SUSTAINED ATRIAL FIBRILLATION INDUCED BY CARBACHOL, METHACHOLINE AND BETHANECHOL

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In 1955, Burn et al. (1) succeeded in the maintenance of atrial fibrillation by continuous infusion of acetylcholine (ACh) in the dog heart-lung preparation when atrial fibrillation was induced by electric stimulation of auricle. Recently, Hashimoto et al. (2) arranged an adequate method for the selective use of drugs into the sinus node artery in situ by modifying the original preparation developed by James and Nadeau (3). Hashimoto et al. (4) observed that atrial fibrillation was regularly induced by the administration of ACh into the sinus node artery under constant pressure perfusion. They analyzed the mechanism for induction of atrial arrhythmia with ACh, assuming the necessary participation of adrenergic mechanism with essential cholinergic effect of ACh. More recently, Nakayama et al. (5) obtained sustaining atrial fibrillation by continuous infusion of a relatively small amount of ACh directly into the sinus node artery.

In the present study, the authors observed regularly an occurrence of a sustaining atrial fibrillation by single injection of carbachol, methacholine or bethanechol into the sinus node artery, and compared with the atrial fibrillation induced by ACh.

METHODS

Twenty-three mongrel dogs of either sex weighing between 9 and 18 kg were used and anesthetized with i.v. sodium pentobarbital, 30 mg/kg. A tracheal tube was inserted and artificial respiration was performed by use of a Bird respirator model 8 with air. Both vagi were cut at the mid-cervical level. The heart was exposed by a mid-sternal or 4th intercostal incision. The direct perfusion of the sinus node artery was modified by the authors as follows. All experiments were performed under constant perfusion pressure at 100 mm Hg because sinoatrial (SA) node is responsive to the pressure change. The change in the flow rate in the sinus node artery was measured by use of an electromagnetic flowmeter (Nihon Kohden MF-2) which is sensitive to a change in the blood flow of 1 ml/min. The polyethylene cannula, the tip of which had an outer diameter of about 0.5 mm, was prepared by tapering a larger polyethylene tube (o.d. 2-3 mm). A rubber tube was connected to the shank of the cannula for the purpose of injecting drug solutions. A magnetic flowmeter was arranged between the cannula and Sigma-motor pump, to which the arterial blood was conducted from the femoral artery. An additional channel was arranged in parallel with the perfusion system for shunting the excess blood to the femoral vein through a pneumatic resistance of 100 mm Hg. The pumping volume was adjusted to shunt the excess blood to the femoral vein even at the maximum vasodilatory response.
induced by cholinergic drugs. Two electromanometers were arranged to measure perfusion pressure and systemic arterial pressure. ECG (lead II and right auricular lead) was recorded on an electrocardiograph (Nihon Kohden ME-20-TR) at a chart speed of 1.5 cm or 3.0 cm/sec. The heart rate was continuously recorded by a cardiotachograph (Nihon Kohden RT-2) which was triggered by the R wave of the ECG. Sodium heparin used in these experiments was a preparation extracted from the lung and intestinal mucous membrane of whales, 500 U/kg of which was given at the beginning of perfusion and 200 U/kg at 1-hr intervals.

The drugs used in these experiments were acetylcholine chloride (Daiichi Seiyaku), carbachol chloride (Chemwey), bethanechol chloride (Fujita Seiyaku), methacholine chloride (Merck), dl-epinephrine (Sankyo), dl-propranolol (Sumitomo Chemicals), dl-alprenolol (H 56/28, 1-(O-allylphenoxy)-3-isopropylamino-2-propanol) (Hässel) and tetrodotoxin (Sankyo).

Microinjectors, “AGLA” (Burroughs Wellcome Co.) and “Jintan” (Jintan Terumo Co.) were used for injection of 0.01 ml of a drug solution into the sinus node artery. Each microinjector was used exclusively for each drug solution in order to avoid contamination among different drug solutions.

