenaline

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (M)</th>
<th>t\textsubscript{1} value (ms)</th>
<th>t\textsubscript{2} value (ms)</th>
<th>S\textsubscript{1} value (mN/s)</th>
<th>S\textsubscript{2} value (mN/s)</th>
</tr>
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<tbody>
<tr>
<td>—</td>
<td>—</td>
<td>62.5 ± 2.0</td>
<td>106.6 ± 2.1</td>
<td>34.8 ± 1.7</td>
<td>21.1 ± 1.6</td>
</tr>
<tr>
<td>Carteolol</td>
<td>10\textsuperscript{-7}</td>
<td>60.1 ± 3.0</td>
<td>105.0 ± 0.9</td>
<td>40.2 ± 5.7</td>
<td>23.0 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>10\textsuperscript{-6}</td>
<td>59.5 ± 0.4</td>
<td>105.0 ± 0.5</td>
<td>41.2 ± 3.1</td>
<td>25.2 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>10\textsuperscript{-5}</td>
<td>60.7 ± 0.2</td>
<td>100.2 ± 0.9*</td>
<td>50.7 ± 2.4***</td>
<td>30.7 ± 2.3**</td>
</tr>
<tr>
<td></td>
<td>10\textsuperscript{-4}</td>
<td>61.8 ± 2.1</td>
<td>100.6 ± 0.2*</td>
<td>50.3 ± 2.4***</td>
<td>30.9 ± 2.4**</td>
</tr>
<tr>
<td>Isoprenaline</td>
<td>10\textsuperscript{-7}</td>
<td>53.9 ± 3.1*</td>
<td>93.0 ± 4.2*</td>
<td>59.7 ± 3.9***</td>
<td>37.4 ± 1.2***</td>
</tr>
</tbody>
</table>

Isometric contraction curves were analyzed for t\textsubscript{1} (time to peak force), t\textsubscript{2} (relaxation time), S\textsubscript{1} (mean velocity of force development, F\textsubscript{t}/t\textsubscript{1}), and S\textsubscript{2} (mean velocity of relaxation, F\textsubscript{c}/t\textsubscript{2}) values, according to Reiter (22). Experimental conditions are described in Materials and Methods. Results are means ± S.E.M. from nine different experiments carried out on different atrial preparations. *P<0.05, **P<0.01 and ***P<0.001 vs control values.

![Fig. 3. Concentration-effect curves for positive inotropic effect of carteolol in absence and presence of 1 or 10 μM propranolol or 10 μM timolol. Effect of carteolol, defined as difference between force of contraction before and after its addition to bathing fluid, expressed as percentage of response induced by 0.1 μM isoprenaline in the same preparation. Results are means ± S.E.M. (n = 10). *P<0.05 vs respective controls.](image-url)
Effect of carteolol on vascular preparations

The effect of carteolol was also studied in vascular preparations, such as rat femoral and tail arteries, in which \( \beta \)-adrenoceptor stimulation is well-known to mediate vascular relaxation (32). In rat femoral arteries pre-contracted by the \( \alpha \)-adrenergic agent phenylephrine (1 \( \mu \)M), the addition of carteolol partially inhibited the long-lasting contractile response to the amine in a concentration-dependent manner. The relaxing effect of carteolol appeared at 1 \( \mu \)M and peaked at 0.1 mM (Fig. 4A). The relaxation induced by carteolol (0.1 mM) was significantly decreased and almost abolished by pre-treatment of vascular rings with a high concentration (10 \( \mu \)M) of propranolol (Fig. 4B).

The vasodilator effect of carteolol was even more evident in rat tail artery preparations pre-contracted by phenylephrine (Fig. 5). Figure 5A shows that the relaxing effect of carteolol was identical in rings of both endothelium-intact and endothelium-denuded tail artery, thus excluding any involvement of endothelium-dependent relaxing factors in the vascular action of carteolol. The effects of the lowest concentrations of carteolol (up to 100 \( \mu \)M) were sensitive to timolol (10 \( \mu \)M), as clearly demonstrated in Fig. 5B.

