Doxorubicin Affects the Cardiac Muscarinic System in the Rat

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ABSTRACT. During the study on the mechanism of doxorubicin-induced cardiotoxicity, we observed that a long incubation (4 hr) with doxorubicin reduced the maximal negative inotropic effects of muscarinic receptor agonist, carbachol. The mechanism responsible for this doxorubicin-induced reduction of the efficacy of carbachol was examined in isolated guinea pig hearts. In isolated left atrial muscle preparations, 1 hr incubation with 100 µM doxorubicin caused a parallel right-ward shift of the concentration-response curves for carbachol, but a longer (4 hr) incubation with this agent (30, 100 or 200 µM), caused a significant reduction of the magnitude of the negative inotropic effect of carbachol in addition to the concentration-dependent parallel rightward shift. The 4-hr incubation with these concentrations of doxorubicin also reduced the maximal negative inotropic effect of an adenosine A1 receptor agonist, R-phenylisopropyl adenosine (R-PIA), without affecting the potency of this agonist. Doxorubicin (1 to 100 µM) reduced [3H]quinuclidinyl benzilate (QNB) binding in a concentration dependent manner, but failed to alter [3H]R-PIA binding. The decrease in the magnitude of the maximal negative inotropic effect by doxorubicin was caused by changes in the muscarinic system at steps common to the transduction of muscarinic and adenosine A1 receptor mechanisms.

KEY WORDS: adenosine receptor, cardiotoxicity, doxorubicin, muscarinic receptor, rat heart.

The anthracycline antibiotic, doxorubicin, is one of the most potent anticancer agents currently available for cancer chemotherapy. Its anticancer action is attributed to the interaction of the drug between base pairs of DNA helices and the subsequent inhibition of DNA replication and/or RNA synthesis [12]. However, it is well known that doxorubicin produces acute or dose-related chronic cardiotoxicity. The chronic cardiotoxicity is irreversible, appears several years, or sometimes as long as 20 years after the discontinuation of chemotherapy, and is life threatening [11, 14, 20, 25, 27]. Therefore, the use of doxorubicin is limited primarily by its chronic cardiotoxicity.

The mechanisms responsible for the cardiotoxic actions of doxorubicin, have been extensively studied. It has been demonstrated that the negative inotropic effect of doxorubicin results from alterations in Ca2+ handling by the sarcoplasmic reticulum [15, 28–32]. This mechanism, however, cannot explain all the cardiac actions of doxorubicin. Several investigators have reported that doxorubicin alters cardiac adrenergic mechanisms [4, 6, 13, 26, 33]. We have shown that doxorubicin shifts the concentration-response curves for acetylcholine to the right, and reduces the binding of [3H]quinuclidinyl benzilate (QNB) in guinea-pig hearts [28, 29]. Additionally, while studying the cause of the antimuscarinic action, we observed that a relatively long exposure (4 hr) to doxorubicin reduced the maximal negative inotropic effect of carbachol in left atrial muscle preparations isolated from rat hearts.

The present study was undertaken to examine the mechanisms responsible for the doxorubicin-induced reduction in the maximal negative inotropic effect of carbachol. The effects of doxorubicin on the muscarinic agonist were compared with those of an adenosine A1 receptor agonist, R-phenylisopropyl adenosine (R-PIA). Muscarinic and adenosine A1 receptors share a common signal transduction pathway in the atrial muscle. Both receptors cause activation of a GTP-binding protein (Gi) to modulate ligand-regulated K+ current [17, 23]. The results show that doxorubicin competitively antagonizes binding to the muscarinic receptors and alters the muscarinic mechanisms in step(s) common to adenosine A1 receptor mechanisms.

MATERIALS AND METHODS

All protocols in this study were approved by the Institutional Animal Care and Use Committee at the Kitasato University School of Veterinary Medicine and Animal Sciences and were in accordance with the Guide to the Use of Laboratory Animals issued by the U. S. Department of Health and Human Services.

