Dominant Anti-vagal Effect of Pentobarbital on Cardiac Responses to Intracardiac Autonomic Nerve Stimulation in the Dog

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ABSTRACT—The isolated canine atrium was perfused by heparinized blood of the donor dog. An adequate dose of pentobarbital that induced a potent hypotension in the donor did not produce any significant change in the atrial rate and developed tension in the isolated atrium perfused with donor’s blood. Pentobarbital in doses that modified neither cardiac responses to intracardiac adrenergic nerve stimulation nor exogenously given norepinephrine or acetylcholine significantly inhibited intracardiac vagal responses. From these results, it is concluded that a large dose of pentobarbital has a dominant antivagal effect in the heart.

Keywords: Dog atrium, Intracardiac nerve stimulation, Chronotropism

It has been well known that an intravenous administration of pentobarbital causes a hypotension and tachycardia. Since pentobarbital caused a bradycardia when injected selectively into the cannulated sino node artery in the isolated atrium and in vivo heart of the dog (1, 2), it was recognized that it has direct depressant properties on the sinoatrial (SA) node. However, it is still not precisely clear whether pentobarbital in concentrations that induced an adequate anesthesia influences the SA nodal pacemaker activity. Moreover, it is yet not clear whether pentobarbital dominantly influences adrenergic and vagal nerves or postsynaptic muscarinic and adrenergic receptors. Thus, in the present study, we investigated effects of intravenous pentobarbital to the donor dog and effects of intraarterial pentobarbital on responses to intracardiac autonomic nerve activation in isolated atrium.

The animal experiments were approved by the Shinshu University School of Medicine of Medicine Animal Studies Committee. Eight adult beagle dogs (CSK Research Park, Suwa) weighing 6 – 10 kg were anesthetized with sodium pentobarbital (Tanabe, Osaka) at 30 mg/kg, i.v. After sodium heparin treatment (500 U/kg), the right atrium was quickly removed and plunged into Tyrode solution at about 4°C. The sinus node artery of the excised atrial muscle was cannulated via the right coronary artery and perfused under a constant pressure of 100 mmHg with heparinized arterial blood led from a carotid artery of a donor dog by the aid of a peristaltic pump (Model 1210; Harvard Apparatus, Millis, MA, USA). The atrium was suspended and subjected to a tension of 2 g in the bath filled with blood at a constant temperature of 37°C.

A bipolar silver electrode with an inter-electrode distance of 1.5 mm was placed on the epicardial site of the atrial free wall for recording the electrogram. An another silver electrode with an inter-electrode distance of 2 mm was placed on the epicardial fat pad at the posterior portion in the caval margin for stimulating the intracardiac nerve fibers (3, 4). In the donor dog, the artificial respiration was continuously performed with a respirator (Model 607, Harvard Apparatus), and the systemic blood pressure and heart rate was continuously recorded. In the isolated atrium, spontaneous atrial rate was measured with a tachometer (AT-600G; Nihon Kohden, Tokyo), which was triggered by potential waves of atrial electrogram. The isometric tension development was measured with a force displacement transducer (TB-611T, Nihon Kohden) and recorded on a thermo-writing rectigraph (RTA-1200, Nihon Kohden). Electrical stimulation was performed with rectangular pulses of 1-ms duration at 30 Hz and 4 V by use of an electric stimulator (SEN-7103, Nihon Kohden) through an isolation unit (MSE-3, Nihon Kohden). The preparation was described in detail previously (5).

Drugs used were acetylcholine chloride (ACH; Daiichi, Tokyo), dl-norepinephrine hydrochloride (Sankyo, Tokyo) and sodium pentobarbital (Tanabe, Osaka). In four dogs, pentobarbital was intravenously administered. In four isolated atrial preparations, the drug solution was injected into the cannulated sinus node artery by use of a microinjector (Terumo, Tokyo) with the volume of 0.01 – 0.03 ml in a period of 4 s.

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All data are shown as the maximum change in response to each drug and expressed as the mean ± S.E.M. Student’s t-test for paired data was used for comparison between the two groups. P values of less than 0.05 were considered statistically significant.

When pentobarbital was intravenously administered to the donor dog in a dose range of 1 – 30 mg/kg, hypotensive effects were consistently introduced in a dose-related manner. On the other hand, even at 30 mg/kg, at 2 to 3 min after administration of i.v. pentobarbital, the atrial rate and developed tension were not significantly modified in the isolated atrium which was perfused with the donor’s blood, although they were slightly decreased. Figure 1 shows summarized data of effects of i.v. pentobarbital on the systemic blood pressure and heart rate in the whole animal and the atrial rate and developed tension in the isolated atrium.

