Inhibition of Constrictor Responses of Dog Coronary Artery by Atropine

A Possible Effectiveness of Atropine on Variant Form of Angina Pectoris

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SUMMARY

A possible effectiveness of atropine on variant form of angina pectoris was investigated using the left circumflex coronary arterial strips of dogs. Acetylcholine 10^{-5}–10^{-3} Gm/ml dose-dependently constricted the isolated arterial strips during potassium-contracture in 6 cases, and repetitive applications of acetylcholine could produce the similar contractions to the control. In 18 strips atropine 10^{-6} Gm/ml significantly depressed the contractions of coronary arteries induced by acetylcholine 10^{-5}–10^{-3} Gm/ml. In 5 arterial strips atropine 10^{-6} Gm/ml significantly inhibited norepinephrine-induced responses of these arteries, and by 10^{-5} Gm/ml further suppression of these responses was obtained. The results suggest that atropine may suppress the contractile responses of the coronary artery induced by acetylcholine and norepinephrine through a muscarinic-receptor blocking action and simultaneously partly through an adrenergic alpha-receptor blocking action.

Additional Indexing Words:

Acetylcholine Contractions of coronary artery Norepinephrine
Muscarinic receptor Adrenergic alpha-receptor

IT is well known that the spasm of large coronary vessels causes Prinzmetal's variant form of angina pectoris, and that the autonomic nervous system contributes to these vascular spasms.[1)–7)] These phenomena are confirmed by the observations by means of coronary angiographic methods,[8)–12)] in which it is proved that muscarinic agonists, methacholine and pilocarpine, and adrenergic alpha-receptor stimulating agents, norepinephrine and epinephrine, and/or ergot alkaloids induce an attack of angina pectoris. These results suggest that the development of Prinzmetal's angina is related to both cholinergic and adrenergic interactions. However, even now these interactions have

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been confused, and the mechanism of variant angina is not confirmed.

On the other hand, as therapeutic agents following drugs are used: a muscarinic receptor blocking agent, atropine, and an adrenergic alpha-receptor blocking agent, phenoxybenzamine, and Ca^{++}-antagonists, diltiazem, nifedipine et al. And there are many clinical reports on the effectiveness of these drugs.[4),6),7),13),14)] In this study, to confirm the possible effectiveness of atropine on Prinzmetal's angina pectoris we investigated the effects of atropine on the constrictor responses of dog coronary artery induced by acetylcholine and norepinephrine.

Methods

Twenty-nine mongrel dogs weighing 8–15 Kg of either sex were anesthetized with pentobarbital sodium 30 mg/Kg i.v. The heart was isolated and 4 portions of coronary artery were removed. Helical strips cut from the coronary artery were suspended in a 20-ml muscle chamber filled with Krebs-Ringer bicarbonate solution of the following millimolar composition; NaCl 117.7, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 24.4, glucose 10.0, and calcium disodium ethylenediaminetetraacetate 0.026 (this was added to chelate trace amounts of heavy metals which oxidize catecholamines). The solution in the bath was maintained at 37°C and aerated with a gas of 95% O_2 and 5% CO_2. The oxygen tension (PO_2) of the solution averaged 650 mmHg and the pH was 7.4. The coronary strips were connected to an isometric transducer (Nihonkoden SB-1T) and tension developments were recorded on an ink-writing polygraph (Nihonkoden RJG-3024). Resting tension was adjusted to 1.0 Gm, and the arterial strips were allowed to equilibrate for 2 hrs before any experiments were begun.

The drugs employed were as follows: acetylcholine chloride (Daiichi), atropine sulfate (Merck), and l-norepinephrine tartrate (Merck). The drugs were dissolved in Krebs-Ringer bicarbonate solution, and added to the bath with a volume of 0.2 ml. All doses are expressed as final bath concentrations of the salts. The drugs were removed from the bath by overflowing the preparations with oxygenated Krebs-Ringer bicarbonate solution at 37°C.