Left atrial muscle preparations: Rats were sacrificed by cervical dislocation. Hearts were immediately removed and perfused via the aorta using a Langendorff apparatus with Krebs-Henseleit bicarbonate buffer solution with the following millimolar composition: 118 NaCl, 27.2 NaHCO3, 4.8 KCl, 1.0 KH2PO4, 1.2 MgSO4, 1.2 CaCl2 and 11.1 glucose. The solution was saturated with a 95% O2 and 5% CO2 gas mixture, yielding a pH value of 7.4 at 30°C. After

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all visible blood was washed out, left atrial muscles were excised.  Left atrial muscle preparations were suspended vertically in a temperature-controlled bath containing 20 ml of the above-mentioned Krebs-Henseleit bicarbonate buffer solution. The preparations were electrically stimulated at 2 Hz with square-wave pulses of 3 msec duration at a voltage approximately 15% above the threshold using a pair of platinum field-stimulation electrodes. The resting tension was adjusted to 1.0 g. Developed tension was continuously monitored using a force-displacement transducer and a polygraph recorder (model TB 651T and WB840G, respectively, Nihon Kohden Kogyo, Tokyo). Experiments were started after a 90 to 120 min equilibration period. Cumulative concentration-response curves for the negative inotropic effect of carbachol or R-PIA were generated by increasing the drug concentration at 2-min intervals or when the effect reached a steady state at each concentration.

\[ ^{3}H \]QNB binding: QNB specifically binds to muscarinic receptors, and inhibits these receptors. The characteristics of muscarinic receptors in the rat heart were examined in the \[^{3}H\]QNB binding study [2].

In this study, to collect a relatively large volume of homogenates for \[^{3}H\]QNB binding study, ventricular muscles were employed. Ventricular muscles of rat hearts were minced and homogenized using a Dounce ball-type homogenizer for \[^{3}H\]QNB binding study [2].

The binding reaction was started by the addition of a 25-µl aliquot of filtered homogenate to 225 µl of an incubation solution (final protein concentration, 0.1–0.3 mg/ml) containing 1 nM \[^{3}H\]QNB, 75 mM MgCl₂, 25 mM Tris-HCl buffer (pH 7.5 at 26°C) and doxorubicin (1, 10 or 100 µM) with or without 2 µM atropine. After the mixture was incubated for 2 hr at 26°C, the reaction was terminated by the addition of 3 ml of an ice-cold “stopping” solution containing 75 mM MgCl₂ and 25 mM Tris-HCl buffer (pH 7.5). The homogenate was passed through a medical-grade gauze mesh to remove large debris.

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\[ ^{3}H \]R-PIA binding: In the \[^{3}H\]QNB binding study, homogenates were used. In the \[^{3}H\]R-PIA binding study, however, membrane preparations partially purified from the ventricular muscle of rat heart using the method reported by Endoh et al. [10] were used to reduce non-specific binding. Ventricular muscles were homogenized with 10 volumes of an ice-cold solution containing 250 mM sucrose, 1 mM MgCl₂ and 5 mM Tris-HCl (pH 7.5) using a Polytron homogenizer for 15 sec at the setting of 7. The homogenate was centrifuged at 900 x g for 10 min at 4°C, and the resultant supernatant solution was centrifuged at 50,000 x g for 20 min at 4°C. The pellet was washed twice with an ice-cold “resuspending solution” containing 10 mM MgCl₂ and 50 mM Tris-HCl (pH 7.5 at 4°C).

The binding reaction was started by the addition of a 50-µl aliquot of the membrane preparation (final concentration of protein, 0.07–0.15 mg/ml) to a prewarmed (450 µl) incubation mixture, yielding final concentrations of 10 mM MgCl₂, 5 nM \[^{3}H\]R-PIA and 50 mM Tris-HCl (pH 7.5 at 25°C) in the absence or presence of 1, 10 or 100 µM doxorubicin. After 2 hr incubation, the reaction was terminated by the addition of a 4-ml ice-cold aliquot of the above resuspending solution. Bound \[^{3}H\]R-PIA was collected using a filter (GF/C, Whatman, Clifton, NJ, U.S.A.) under vacuum. Non-specific binding was determined in the presence of 10 µM unlabeled R-PIA [10].

