COMPARISON OF THE EFFECTS OF NOREPINEPHRINE AND ACETYLCYLCHOLINE BETWEEN INTRAARTERIAL AND EXTRAVASCULAR ADMINISTRATION TO THE ISOLATED, BLOOD-PERFUSED CANINE ATRIUM

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Abstract—Effects of acetylcholine (ACh) and norepinephrine on atrial contractility and pacemaker activity were investigated in isolated and blood-perfused canine atrium preparations with a support dog which were suspended in the blood-filled bath kept at 37°C. The drug was given in two forms of administration, i.e., intraarterial injection into the cannulated sinus node artery or direct administration into the bath. ACh administered into the bath produced a significant decrease in the developed tension from a concentration of $10^{-5}$ g/ml and norepinephrine produced a significant increase in the developed tension from $3 \cdot 10^{-6}$ g/ml. An injection via the sinus node artery resulted in 300 and 100 times greater response to ACh and norepinephrine, respectively, in the tension development. In atrial pacemaker activity, ACh given into the bath did not produce a dose-related decrease while norepinephrine produced a dose-related increase frequently accompanied by an irregularity of rhythmicity.

The isolated atrium suspended in the oxygenated Tyrode's solution has been used to evaluate drug effects on inotropic and chronotropic activities. In the mammalian heart, rabbit's cat's and guinea pig's atrium preparations have been widely used in the past (1-4), while there is no available report on the isolated dog atrium preparation. The reason may be that while the atrium wall of the dog may permit less diffusion of not only oxygen and metabolites, but also various exogenous substances, the dog atrium bathed in a physiological solution may readily produce anoxia and disappearance of pacemaker activity and contractility. Most recently, the present authors arranged the canine atrium preparation which suspended in the bath filled with blood and perfused with blood introduced from a support dog (5). It was demonstrated that this canine atrium preparation maintained a very stable rate and tension development for over 5 hr in 5 control experiments. Atrial rate of this preparation was very similar to the heart rate of the conscious dog.

In this study, chronotropic and inotropic response to ACh or norepinephrine was compared when administered intraarterially via the cannulated sinus node artery or directly into the bath filled with blood.

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MATERIALS AND METHODS

Eleven mongrel dogs of either sex, weighing from 8 to 14 kg, were anesthetized with sodium pentobarbital, 30 mg/kg, given intravenously. After treatment with 500 units/kg i.v. of sodium heparin, the right atrium was quickly excised and plunged into a cold Tyrode's solution at about 4°C. The sinus node artery was cannulated via the right coronary artery and perfused with blood led from a carotid artery of the support dog by the aid of a pump (Harvard Apparatus model 505–1200). A Starling resistance was placed in parallel with the perfusion system so that a constant perfusion pressure at 100 mm Hg was maintained. The atrial muscle was fixed to a stainless steel bar at two parts of the ventricular margin and put into a cup shaped glass container which was warmed at a constant temperature of 37°C by circulating warm water pumped from a water bath. The upper part of the atrium was connected directly to the force displacement transducer (Grass FT03B) by a fine silk thread. The volume of the container was 65 ml and initially filled with the warmed Tyrode's solution which was gradually displaced with the blood passed through the atrium during the progress of the experiment. The arterial blood passing through a Starling resistance was also poured into the bath in order to stir up the drug in the bathing blood. The stream of blood overflowing from the container was collected in a blood reservoir and returned to the support dog through the jugular vein as illustrated in Fig. 1. However, when the drug was given directly into the bath, the overflowing blood was taken out of circulation in order to avoid the drug effect on the support dog. The support dogs, weighing from 11 to 20 kg, were anesthetized with 30 mg/kg of sodium pentobarbital i.v. Sodium heparin, 500 units/kg, was administered at the beginning of the perfusion and 200 units/kg was added at 1-hr intervals. The rate of perfusing blood flow was measured with an

![Diagram of the perfusion setup](image-url)
electric flowmeter (Nihon Kohden MF-2T). A bipolar platinum electrode was brought into contact on the atrial epicardium. The sinus rate was measured with a cardiotachograph which was triggered by an electrogram. The muscle was loaded with a tension of 2 g. The isometric tension and the tension development (dT/dt) were recorded on an ink-writing rectigraph (San’ei Sokki Instrument) through a carrier preamplifier (Nihon Kohden RP-2), RC circuit and a high gain amplifier (Nihon Kohden RDH-2). The diagram of the atrium preparation is shown in Fig. 1.

The drugs used in this study were acetylcholine chloride (Daiichi) and DL-norepinephrine hydrochloride (Sankyo). The drug solution was given in two forms of administration i.e., intraarterially in a volume of 0.01 to 0.03 ml over a 4 sec period into the perfusion system to the sinus node artery and extravascularly in a volume of 1 to 10 ml over 4 sec into the bath.

RESULTS

Chronotropic and inotropic responses of the canine atrium preparation to the occlusion of the sinus node artery

Even though the canine atrium was suspended in the bath filled with blood, both chronotropic and inotropic activities were immediately suppressed, when the blood supply via the sinus node artery was interrupted. Contractility was depressed continuously, while depression of pacemaker activity reached a certain plateau and several minutes later caused a sudden sinus arrest as shown in Fig. 2. Thus, oxygen may not diffuse adequately to the myocardium of the canine atrium. Oxygen supply through the sinus node artery was thus requisite for maintainance of SA nodal pacemaker activity.