RESULTS

1) Responses of the SA node to i.a. injections of carbachol, methacholine and bethanechol (Fig. 1, Table 1)

As James and Nadeau and Hashimoto et al. reported, the SA node is sensitive to the change in perfusion pressure. Although the perfusion pressure was kept constant, the rate of injection of saline over 0.05 ml per second caused deceleration of the sinus rhythm. Thus, a volume of 0.01 ml of drug solution was injected in a period of 4 seconds by use of Agla or Jintan microinjector. A smaller dose of carbachol (0.01 μg), methacholine (0.1 μg) or bethanechol (1 μg) into the sinus node artery depressed the pacemaker activity of the SA node and caused a sinus bradycardia. A larger dose of carbachol above 0.1 μg, methacholine above 1 μg or bethanechol above 10 μg occasionally induced atrial fibrillation just when the pacemaker activity was depressed. Carbachol, methacholine and bethanechol induced atrial fibrillation in nearly all animals at doses above 1, 10 and 30 μg respectively. Atrial fibrillation induced by these compounds lasted over 2 minutes. Although the dosage for inducing atrial fibrillation varied from animal to animal, a given dose of these cholinergic drugs could cause repetitively sustaining atrial fibrillations in the same animal. The summarized data was shown in Table 1. As reported previously, ACh always caused atrial fibrillation in 66 per cent at a dose of 1 μg, and 95 per cent at a dose of 10 μg. The frequency of incidence of fibrillation with ACh was similar to that with methacholine. The duration of ACh-induced atrial fibrillation was about 30 seconds regardless of various doses of ACh. When atrial fibrillation was induced by use of carbachol, methacholine or bethanechol, it lasted for several minutes, however, methacholine-induced fibrillation was shorter than bethanechol- or carbachol-induced one.
FIG. 1. Negative chronotropic effect and atrial fibrillation induced by increasing doses of methacholine (A), bethanechol (B) or carbachol (C) given into the sinus node artery. SBP, systemic blood pressure; HR, heart rate.

TABLE 1. Frequency of incidence of atrial fibrillation induced by doses of carbachol, methacholine, bethanechol and ACh given into the sinus node artery.

| Drug       | Dose (µg) | 0.01 | 0.1 | 1    | 10   | 30   |
|------------|-----------|------|-----|------|------|------|
| Carbachol  |           | 2/4  | 5/7 | 4/4  | 3/3  | 5/11 |
| Methacholine|          | 2/4  | 5/8 | 3/3  | 3/3  | 3/3  |
| Bethanechol|           | 0/3  | 0/8 | 5/11 | 3/3  | 3/3  |
| ACh        |           | 16/49| 39/59| 59/62| 59/62| 59/62|

(*)*: A number in parenthesis represents duration of atrial fibrillation on an average in minutes.

2) Synergistic effect of ACh or epinephrine for induction of atrial fibrillation by cholinergic drugs (Fig. 2)

Though a smaller dose of these cholinergic compounds induced a long-lasting sinus bradycardia but not atrial fibrillation as shown in Table 1, a successive administration of ACh, in a dose of which induced only a sinus bradycardia, produced a sustained atrial fibrillation for several minutes. Not only ACh but also epinephrine in a single shot played the role as a trigger for induction of fibrillation in three out of five cases as shown in Fig. 2.
3) Blocking action of atropine, but absence of such effect with adrenergic beta-receptor blocking agents and tetrodotoxin (Figs. 3 and 4)

Atrial fibrillation induced by 1 μg of carbachol usually sustained for 5 minutes. Fig. 3 demonstrated that a sustained atrial fibrillation induced by carbachol was readily blocked
Fig. 4. Blocking effect of 10 \( \mu \text{g} \) of propranolol on carbachol-induced atrial fibrillation.  
A; just after 1 \( \mu \text{g} \) of carbachol (CAR.).  
B; atrial fibrillation following sinus depression.  
C; sustained atrial fibrillation.  
D; AV nodal escape rhythm appeared after 10 \( \mu \text{g} \) of propranolol.

by a single dose of 10 \( \mu \text{g} \) of atropine given into the sinus node artery. However, 1 to 10 \( \mu \text{g} \) of tetrodotoxin could not block its sustaining fibrillation in four out of 5 cases. Alprenolol and propranolol in a dose to be able to block 1 \( \mu \text{g} \) of norepinephrine hardly blocked the sustained atrial fibrillation induced by carbachol. Large doses over 10 until 30 \( \mu \text{g} \) of beta-adrenergic blocking agents injected into the sinus node artery could block sustained atrial fibrillation in two of 6 cases, but nodal rhythm took place as shown in Fig. 4. It suggests that atrial fibrillation is blocked by complete depression of SA pacemaker activity by use of a large amount of propranolol.