Chemicals and statistical analyses: \[^{3}H\]QNB (specific activity 50 Ci/mmole) and \[^{3}H\]R-PIA (specific activity 38 Ci/mmole) were purchased from Amersham Japan Corp., (Tokyo). Doxorubicin hydrochloride (Adriacin®) was purchased from Kyowa Hakko Kogyo Co., Ltd. (Tokyo). Atropine sulfate, carbachol (carbamylcholine chloride) and R-PIA were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of reagent grade.

Data are expressed as the mean ± SEM. Statistical significance was determined using one-way analysis of variance (ANOVA) for multiple comparison of data. When appropriate, Student’s unpaired t-test was used. Values of P < 0.05 were considered significant. Protein concentration was determined by the method of Lowry et al. [21] using bovine serum albumin as the standard.

RESULTS

Effects of doxorubicin on the negative inotropic action of carbachol: We have shown previously that doxorubicin causes a reversible rightward shift of the concentration-response curves for the negative inotropic effect of acetylcholine in left atrial muscle preparations isolated from guinea-pig hearts [28, 29]. Whether doxorubicin produces the same effect on rat left atrial muscle was examined first.

A low concentration of doxorubicin (final concentration 30 µM) caused biphasic positive inotropic effects. An early peak was reached within 10 min (3.2 ± 0.9% above the initial value, n=6). The late phase was apparent 120 min later and developed gradually thereafter. Developed tension observed 4 hr after the doxorubicin addition was 30.1 ± 2.8% (n=6) above the corresponding values observed in control preparations. The peak of the early phase was greater with 100 µM (16.0 ± 5.3% above the initial value, n=10) or 200 µM (25.5 ± 8.5% above the initial value, n=6) doxorubicin than the value observed with 30 µM doxorubicin. These higher concentrations of doxorubicin failed to produce the late phase positive inotropic effect. With 100 or 200 µM doxorubicin, developed tension decreased during the 4-hr period after the early phase peak which was
observed within 10 min. The negative inotropic effects caused by 200 µM doxorubicin were significantly greater than those observed with 100 µM doxorubicin. Developed tension observed at the end of the 4-hr incubation in the presence of 200 µM doxorubicin was 10.8 ± 6.0% (n=6) below the corresponding value observed in control preparations, while the value observed in the presence of 100 µM doxorubicin was 18.1 ± 4.1% above control (n=9). These effects of doxorubicin are consistent with our earlier observations in left atrial muscle preparations obtained from rat hearts [30].

The concentration-response curves for carbachol were generated after 4 hr incubation at 30°C in the absence or presence of 30, 100 or 200 µM doxorubicin. Doxorubicin caused a right-ward shift of the dose-response curves for the negative inotropic effect of carbachol (Fig. 1A and 1B). The ED₅₀ value, the concentration of carbachol, which causes a half maximal negative inotropic effect, was increased by doxorubicin in a concentration-dependent manner (Table 1). Moreover, the maximal negative inotropic effect observed with high concentrations of carbachol was significantly attenuated by doxorubicin (Fig. 1A and Table 1). The increase in the ED₅₀ value and the decrease in the maximal negative inotropic effects were significantly reversed by 2 hr incubation in a drug-free solution (Table 1). In atrial muscle preparations exposed to 100 µM doxorubicin for 1 hr, the ED₅₀ value for carbachol increased a value similar to that observed with a 4-hr exposure; the attenuation of the maximal negative inotropic effects of carbachol, however, was not observed after 1 hr incubation with doxorubicin (Fig. 2A, 2B and Table 1).