The number of arterial strips in the text is also the number of dogs used. The data show the per cent changes from the contraction induced by potassium 30 mM (=100%) and expressed as means±SE. The test for the statistical analysis of the data was done by Student's *t*-test.

Results

Effects of atropine on acetylcholine-induced contractions of coronary artery:

Fig. 1 shows typical recordings of 4 portions of isolated coronary arterial strips induced by acetylcholine 10^{-8}–10^{-3} Gm/ml during potassium-contracture. Four portions of coronary artery correspond to a portion before bifurcation of the left circumflex and the left anterior descending coronary artery

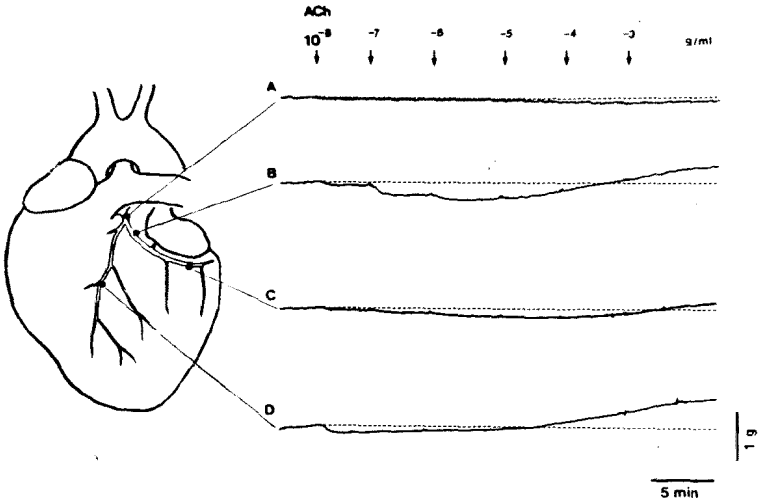

Fig. 1. Typical recordings of each portion of isolated coronary artery during potassium (30 mM)-contracture.

A: a portion before bifurcation of the left circumflex and the left anterior descending coronary artery.

B: a portion of the left circumflex coronary artery proximal to the first obtuse marginal branch.

C: a portion of the left circumflex coronary artery proximal to the left posterior descending branch.

D: a portion of the left anterior descending coronary artery proximal to the second diagonal branch.

ACh: acetylcholine.

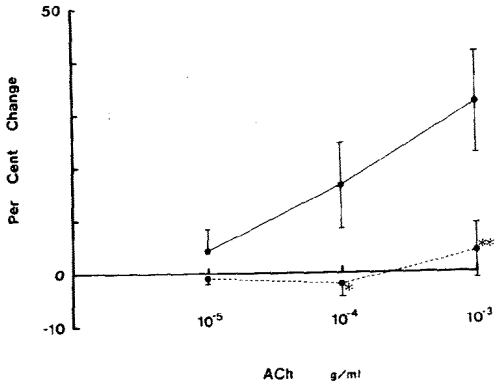

Fig. 2. Effect of atropine on acetylcholine-induced contractions of coronary arteries. Solid circles represent the control values, and open circles the values after atropine 10^{-6} Gm/ml. $100\% = 1.24 \pm 0.05$ Gm for 18 strips. * and ** show that differences from control value are statistically significant ($P<0.05$ and $P<0.02$, respectively).

(portion "A"), a portion of the left circumflex coronary artery proximal to the first obtuse marginal branch (portion " B "), a portion of the left circumflex coronary artery proximal to the left posterior descending branch (portion " C "), and a portion of the left anterior descending coronary artery proximal to the second diagonal branch (portion " D "), respectively. A portion "A" hardly responded to acetylcholine applied. Other 3 (portions " B, C, and D ") showed biphasic responses, the relaxation followed by the contraction, in proportion to the doses employed. We used the portion " B " in the following experiments. In 6 cases, acetylcholine 10^{-5}–10^{-3} Gm/ml dose-dependently constricted the portion " B " during potassium-contracture, and repetitive applications of acetylcholine (10^{-5}–10^{-3} Gm/ml) could produce the similar contractions of these strips to the control.

