Differences in Chronotropic and Inotropic Responses of Canine Atrial Muscle and SA Node Pacemaker Activity to Adenosine and ACh

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SUMMARY

The right atrium isolated from the dog heart was perfused through the cannulated sinus node artery with heparinized arterial blood led from a support dog anesthetized with 30 mg/Kg of sodium pentobarbital. When adenosine was administered into the sinus node artery, negative chronotropic and inotropic effects were dose-relatedly induced. The threshold dose for inducing the negative ones was 0.3 µg. Even a large dose level of 100 µg of adenosine did not cause sinus arrest although a profound sinus deceleration was induced. Adenosine action was suppressed by treatment with caffeine both in chronotropism and in inotropism. On the other hand, ACh induced only negative inotropic effect at a dose range of 0.01–0.03 µg. At 0.1 µg, ACh produced a significantly negative chronotropic effect. A large amount of 3–10 µg of ACh usually caused sinus arrest. Atropine treatment inhibited a negative chronotropic effect much more readily than a negative inotropic one. Although ACh action was enhanced by physostigmine, the difference of threshold doses remained unchanged even after physostigmine treatment.

From these results, either adenosine or ACh depresses both SA nodal pacemaker activity and atrial contractile force and there may be the difference of receptor density for ACh between the SA node and atrial tissue.

Additional Indexing Words:
Isolated blood-perfused atrium Isometric tension development Chronotropism Inotropism Caffeine Atropine Physostigmine

In a previous paper,1) it was demonstrated in the dog heart that 1) adenosine and acetylcholine (ACh) both of which are intrinsic biogenic substances readily suppressed the cardiac automaticity of both the sino-atrial (SA) and atrio-ventricular (AV) nodes, while 2) adenosine did not so suppress the AV conductivity, which definitely differed from ACh. In the present study, an attempt was made to compare inotropic and chronotropic responses to

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adenosine with those to ACh in the isolated, blood-perfused atrium preparation of the dog. The isolated atrium preparation originally developed by Chiba et al\(^2\) in 1972 was improved for the simultaneous measurement of the atrial contractile force.\(^3\),\(^4\)

**METHODS**

Twelve mongrel dogs, weighing 12 to 16 Kg, were anesthetized with sodium pentobarbital, 30 mg/Kg i.v. The right atrium was excised after treatment with arterial blood led from a carotid artery of the heparinized support dog. The details of the atrium preparation were reported in previous papers.\(^3\)\(^-\)\(^5\) Electrograms, the isometric tension development, the maximum rate of the tension development (dT/dt) and the perfusion flow rate were continuously recorded on an ink-writing rectigraph (Sanei Sokki Instrument). The drugs used in these experiments were acetylcholine chloride (ACh) (Takeda), adenosine (Boehringer & Sohn), atropine sulfate, physostigmine salicylate (Merck), and caffeine sodium benzoate (Takeda). The volume of drug solution injected was 0.01 to 0.03 ml and was given in a period of 4 sec by a microsyringe (Terumo Co).

**RESULTS**

1. **Effects of ACh and adenosine on the canine atrium**

   When ACh was administered into the cannulated sinus node artery, a negative inotropic effect was clearly induced at a dose range of 0.01 to 0.03 \(\mu\)g but this dose level of ACh did not clearly exert its effect on atrial rate. The threshold doses of ACh for inducing a negative chronotropic effect were approximately 0.1 \(\mu\)g as previously reported.\(^5\) At a higher dose range, ACh induced marked negative chronotropic and inotropic responses. At a dose of 3–10 \(\mu\)g, ACh usually caused complete depression of the tension development and sinus arrest. In the recovery course, pacemaker activity restored more quickly. However, the developed tension recovered slower and usually accompanied the enhancement of the tension development as previously reported.\(^5\)

   On the other hand, the threshold doses of adenosine for inducing negative chronotropic and inotropic effects were almost the same. The i.a. injection of adenosine produced similar dose-related negative chronotropic and inotropic effects.\(^6\),\(^7\) Even with the highest dose of 100 to 300 \(\mu\)g, adenosine could not induce sinus arrest. Moreover, adenosine did not cause the enhancement of the tension development following the depression. Fig. 1 shows typical responses to ACh and adenosine which clearly represent the definite difference mentioned above in the text. Summarized data are shown
Fig. 1. Effects of increasing doses of ACh (A) and adenosine (AD) on the isolated atrium preparation of the dog.