These results show that doxorubicin causes a right-ward shift of the concentration-response curves for carbachol in rat heart muscle preparations similar to that previously reported for guinea-pig heart muscle [28, 29]. In addition, doxorubicin reduced the maximal negative inotropic effects of carbachol, a muscarinic agonist, after a 4-hr exposure.

Effects of doxorubicin on the negative inotropic action of R-PIA: The addition of R-PIA to the incubation medium also caused a concentration-dependent negative inotropic effect (Fig. 3A). Doxorubicin significantly reduced the maximal negative inotropic effect of R-PIA. In the presence of 30, 100 and 200 µM doxorubicin, 10 µM R-PIA caused 74.3 ± 2.5% (n=4), 56.5 ± 6.6% (n=4) and 46.0 ± 15.8 % (n=3) reductions, respectively, of developed tension compared to the 82.8 ± 1.7% (n=4) reduction observed in the absence of doxorubicin (Table 1). Doxorubicin, however, did not cause a significant increase in the ED₅₀ values of the concentration-response curve for R-PIA (Fig. 3B and Table 1). The reduction of the maximal negative inotropic effect of R-PIA by doxorubicin was significantly decreased by a 2-hr incubation in the absence of doxorubicin (Fig. 4).

Doxorubicin-induced reversal of the negative inotropic effects carbachol or R-PIA: Carbachol (final concentration, 0.1 µM) caused a 54.8 ± 2.2% (n=5) decrease in developed tension (Fig. 5). The developed tension reached the max-
mal effect within 2 min, and then stabilized at the lowest level. The addition of 100 µM doxorubicin reversed the negative inotropic effects of carbachol; the developed tension rapidly increased after the addition of doxorubicin reaching the peak 4 min later. Developed tension observed at this time was 77.0 ± 6.2% (n = 11) of the initial value. Doxorubicin, however, failed to alter the negative inotropic effects of R-PIA (Fig. 6). R-PIA (final concentration, 0.1 µM) decreased developed tension to 35.7 ± 4.5% (n = 4) of the initial value. The addition of 100 µM doxorubicin 15 min after the addition of R-PIA increased the developed tension only slightly (10.5 ± 2.7%, n = 4). The R-PIA-induced increases in developed tension in the absence (16.0 ± 5.3%, n = 10) or presence of 100 µM doxorubicin (10.5 ± 2.7%, n = 4) were not significantly different.

Effects of doxorubicin on [3H]QNB or [3H]R-PIA binding: The interaction of doxorubicin with muscarinic receptors of rat ventricular muscle was examined by [3H]QNB binding. In this study, homogenates from ventricular muscle of rat heart were used. Under the present conditions, specific [3H]QNB binding (389 ± 51 fmol/mg protein, n = 4) accounted for 42% of total binding (924 ± 89 fmol/mg protein).

Doxorubicin, in concentrations ranging from 1 to 100 µM, reduced [3H]QNB binding in a dose-dependent manner (Fig. 7). The IC50 value for doxorubicin was 49.9 ± 19.0 µM (n = 4).

The interaction of doxorubicin with adenosine receptors of rat hearts was examined by [3H]R-PIA binding. The specific binding of [3H]R-PIA to partially purified membrane preparations obtained from ventricular muscle (43.7 ± 4.1 fmol/mg protein, n = 5) accounted for 38% of total binding (115 ± 4 fmol/mg protein, n = 5). Doxorubicin, in concentrations ranging from 1 to 100 (M, failed to significantly alter specific [3H]R-PIA binding (Fig. 7). These results show that doxorubicin directly interacts with muscarinic receptors but fails to react with adenosine A1 receptors.