When the fat pad close to the SA node was electrically stimulated, negative chrono- and inotropic responses followed by positive ones were consistently induced (4). Since chrono- and inotropic responses to intracardiac nerve stimulation were blocked by treatment with tetrodotoxin, these are likely due to excitation of autonomic nerve fibers (4). It was also reported that negative and positive responses were blocked by atropine and propranolol, respectively (4), showing that they are due to activation of muscarinic and adrenergic receptors. The negative responses to nerve stimulation were significantly inhibited by a relatively large dose of pentobarbital. However, the treatment with pentobarbital used in this study did not modify the responses to exogenously administered norepinephrine or ACh. Summarized data are shown in Fig. 2.

In the past, we investigated effects of pentobarbital on the canine SA nodal pacemaker activity by use of the in vivo direct perfusion method of the sinus node artery. In the isolated atrium, when pentobarbital was injected into the cannulated sinus node artery, the negative chrono- and inotropic responses were dose-relatedly induced as reported previously. In this study, we tried to confirm effects of

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**Fig. 1.** Cardiovascular effects of intravenous injection of 30 mg/kg pentobarbital on systemic blood pressure (BP) and heart rate (HR) of donor dogs (A); and atrial rate (AR) and developed tension (DT) in isolated atria (B). NS, not significantly different.

**Fig. 2.** Effects on atrial rate (AR) and developed tension (DT) of pentobarbital intra-arterially injected into the sinus node artery in the isolated canine atrium on responses to intracardiac autonomic nerve stimulation, by exogenously injected 0.06 µg ACh and 0.03 µg norepinephrine. NS, not significantly different.
pentobarbital on cardiovascular functions. This study shows that at doses causing a severe hypotension in donor dogs, pentobarbital did not produce direct negative chronotropic or inotropic effects. Furthermore, it was demonstrated that pentobarbital has a dominant negative inotropic effect compared to its negative chronotropic one in the isolated canine atrial preparation (2). It was also shown that in the isolated canine ventricular preparation, pentobarbital induced a strong negative inotropic effect in a dose-related manner with extremely large doses (6). In this study, pentobarbital that was administered to the donor dog did not produce significant negative inotropic effect in the isolated atrium. Therefore, it demonstrates that therapeutic doses of pentobarbital (≤30 mg/kg) do not exert its depressive effect on the SA nodal pacemaker activity and cardiac contractility, except in the damaged heart such as in the case of congestive heart failure. It has well recognized that anesthetic concentrations of barbiturates have direct electrophysiological effects in the heart. Although they reduce the function of at least two types of K+ channels (7–9) in addition to depressing Na+ channels, their effects might be due to extremely large concentrations of barbiturates in isolated cardiac muscle preparations.

In the past, it has been reported that pentobarbital antagonized norepinephrine-induced tachycardia in the dog heart-lung preparation (10) and in situ perfusion preparations of the canine sinus node artery (2) and in isolated canine atrial preparations (1). Moreover, Chiba and Nakajima (11) reported that pentobarbital antagonized the effect of norepinephrine and epinephrine in the atrioventricular (AV) node, showing that pentobarbital inhibited AV nodal tachycardia induced by injection of norepinephrine and epinephrine into the AV node artery of the dog in situ. It was also reported that pentobarbital-norepinephrine antagonism was less evident with inotropism than with chronotropism (1). In this study, pentobarbital at doses inducing a marked hypotension did not antagonize norepinephrine-induced positive chronotropic and inotropic effects. Therefore, it is concluded that concentrations of pentobarbital (≤30 mg) may not antagonize norepinephrine-induced cardiac effects. It is considered that an extremely large dose of pentobarbital has an antiadrenergic property in cardiac function.

In this study, it was shown that pentobarbital has dominantly antivagal properties because pentobarbital significantly inhibited intracardiac nerve electric stimulation-induced negative chronotropic and inotropic effects but not positive effects, and it did not modify negative or positive cardiac responses induced by exogenous ACh or norepinephrine, respectively. This indicates that pentobarbital inhibits a vagal component but not an adrenergic component induced by intracardiac nerve stimulation. In the heart, there are abundant parasympathetic ganglia, but no sympathetic ones. As reported previously, intracardiac cholinergic nerve stimulation caused an activation of preganglionic vagal fibers because a potent ganglionic blocking agent, hexamethonium, inhibited the negative chronotropic and inotropic responses to electrical stimulation but not the positive ones (3). Pentobarbital may cause partial inhibition of ganglionic transmission (12), which in turn may impair the cholinergic component.

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