Fig. 2. Effects of the occlusion of the sinus node artery on atrial contractility and pacemaker activity. The underlined area indicates the period of occlusion of the sinus node artery.
Comparison of isotropic and chronotropic effects of ACh between intraarterial and extravascular application to the canine right atrium

Since the flow rate of the sinus node artery was 3.8±0.4 ml/min (mean ± S.E., N=5), the maximum concentrations of ACh were approximately calculated as $3 \times 10^{-8}$, $10^{-7}$ and $3 \times 10^{-7}$ g/ml, respectively, when doses of 0.01, 0.03 and 0.1 µg were injected in a period of 4 sec. When 0.01 µg of ACh was injected into the sinus node artery, it induced a significantly negative inotropic effect but not a negative chronotropic one. Increasing doses of ACh from 0.03 to 0.1 µg caused a dose-related effect on the negative inotropism and chronotropism. When ACh was extravascularly administered into the bath filled with blood, ACh in a concentration of $10^{-5}$ g/ml induced a negative inotropic effect equivalent to 0.01 µg given intraarterially. Increasing concentrations caused also dose-relatedly negative inotropic responses. Concerning negative chronotropism, responses to ACh administered into the bath were not uniform i.e., there were sudden changes in heart rate, as shown in Fig. 3. Thus, the negative inotropic responses to ACh were compared between intraarterial and extravascular application to the canine right atrium as shown in Fig. 4.

Comparison of inotropic and chronotropic effects of norepinephrine between intraarterial and extravascular application

When 0.01 µg of norepinephrine was injected into the sinus node artery which was approximately calculated as $3 \times 10^{-8}$ g/ml, positive inotropic and chronotropic effects were simultaneously induced. These effects were dose-related with increasing doses of norepinephrine. When norepinephrine was administered extravascularly into the bath filled with blood, both positive inotropic and chronotropic effects were clearly induced at a concentration of $3 \times 10^{-6}$ g/ml as shown in Fig. 5. These responses were long-lasting and
Fig. 5. Effects of norepinephrine (NE) on the isolated canine atrium preparation by an injection into the sinus node artery or an administration into the bath.

hardly abolished even after norepinephrine contained-blood was removed from the bath and replaced with the fresh arterial blood of the support dog. Thus, increasing doses of norepinephrine were given extravascularly in a cumulative manner. The extravascular administration of norepinephrine frequently caused an irregularity of rhythmicity different from the intraarterial injection. Moreover, it usually induced more marked increases in atrial rate than those in the developed tension. Summarized data are represented in Fig. 6.

Fig. 6. Dose-effect curves of norepinephrine on atrial contractility and atrial rate. The ordinate represents the changes in the developed tension and atrial rate. The abscissa represents the concentration of norepinephrine. Open circles: effect of norepinephrine when administered into the bath. Closed circles: effect of norepinephrine when injected into the sinus node artery. Vertical bracketed lines indicate S.E.
DISCUSSION

Since the blood-perfused atrium preparation is totally dipped in the blood-filled bath, it is expected that the atrial function would be well preserved with enough oxygen which may readily diffuse through the atrial myocardium. However, not only contractile force of the atrial myocardium but also the pacemaker activity of the SA node diminished soon after interruption of blood supply through the sinus node artery. Thus the uptake of oxygen by the atrial myocardium may not be adequate for maintaining the physiological activities of the atrium. Effect of anoxia was progressively induced in the inotropism while atrial arrest was suddenly induced after reaching a plateau of reduced sinus rate. This arrest may be caused by the block of the sino-atrial conduction, because the sinus rate reappeared suddenly with a relatively higher rate when the blood circulation was reopened. Thus the contractile force must be more susceptible to anoxia than the pacemaker activity. Furthermore, the effect of the drug on the contractile force was readily induced and more dose-dependent than that on the sinus pacemaker activity, and chronotropic responses to either ACh or norepinephrine were not so consistent and often caused sudden irregular beats especially when the drug solution was given extravascularly into the bath. The susceptibility of the blood-perfused canine atrium to ACh and norepinephrine when given into the bath is not so different from that of the isolated guinea-pig atrium, because the threshold concentration is $3 \times 10^{-6}$ g/ml for the former in this study and $10^{-5}$ g/ml or less for the latter (6). There was sufficient diffusion of the drug to produce responses in the canine atrium as well as in the guinea-pig.

In the present study, it was demonstrated that the contractile force was approximately 300 times stronger when the drug was intraarterially injected into the sinus node artery than when it was extravascularly administered into the bath. Furthermore, norepinephrine was also approximately 100 times more effective in the intraarterial administration than in the extravascular application into the bath while only 10 times more effective concerning the effect on the sinus rate. Even though the isolated canine atrium preparation is dipped into the blood, the condition is unlike other isolated atrium preparations, where the former is perfused with blood through the sinus node artery. From these results, it appears that 1) it may be difficult for the drug to diffuse through the richly preserved connective tissue of the pacemaker area, 2) the blood supply through the sinus node artery may prevent the drug diffusion from the bath, and 3) in the case of norepinephrine application, pacemaker shift may occur from the SA node to the atrial myocardium especially when the drug is given extravascularly into the bath.

It was confirmed that either an intraarterial injection or an extravascular administration of the drug into the bath exerts its effects on the isolated canine atrium preparation, but with an intraarterial injection, the response is from 100 to 300 times greater than when the drug is administered into the bath.

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