DISCUSSION

In left atrial muscle isolated from rat heart, doxorubicin caused a right-ward shift of the concentration-response curves for the negative inotropic effects of carbachol, and caused a concentration-dependent reduction of [3H]QNB binding in homogenates obtained from ventricular muscles of rat hearts. It has been reported that muscarinic receptors

### Table 1. Influence of doxorubicin on the negative inotropic effect of carbachol or R-PIA

<table>
<thead>
<tr>
<th>Incubation Time</th>
<th>Carbachol</th>
<th>R-PIA</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>ED50 (µM)</td>
<td>Maximal response (%)</td>
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<tr>
<td>1 hr incubation with doxorubicin</td>
<td></td>
<td></td>
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<tr>
<td>Carbachol</td>
<td></td>
<td></td>
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<tr>
<td>Control (4)</td>
<td>0.064 ± 0.009a</td>
<td>87.8 ± 1.5b</td>
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<tr>
<td>100 µM doxorubicin (4)</td>
<td>0.465 ± 0.057c</td>
<td>88.1 ± 11.2</td>
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<tr>
<td>4 hr incubation with doxorubicin</td>
<td></td>
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<tr>
<td>Carbachol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (7)</td>
<td>0.056 ± 0.006</td>
<td>90.9 ± 0.8</td>
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<tr>
<td>30 µM doxorubicin (6)</td>
<td>0.169 ± 0.024</td>
<td>84.2 ± 3.1</td>
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<tr>
<td>100 µM doxorubicin (6)</td>
<td>0.522 ± 0.076d</td>
<td>73.8 ± 5.0h</td>
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<tr>
<td>200 µM doxorubicin (6)</td>
<td>1.267 ± 0.152e</td>
<td>55.0 ± 5.4h</td>
</tr>
<tr>
<td>200 µM doxorubicin, followed by 1 hr washout (6)</td>
<td>0.232 ± 0.047f</td>
<td>77.2 ± 3.0d,e</td>
</tr>
<tr>
<td>2 hr washout (5)</td>
<td>0.158 ± 0.023g</td>
<td>83.0 ± 1.34c</td>
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<tr>
<td>R-PIA</td>
<td></td>
<td></td>
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<tr>
<td>Control (4)</td>
<td>0.041 ± 0.007</td>
<td>82.8 ± 1.7</td>
</tr>
<tr>
<td>30 µM doxorubicin (4)</td>
<td>0.060 ± 0.009</td>
<td>74.3 ± 2.5</td>
</tr>
<tr>
<td>100 µM doxorubicin (4)</td>
<td>0.047 ± 0.017</td>
<td>56.5 ± 6.6</td>
</tr>
<tr>
<td>200 µM doxorubicin (3)</td>
<td>0.065 ± 0.013</td>
<td>46.0 ± 15.8d</td>
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</table>

- a) Values represent the carbachol or R-PIA concentrations that cause a half-maximal negative inotropic effect. b) Values for the maximal response are the reduction in developed tension observed in the presence of high concentrations of either carbachol or R-PIA, and are expressed as the percentage reduction of the developed tension compared to the values observed at the end of 1 or 4 hr incubation with doxorubicin and before the additions of carbachol or R-PIA. c) Significantly different from control values (Student’s t-test, p<0.05). d) Significantly different from control values (ANOVA, p<0.05). e) Significantly different from the value observed in atrial muscle preparations incubated in the presence of 200 µM doxorubicin (ANOVA, p<0.05). Numbers in parentheses indicate the number of experiments. See legend to Fig. 1 for further details.
Fig. 2. Influence of doxorubicin (1 hr incubation) on the negative inotropic effect of carbachol. Left atrial preparations were incubated for 1 hr in the absence (O, control) or presence of 100 μM (▼) doxorubicin. The concentration-response curves for carbachol were then generated. Each point represents the mean of 4 experiments. Vertical lines indicate the standard error. See legend to Fig. 1 for further details. A: The values at each point are calculated as the percentage of the initial force of contraction observed immediately before the addition of carbachol. B: The values at each point are expressed as percentages of the maximum reduction caused by carbachol.