Fig. 2. Comparative negative chronotropic and inotropic responses to ACh and adenosine in the isolated atrium of the dog. Control sinus rate is 105 ± 4.8 beats/min (mean ± S.E.) in 6 preparations.

in Fig. 2.

2. Effects of atropine and physostigmine on action of ACh

Negative chronotropic and inotropic effects of ACh were inhibited by treatment with atropine. However, at this time, increasing doses of ACh produced a negative inotropic effect without influence on sinus rhythm. Fig. 3 shows a typical experiment.

When a large dose of ACh was injected after treatment with relatively large amounts of atropine, positive chronotropic and inotropic effects were usually induced as previously reported. However, a negative inotropic
Fig. 3. Effect of a relatively small dose of atropine on ACh-induced negative chronotropic and inotropic responses.

Fig. 4. Effect of a relatively large dose of atropine on action of ACh. Although a negative chronotropic effect was initially induced although a negative chronotropic effect was never produced. Fig 4 shows a typical experiment.

After physostigmine treatment, a negative inotropic effect of ACh was slightly potentiated. But the difference of the threshold doses between the negative chronotropic and inotropic responses to ACh did not disappear as shown in Fig. 5.
3. Effect of caffeine on action of adenosine

When caffeine was given into the sinus node artery, positive chronotropic and inotropic effects were dose-relatedly induced. Action of adenosine was manifestly suppressed by treatment with caffeine. Fig. 6 shows a typical effect of caffeine. In this case, a continuous infusion of caffeine suppresses adenosine action similarly in chronotropism and in inotropism. On the contrary, ACh action is rather potentiated by treatment with caffeine.
Discussion

In 1968, Blumenthal et al. reported that ACh infused into the canine coronary circulation produced only coronary dilatation at a dose of 0.01 to 0.1 µg without any cardiodynamic effect but a negative inotropic effect on the ventricle was observed at a 10-fold greater dose and a negative chronotropic effect at a 100-fold greater dose. Recently, it was also demonstrated that ACh was more potent for inducing a negative inotropic response than a negative chronotropic one in the isolated atrium preparation of the dog. Even in this study, it was confirmed the same results of action of ACh. Concerning the difference among the threshold doses for the cardiovascular effects of ACh, Higgins et al. reviewed that in addition to dependence on regional variation of cholinesterase activity, the order of responsiveness undoubtedly depends also upon the distribution of cholinergic nerve endings and receptor density in various portions of the heart.

In the isolated atrium preparation, the difference between the threshold doses of ACh for inducing a negative chronotropic and inotropic action exists even after treatment with physostigmine. Furthermore, it was also demonstrated that atropine inhibited more readily the negative chronotropic response to ACh than the negative inotropic one. Therefore, it seems that difference between the threshold doses is due to difference of receptor density for ACh between the SA node and atrial tissue.

In the present study, the differences of response patterns between adenosine and ACh were also observed. Adenosine caused both the negative chronotropic and inotropic responses which were almost the same not only in the doses but also in the time course of the response. A large amount of ACh readily caused sinus arrest. However, adenosine did not cause sinus arrest even at an extremely large dose although a marked sinus deceleration was produced. Since the mechanism of the effect of adenosine has been understood as the direct effect on the excitable membrane, the difference between effects of adenosine and ACh may suggest indirectly the unequal distribution of ACh receptors on the different parts of the myocardium.

In previous papers, it was reported that caffeine antagonized adenosine effect in the in situ SA node preparation of the dog heart. In this study, it was also demonstrated that caffeine antagonized effect of adenosine both in chronotropism and in inotropism. On the other hand, action of ACh was rather potentiated by treatment with caffeine. The potentiation may be due to adrenergic component of action of caffeine. Because caffeine effect was partially suppressed by an adrenergic beta-blocking agent as previously reported, and it has been reported that ACh effect was enhanced
by participation of the adrenergic mechanism.\textsuperscript{13$-$15)}

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