**DISCUSSION**

Recently, we reported that atrial fibrillation was induced by the administration of ACh at doses between 1 to 10 \( \mu \text{g} \) into the sinus node artery (4). And also, Nakayama et al. (5) reported sustaining atrial fibrillation by ACh infusion, using a direct perfusion method of the canine sinus node artery (2). In 1953 Hoffman and Suckling (6) determined the effect of ACh on the action potential of the atria of the dog; they found ACh greatly hastened repolarization in the duration of the action potential. The same effect was observed by use of carbachol and methacholine (Nahum and Hoff (7), Burgen and Terroux (8), Marshall and Katsh (9), and Webb and Hollander (10)). The reduction of the duration of the action potential in the atrial fiber must result in a marked shortening of the absolute refractory period. If these changes are not uniform and cause a considerable inhomogeneity in excitability in atrial fibers, atrial fibrillation might occur. In the previous paper, we concluded that adrenergic mechanism through pharmacological investigation may probably participate for induction of atrial fibrillation. Such inhomogeneity in excitability expected by the local action of ACh will be aggravated by the effect of re-
leased catecholamine by the selective use of ACh into the sinus node artery. In this study we investigated effects of carbachol, methacholine and bethanechol on the pacemaker activity of the sinoatrial node. These compounds produced a negative chronotropic effect in a smaller dose but an increasing dose induced atrial fibrillation following a negative chronotropic response. The mechanism for induction of atrial fibrillation by use of these cholinergic agents must be the same to that with ACh. However, atrial fibrillation induced by these agents sustained for several minutes in contrast with a short duration of ACh-induced atrial fibrillation. Among these compounds, carbachol is the most potent for inducing atrial fibrillation, methacholine is next and bethanechol is the weakest, the potencies of which are roughly estimated as 1: 1/10: 1/100. It is well known that carbachol has a prominent nicotinic but weaker muscarinic action on the heart. On the contrary bethanechol is predominantly muscarinic. Nicotinic action of these compounds is assumed to be closely related with potency for induction of atrial arrhythmia, which is expected in the previous paper as participation of adrenergic mechanism. Even with carbachol, atrial fibrillation could not be induced in few cases, however a successive administration of epinephrine triggered atrial fibrillation. Thus these results may probable support the previous explanation for induction of atrial fibrillation.

Concerning the mechanism of sustaining fibrillation, atropine always blocked fibrillation induced by these compounds, while even a large dose of beta-adrenergic blocking agents, alprenolol and propranolol, or tetrodotoxin did not block it. Accordingly, little chance for participation of adrenergic mechanism is expected for maintenance of atrial fibrillation. Nakayama et al. (5) reported that the dose of ACh necessary to maintain fibrillation is much smaller than the dose for induction of fibrillation. As a matter of fact, once atrial fibrillation occurred, its duration was longer in cases of carbachol or bethanechol than in those of methacholine. Methacholine is hydrolyzed by acetylcholinesterase, while carbachol and bethanechol are virtually resistant not only to acetylcholinesterase but also to non-specific cholinesterase. Therefore, the duration of fibrillation may probably indicate the continuation of the muscarinic effect on the atrial fibers including pacemaker of the SA node, that is, the reduction of duration of the action potential and shortening of refractory period.

Sustaining atrial fibrillation demonstrated by use of cholinergic compounds in this study may be available for the evaluation of anti-arrhythmic property of drugs.

SUMMARY

The mode of atrial fibrillation induced by synthetic cholinergic compounds, methacholine, carbachol and bethanechol, was compared with ACh-induced fibrillation when these drugs were selectively injected into the sinus node artery of 23 mongrel dogs.

1. Among three synthetic compounds, the potency for induction of fibrillation was in the following order; carbachol > methacholine > bethanechol. The doses which caused fibrillation in almost all cases were about 1, 10 and 30 µg respectively. The potency for induction seems to be in parallel with nicotinic effect of these compounds.
2. The fibrillation induced by these compounds sustained more than two minutes, while ACh-induced fibrillation spontaneously ceased within a period of 30 seconds. The duration of atrial fibrillation is assumably related with the susceptibility to different kinds of esterases. The duration of fibrillation was shorter in the following order; ACh > methacholine > bethanechol and carbachol which maintained fibrillation roughly for 30 seconds, 3 and over 8 minutes, respectively.

3. The maintenance of atrial fibrillation was readily blocked by atropine but hardly blocked by tetrodotoxin and propranolol. When the sustaining fibrillation was blocked by tetrodotoxin or propranolol, its rhythmicity became nodal or strongly depressed sinus rhythm.

4. The authors concluded that the participation of adrenergic mechanism is requisite for induction of fibrillation with cholinergic drugs while it is not necessary for its maintenance.

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