Some experiments were performed in rat tail artery rings pre-contracted by 90 mM KCl. In these vascular preparations, carteolol up to 10 mM was devoid of any effect (data not shown). In the same experimental conditions, the \( \beta \)-adrenoceptor agonists, isoprenaline and salbutamol, also did not inhibit the \( K^+ \)-induced contractile effect (data not shown).

Biochemical assays

Some experiments were carried out to evaluate the affinity of carteolol for \( \beta \)-adrenoceptors of rat heart membranes. The affinity of the drug was measured by its ability to specifically displace \(^{3}H\)CGP12177 (20 nM) from its binding sites. Carteolol showed a very high affinity for \( \beta_1/\beta_2 \)-adrenoceptors of rat heart, its \( K_i \) value being 1.5 \( \pm \) 0.3 nM, in the same order of magnitude as those we measured for the classic \( \beta \)-blockers, propranolol (K\( _i \) value of 8.5 \( \pm \) 0.7 nM) and timolol (K\( _i \) values of 2.3 \( \pm \) 0.3 nM).

As a control, the ability of carteolol to displace \(^{3}H\)prazosin from \( \alpha \)-adrenoceptors present in the same rat heart membranes was also evaluated. Carteolol was completely devoid of any effect on the binding of \(^{3}H\)prazosin, confirming its lack of affinity for \( \alpha \)-adrenoceptors (data not shown). Increasing concentrations of carteolol (from 1 nM to 10 \( \mu \)M) were also tested on the \( \beta \)-adrenoceptor-related adenylate cyclase activity present in rat heart membranes. In our experimental conditions, carteolol caused a concentration-dependent increase in cAMP production. From the concentration-effect curves (data not shown) an EC\( _{50} \) value of 26 \( \pm \) 4 nM was calculated for carteolol.

Discussion

This study provides new insights in the ISA-related cardiovascular effects of carteolol, a \( \beta \)-blocking agent currently used to treat cardiovascular and non-cardiovascular diseases. We confirm that carteolol is endowed with cAMP-dependent intrinsic sympathomimetic activity both at the cardiac and vascular level, but we also propose, for the first time, that carteolol acts as a non-conventional partial agonist of \( \beta \)-adrenoceptors. Many evidences support this conclusion. First of all,
the experiments on spontaneously beating atria show that carteolol concentrations blocking catecholamine effects are two or three orders of magnitude lower than those causing cardiostimulation, this behavior being typical of non-conventional partial agonists (5–10). Carteolol at 0.01–0.1 μM antagonizes the cardiac effects of the full β-adrenoceptor agonist, isoprenaline, indicating that it is provided with very high affinity for β-adrenoceptor binding sites, to which agonists (i.e., isoprenaline) and/or conventional antagonists (propranolol or timolol) bind. In fact, when we assessed carteolol affinity for cardiac β₁/β₂-adrenoceptors as displacement of [³H]CGP12177, we obtained a Kᵢ value of 1.5 ± 0.3 nM, clearly related to Kᵢ values measured for conventional β₁/β₂-antagonists, propranolol, and timolol (present results; 33) and also rather well-related to the concentrations that block the effect of isoprenaline in isolated atria. The discrepancy between carteolol concentrations found active in biochemical (binding study) and functional (agonism towards isoprenaline) assays is easily explained considering that the results from biochemical assays are obtained in cell-free systems, whereas cardiac responses are evaluated in an intact tissue and are certainly affected by the low permeability of the cells to carteolol, which is a hydrophilic molecule. The inotropic and chronotropic effects induced by carteolol appear only at higher concentrations. In our experimental conditions, they peak at 0.1 mM and decrease at 1 mM, this reduction at the highest concentration probably reflecting a direct membrane-stabilizing effect of carteolol (34).

Another interesting and original result of our study is the partial resistance of the cardiac action of carteolol to the inhibition by conventional antagonists, propranolol and timolol. The inotropic effect of carteolol is prevented by a concentration (10 μM) of β-blockers two orders of magnitude higher than that (0.1 μM) completely abolishing the effect of isoprenaline in the same preparations (data not shown). This low sensitivity of carteolol towards conventional β-blockers resembles that of non-conventional partial agonists which are largely resistant to the usual concentrations of propranolol and other β-blockers (5–9, 35), since their effects are due to interaction with a propranolol-insensitive state of the β₁-adrenoceptor (6, 8, 9).