In 18 isolated coronary arterial strips contracted by potassium 30 mM, atropine 10^{-6} Gm/ml could suppress the contractions of these arterial strips induced by acetylcholine 10^{-5}–10^{-3} Gm/ml. When the mean values for 18 cases were calculated, constrictor responses to acetylcholine 10^{-5}–10^{-4} Gm/ml were converted to the relaxations by atropine. The suppressions to acetylcholine (10^{-4}–10^{-3} Gm/ml)-induced contractions were statistically significant ($p<0.05$ and $p<0.02$, respectively. Fig. 2). Atropine 10^{-6} Gm/ml itself did not affect the potassium-contracture of these strips.

Effects of atropine on norepinephrine-induced contractions of coronary artery:

Two out of 5 coronary arteries (portion " B ") developed the contractile responses to norepinephrine 10^{-8} Gm/ml, and remaining 3 strips did the relaxations. By norepinephrine 10^{-7} Gm/ml vascular responses of 5 arterial strips were converted from the relaxation to the contraction, and by 10^{-6} Gm/ml further contractions of these strips were observed. Fig. 3 shows the typical recordings induced by norepinephrine 10^{-8}–10^{-6} Gm/ml, in which

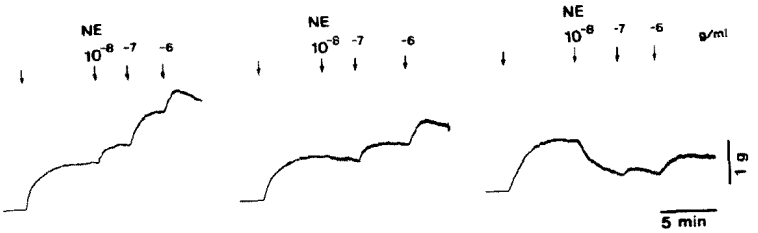

Fig. 3. Typical recordings of coronary arterial strip responded to norepinephrine (NE) and the effects of atropine on them. The left panel shows the control recording, the middle panel, recording after atropine 10^{-6} Gm/ml, and the right panel, recording after atropine 10^{-5} Gm/ml, respectively. First arrow in each panel indicates the application of potassium 30 mM.

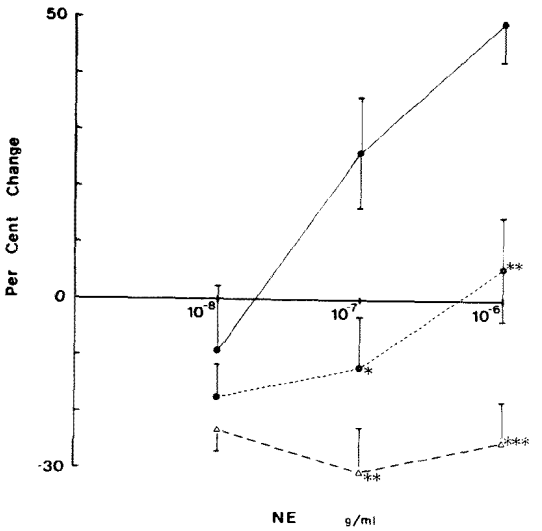

Fig. 4. Effects of atropine on norepinephrine (NE)-induced responses of coronary arteries. Closed circles represent the control values, open circles the values after atropine 10^{-6} Gm/ml, and open triangles the values after atropine 10^{-5} Gm/ml, respectively. $100\% = 0.78 \pm 0.09$ Gm for 5 strips. *, **, and *** show that differences from control value are statistically significant ($p<0.05$, $p<0.01$, and $p<0.001$, respectively).

