Differences between Negative Chrono-, Dromo- and Inotropic Actions of Cholinomimetics in the Dog Isolated Atrium

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SUMMARY
Both bethanechol and physostigmine produced dose-dependent negative chrono-, dromo- and inotropic effects in the dog isolated atrial preparation. Within a relatively small dose of bethanechol and physostigmine, negative inotropic effect was clearly induced but no significant chrono- and dromotropic changes were observed. With a relatively large dose, the two cholinomimetics caused negative chrono-, dromo- and inotropic effects. The order of the negative actions of the drugs in % changes was inotropism > dromotropism > chronotropism. These results indicate that pacemaker activity, sinoatrial conductivity and atrial contractility may be differentially sensitive to actions of cholinomimetics.

Additional Indexing Words:
Bethanechol  Physostigmine  Atrial contractility  Isolated dog atria  Sinoatrial conduction time  Sinus cycle length

THE distribution of cholinergic innervation and muscarinic receptors in the heart is not homogeneous. For example, histochemical studies showed that vagal nerve endings differ from site to site in atria. On the other hand, Barlow et al postulated that there might be 2 types of muscarine-sensitive acetylcholine receptors in guinea-pig atria, one for heart rate and the other for atrial contraction. These findings are compatible with the results of Chiba, who reported that acetylcholine was more potent for inducing a negative inotropic than a negative chronotropic response in the isolated atria of the dog. Furthermore, Kobayashi et al observed, in the canine isolated atrium, that metacholine prolonged sinoatrial conduction time (SACT) more
than sinus cycle length (SCL), while atropine shortened SACT without affecting SCL. Woods et al. also found that acetylcholine suppressed sinoatrial conduction more readily than sinoatrial pacemaker initiation in the puppy atrium. Although these studies suggest that pacemaker activity, sinoatrial conductivity and atrial contractility may be differentially sensitive to cholinergic substances, there are, to our knowledge, no reports available studying the simultaneous effects of cholinomimetics on these three parameters. The present study was designed to compare effects of two cholinomimetic drugs, bethanechol and physostigmine, on SCL, SACT and developed tension of atrial muscles (DT) in the isolated, blood-perfused atrial preparation of the dog developed by Chiba et al.

Methods

Mongrel dogs (9 to 17 Kg) were anesthetized with sodium pentobarbital (30 mg/Kg, i.v.). After pretreatment with sodium heparin (200 units/Kg, i.v.), the right atrium was excised and immersed in Tyrode's solution at 4°C. The atrium was then perfused through the sinus node artery with blood from the carotid artery of a heparinized donor dog using a peristaltic pump. The perfusion pressure was kept constant at 100 mmHg. The atrium, subjected to a tension of 2 Gm, was suspended in a blood-filled bath at a constant temperature of 37°C. Two pairs of bipolar electrodes were placed 1.5 cm apart on the atrial epicardium close to the sulcus terminalis: one for electrical stimulation and another for recording. SACT was estimated by the constant atrial pacing method with these electrodes. Large doses of bethanechol and physostigmine (3–10 µg), in most preparations, caused sinus arrest which

![Fig. 1. A representative experimental tracing of effects of physostigmine injected into the sinus node artery on atrial rate and developed tension in a dog isolated, blood-perfused atrial preparation.](image-url)
usually recovered within 3 min. In these cases, SACT was assessed about 1 min after a spontaneous SCL was triggered by a cardiotachometer (Fig. 1). SCL and DT were measured with a cardiotachometer triggered by the atrial electrogram and with a force displacement transducer, respectively. The details of the methods employed in this study have been described elsewhere. The drugs used were bethanechol chloride (Eisai) and physostigmine (Sigma).

**Results**

1. **Effects of bethanechol on the isolated atrium**

Control SCL, SACT and DT were 582 ± 23 msec, 75 ± 9 msec and 2.7 ± 0.5 Gm, respectively (n=9, mean ± SEM). When injected into the cannulated sinus node artery with a microsyringe, bethanechol exerted dose-related negative chrono-, dromo- and inotropic effects as shown in Fig. 2. Small doses of the drug (0.01–0.03 µg) caused significant negative inotropic effects without producing prominent chronotropic and dromotropic actions. At relatively higher dose levels (more than 0.03 µg), the choline ester tended to be more potent in increasing SACT than SCL, usually accompanied by profound negative inotropic effects.

2. **Effects of physostigmine on the isolated atrium**

Before physostigmine was given, SCL, SACT and DT were 637 ± 36 msec,
82±8 msec and 2.0±0.4 Gm, respectively (n=9). Physostigmine, injected into the sinus node artery, produced dose-dependent negative chrono-, dromo- and inotropic responses as shown in Figs. 1 and 2. The threshold doses of the chrono, dromo- and inotropism were 1, 1 and 0.1 μg, respectively. The right side of Fig. 2 shows the dose-response relation for the negative chrono-, dromo- and inotropic responses to physostigmine.

**Discussion**

It is well documented that strong vagal stimulation or a large amount of acetylcholine induced a shift of dominant pacemaker sites in the sinoatrial node.11) In the present experiments, a large dose of bethanechol and physostigmine (10 μg) produced an abrupt increase or decrease in SACT; this is probably due to the pacemaker shift. We, therefore, compared the effects of the agents at relatively small dose levels.

In our study, at small doses, bethanechol and physostigmine clearly showed profound negative inotropic effects without any significant influences on chronotropism and dromotropism. In a relative large dose range, bethanechol and physostigmine caused much greater negative inotropic effects with slight negative chrono- and dromotropic effects in percent changes. The prolongation of SACT was a little greater than the SCL prolongation in percent changes, but the difference was not significant. Thus, the order of the negative actions was inotropism>dromotropism>chronotropism. These differences in the responses to the cholinomimetic drugs can be explained as follows: First, distribution of vagal nerve endings is not uniform.11,2 Therefore, the differences between the negative actions of physostigmine may be due to a differential distribution of cardiac cholinergic nerve endings. This speculation is supported by the observation of Kobayashi et al5) that atropine reduced SACT but not SCL in the dog atrium. Second, the different sensitivities of inotropism, dromotropism and chronotropism to both physostigmine and bethanechol may result from differences in muscarinic receptor density in the sinoatrial node, perinodal region and atrial muscle. This is likely, because Chiba4) reported that the difference between the threshold doses of acetylcholine for inducing negative inotropic and chronotropic effects may be due to the differences in receptor density for acetylcholine between the sinoatrial node and atrial tissue. Finally, since it has been reported that muscarinic receptors for heart rate may differ from those for atrial contraction in the guinea-pig heart,3) a variation in muscarinic receptors with location in the atrium of the dog cannot be excluded. In conclusion, the present study suggests that the density of muscarinic receptors may be different between the
sinoatrial node, sinoatrial junctional region, and atrial muscle in the dog heart.

References

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