Evidences have been collected that an atypical β-adrenoceptor, sharing many properties with the cardiac propranolol-insensitive state of the β₁-adrenoceptor (for example, the interaction with non-conventional partial agonists and the resistance to propranolol), is present also in rat vascular preparations, where it co-exits with β₁- and β₂-adrenoceptors (35). Our results show that carteolol is endowed with intrinsic activity also in the peripheral artery preparations. The drug relaxes, in a concentration-dependent way, rat femoral and, even more significantly, tail artery rings pre-contracted by the pure α-agonist phenylephrine. In accordance to what was observed in cardiac preparations, also in vascular preparations the relaxing effect of carteolol is sensitive only to a high concentration (10 μM) of conventional β-antagonists; these results strongly suggest the interaction of carteolol with the vascular atypical β-adrenoceptor (35). Some papers report that
the peripheral vasodilator activity of a high concentration (1 mM) of carteolol is apparently modulated by extracellular Ca\(^{2+}\) concentration (36) or is related to release of EDRF (endothelin-derived relaxing factor)-like factors (37). Our results exclude any involvement of endothelium-derived factors in the vascular responses to carteolol, since the effects of increasing concentrations of the drug remains unmodified in endothelium-denuded tail artery rings. In the same manner, in vessels pre- contracted by 90 mM KCl, the relaxing effect of carteolol is lacking, excluding any influence of the drug on Ca\(^{2+}\) influx through voltage-dependent calcium channels (38).

The cardiac insensitive-propranolol state of the \(\beta_1\)-adrenoceptor is associated with a cAMP-mediated pathway (35). In our study, many biochemical and functional evidences indicate that the cardiac effect of carteolol is related to an increase in cAMP concentrations. Firstly, we demonstrated that carteolol induces an increase in adenylate-cyclase activity in rat cardiac membranes. Secondly, in rat spontaneously beating atria, carteolol causes a decreasing trend in the \(t_1\) value of isometric contraction curves, produces a statistically significant reduction in \(t_2\), and increases the mean velocities of both \(S_1\) and \(S_2\) of cardiac fibers. In myocardial preparations, \(t_1\) and \(t_2\) shortening, associated with increases in \(S_1\) and \(S_2\) values, are generally ascribed to cAMP increase (21, 39, 40). Moreover, the cardiac effects of carteolol are significantly modified by the contemporary presence of 1 \(\mu\)M IBMX, a phosphodiesterase inhibitor that blocks cAMP degradation. Both the maximum positive inotropic and the chronotropic effect of carteolol are about 2.5-times enhanced by IBMX. IBMX (1 \(\mu\)M) addition has been used (6) to evidence in vitro the positive inotropic effect of the non-conventional partial agonist CGP12177. In this way, a three-times increase in ISA of CGP12177 has been observed by Sarsero and co-workers in human cardiac preparations (6). Lastly, the positive inotropic effect of carteolol is suppressed by carbachol at a concentration (50 \(\mu\)M) that abolishes the inotropic response to isoprenaline (0.1 \(\mu\)M) in the same heart preparation (present results, ref. 21). CCH is known to selectively abolish, in various heart preparations, the increase in contractility induced by a rise in cAMP levels in response to either stimulation of adenylyl cyclase or inhibition of cAMP-dependent phosphodiesterase (41).

Taken all together, our results clearly show that carteolol is endowed with cAMP-dependent intrinsic sympathomimetic activity both at the cardiac level, where it increases myocardial contractility only slightly affecting the frequency, and at the vascular level, where it causes an endothelium-independent relaxing effect. The novelty emerging from our study is that in cardiovascular preparations, carteolol behaves as a non-conventional partial agonist. At the cardiac level, it may interact with the well-known propranolol-resistant state of the \(\beta_1\)-adrenoceptors (6, 8, 9, 12); and at the vascular level, carteolol may exert its effect by activating the atypical \(\beta\)-adrenoceptor, which co-exists with \(\beta_1\) and \(\beta_2\)-adrenoceptors in the vessels and shares many properties with the cardiac propranolol-resistant state of \(\beta_1\)-adrenoceptors (35).

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