the contractions alone were obtained, and the effects of atropine on them were examined. Atropine 10^{-6} Gm/ml converted the norepinephrine (10^{-8} Gm/ml)-induced contractions to the relaxations in 2 out of 5 cases, and further enhanced the relaxant actions by norepinephrine 10^{-8} Gm/ml in remaining 3 strips. The contractile responses of arterial strips during potassium-contracture to norepinephrine 10^{-7}–10^{-6} Gm/ml were significantly inhibited by atropine 10^{-6} Gm/ml as shown in Fig. 4. When atropine 10^{-5} Gm/ml was applied, the depressant effects of atropine on the norepinephrine-induced responses were more remarkable, and the tensions could not develop to the control level of potassium-contracture. Atropine 10^{-5} Gm/ml itself did not produce any changes in the potassium-contracture of 5 isolated coronary arterial strips.

Discussion

It is generally recognized[1)-7)] that Prinzmetal's angina pectoris is induced by the spasms of coronary arteries, and that the genesis of an anginal attack is deeply concerned with the functional changes in autonomic nervous system. On the other hand, atropine, phenoxybenzamine, calcium antagonists, and

nitrous derivatives have been used as therapeutic agents to Prinzmetal's angina.[4),6),7),13),14)] Judging from the contribution of autonomic nervous system, especially parasympathetic nerves, to Prinzmetal's angina, it is important that the effects of parasympathetic neurotransmitter, acetylcholine, on coronary arteries are fully investigated.

In the present experiments acetylcholine 10^{-5}–10^{-3} Gm/ml produced dose-dependent contractions of isolated dog coronary artery during potassium-contracture, and these responses were also obtained by repetitive applications of acetylcholine. This means that contractile responses of coronary arteries to acetylcholine are reproducible. Atropine significantly inhibited these arterial contractions. This result sugegsts that the mechanisms of coronary arterial contractions induced by acetylcholine may involve a muscarinic receptor activation.

It has been reported that acetylcholine produces norepinephrine release from sympathetic nerve endings by acting on presynaptic nicotinic receptors in the heart and secondarily causes the enhancement of cardiac performance under suppression of muscarinic receptors.[15)] Therefore, in the coronary artery it would be able to be expected that acetylcholine may constrict the artery through a muscarinic action and simultaneously release norepinephrine from sympathetic nerve terminals.

Large coronary arteries have been proposed to be occupied by adrenergic alpha-receptors more dominantly than by beta-receptors.[5),7),16),17)] Therefore, norepinephrine would be anticipated to produce the contractile responses of these coronary arterial strips, and the present data satisfied this anticipation. Recently, Nedergaard and Schrold[18)] and Kawagoe and Sakanashi[19)] have shown that atropine blocks alpha-adrenoceptors in the isolated rabbit pulmonary artery. In the dog coronary artery, such a block of adrenergic alpha-receptor activities would be happened by application of atropine. In fact, it was observed in the present experiments that atropine dose-dependently inhibited the norepinephrine-induced contractions of isolated dog coronary arteries. Furthermore, when the mean values for 18 coronary arterial strips were calculated, acetylcholine 10^{-5}–10^{-4} Gm/ml-induced contractions were converted to the relaxations by atropine. This may be explained by the plausibility that masked adrenergic beta-receptors in coronary arteries reveal their activities when the dominant alpha-receptors are depressed by atropine. Therefore, when relatively large doses of atropine are used clinically, it can be sufficiently expected that atropine is effective in the therapy of Prinzmetal's variant form of angina pectoris.

In conclusion, the data from the present experiments show the possibility that atropine may inhibit the contractile responses of isolated dog coronary

artery induced by acetylcholine and norepinephrine through a muscarinic-receptor blocking action combined with an adrenergic alpha-receptor blocking effect, resulting in a possible effectiveness on Prinzmetal's variant form of angina pectoris.

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