Fig. 3. Influence of doxorubicin on the negative inotropic effect of R-PIA. Left atrial muscle preparations of rat heart were incubated for 4 hr in the presence of 30 μM (▼), 100 μM (□) or 200 μM (▲) doxorubicin. Control preparations were incubated for 4 hr in the absence of doxorubicin (O). Cumulative concentration-response curves for the negative inotropic effect of R-PIA were generated by starting at 1 nM and increasing the concentration stepwise after the effect at each concentration reached steady state (about 2 min). Each value represents the mean of 3 to 4 experiments. Vertical lines indicate the standard error. See legend to Fig. 1 for further details. A: The values at each point are calculated as the percentage of the initial force of contraction observed immediately before the addition of R-PIA. B: The values at each point are expressed as percentages of the maximum reduction caused by R-PIA.
in ventricular muscles are coupled with signal transduction systems differently to those of atrial muscles; for example, muscarinic receptors of atrial muscles are directly coupled with K⁺ channel, as described later, but the receptors of ventricular muscles do not have such a direct interaction in producing a negative inotropic effect [19, 23]. However, the results of the present study may provide sufficient information for understanding the interaction of doxorubicin with receptor sites of muscarinic receptors in atrial muscles. In

**Fig. 4.** Reduction of the maximal negative inotropic effects of R-PIA by doxorubicin, and reversibility of the actions of doxorubicin. Preparations were incubated for 4 hr in the absence (O) or presence of 200 µM doxorubicin (V), or incubated for one (▼) or 2 hr (▲) in a drug-free solution after a 4-hr incubation in the presence of 200 µM doxorubicin. R-PIA (final concentration 10 µM) was then added to the incubation solution. The values are the percentages of developed tension observed immediately before the addition of 10 µM R-PIA. Each value represents the mean of 3 to 5 experiments. Vertical lines indicate the standard error.

**Fig. 5.** Negative inotropic effects of carbachol, and effects of doxorubicin. Fifteen minutes after the addition of 0.1 µM carbachol, 100 µM doxorubicin (V, DXR) or double distilled water (O, for control) was added. Values at each point are the percentages of the developed tension observed immediately before the addition of carbachol. Each point represents the mean of 3 to 5 experiments. Vertical lines indicate the standard error.

**Fig. 6.** Negative inotropic effects of R-PIA, and effects of doxorubicin. Fifteen minutes after the addition of 0.1 µM R-PIA, 100 µM doxorubicin (V, DXR) or double distilled water (O, for control) was added. Values at each point are a percentages of the developed tension observed immediately before the addition of R-PIA. Each point represents the mean of 4 experiments. Vertical lines indicate the standard error.

**Fig. 7.** Effects of doxorubicin on [3H]QNB and [3H]R-PIA binding. Homogenates for [3H]QNB binding study (solid bars) or membrane preparations for [3H]R-PIA binding study (open bars) were obtained from ventricular muscles of rat hearts. Values are expressed as percentages of the specific [3H]QNB or [3H]R-PIA binding observed in the absence of doxorubicin (control). Each point represents the mean of 4 to 5 experiments. Vertical lines indicate the standard error. * Significantly different from control values (ANOVA, p <0.05).
addition, doxorubicin almost completely reversed the negative inotropic effects of carbachol in isolated left atrial muscle preparations. Further, we observed previously that doxorubicin causes a reversible rightward shift of the concentration-response curve for the negative inotropic effects of acetylcholine and competitively inhibits [3H]QNB binding in guinea-pig hearts [28, 29]. Therefore, the rightward shift of the concentration-response curves for carbachol observed in the present study in rat heart, and in the previous study in guinea-pig heart, may be explained by the competitive antagonistic actions of doxorubicin on agonist binding to muscarinic receptors.

A relatively long (4 hr) incubation of atrial muscle preparations with doxorubicin, however, caused an additional effect, namely a reduction in the maximal negative inotropic actions of carbachol. After 4 hr incubation, the ED50 values for carbachol in the presence of 100 µM doxorubicin were not significantly different from the ED50 values observed in atrial muscle preparations exposed to the same concentration of doxorubicin for 1 hr. Thus, it appears that the decreased potency and decreased efficacy of carbachol caused by doxorubicin are mediated by separate mechanisms. Doxorubicin might have multiple actions on the muscarinic system. In our previous study [29], Schild plots constructed for the negative inotropic effects of acetylcholine formed a straight line. The slope of the line, however, was greater than unity. Those results also suggest that the influence of doxorubicin on the negative inotropic effects of the muscarinic agonist cannot be explained solely by competitive antagonism.

The negative inotropic effects of muscarinic agonists are reported to be mediated by activation of ligand-gated K+ channels that are regulated by a GTP binding protein (Gi or Gk) [23, 24]. Therefore, it is possible that the reduction of the maximal negative inotropic effects of carbachol by doxorubicin results from the disruption of this pathway.

Activation of the adenosine A1 receptors also produces negative inotropic effects in the atrial muscle. Similar to muscarinic stimulation, the mechanism for this effect is postulated to involve an increase in K+ conductance through ligand-gated K+ channels that are activated by a GTP binding protein [17, 23]. Thus, muscarinic and adenosine A1 receptor stimulation appears to share a common signal transduction pathway. As observed with carbachol, doxorubicin attenuated the maximum negative inotropic effect of the A1 receptor agonist, R-PIA. The attenuation was significantly reversed by a 2-hr incubation in doxorubicin-free solution. With R-PIA, however, the rightward shift of the concentration-response curves was not observed. In addition, doxorubicin, added to an incubation solution 15 min after the addition of PIA, failed to reverse the negative inotropic effect of R-PIA, and this agent did not affect [3H]R-PIA binding. These data suggest that the doxorubicin-induced attenuation of the maximal negative inotropic effects of muscarinic or adenosine receptor agonists results from its actions on steps that are common to both pathways, such as the G protein or K+ channel activation.

Several investigators [8, 9, 16] have reported that the negative inotropic effects of muscarinic or A1 receptor agonists are reduced by pertussis toxin. This toxin promotes ADP-ribosylation of the GTP binding protein and hence causes the uncoupling of both the muscarinic and adenosine receptor systems. The pattern of inhibition, i.e., attenuation of the maximal negative inotropic effects of carbachol, acetylcholine or R-PIA in left atrial muscle preparations isolated from pertussin toxin-treated rat [8, 9], was similar to that observed with exposure to doxorubicin. These results suggest that doxorubicin has actions similar to pertussis toxin in left atrial muscle of rat heart. It is unknown, however, whether doxorubicin acts on the GTP binding protein or on the K+ channels. In addition, it has been reported that the mechanism responsible for doxorubicin-induced cardiotoxicity may be associated with lipid peroxidation resulting from free radical formation. However, relationships between the anti-muscarinic actions and the free radical formation by doxorubicin, were not investigated in the present study.

The standard dosage of doxorubicin when used clinically as a single agent is 10 to 75 mg/m2 of body surface in a short intravenous infusion [5, 7, 18]. The average initial doxorubicin concentration in the plasma was approximately 1 µM when a 60 mg/m2 of doxorubicin was administered intravenously [1, 3]. Therefore, the plasma concentrations of doxorubicin in patients receiving chemotherapy are at least 30-fold lower than the concentrations used in this study. However, it is conceivable that the heart is exposed to higher concentrations of doxorubicin during intravenous infusion, and it has been reported that anthracycline-induced cardiac dysfunction in isolated heart preparations resembles that observed in patients in many aspects [22].

The results of this study suggest that the rightward shift of the concentration-response curve for carbachol observed in isolated left atrial muscle preparations in the presence of doxorubicin is due to a competitive antagonistic action of doxorubicin at the receptor site. The reduction of the efficacy seems to result from an alteration of the pathway common to muscarinic and adenosine-induced signal transduction in rat atrial muscle.

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