Comparative Study of Cardiovascular Effects of Clonidine and Alinidine in Cross-Perfused Dog Atrial Preparations

Shigetoshi Chiba, M.D., Yasuhiro Ogiwara, M.D., Yasuyuki Furukawa, M.D., and Kunio Akahane, M.D.

SUMMARY

Cross-perfused canine atrial preparations were used to investigate the direct and indirect cardiac actions of clonidine and alinidine. Intravenous injections of clonidine (0.1–3 µg/kg) produced an initial brief pressor response and bradycardia followed by hypotension in the intact dog. Chronotropic and inotropic responses were absent in the isolated atrium perfused with the intact dog’s blood. Intravenous clonidine (10–300 µg) also induced negative chronotropic and inotropic effects in isolated atria. On the other hand, alinidine, at doses which caused a depressor action and bradycardia in the intact dog, consistently produced negative chronotropic and inotropic effects in the isolated atrium. Therefore, it was confirmed that a relatively small dose of clonidine has a selective vascular action, while alinidine has direct cardiac depressant properties at all effective doses.

Negative chronotropic and inotropic effects of peripheral vagal stimulation, carbachol and adenosine were not significantly modified by 100 or 300 µg doses of intraarterial clonidine. On the other hand, the effects of vagal stimulation and carbachol were significantly inhibited by 100 and 300 µg of alinidine, without affecting adenosine-induced cardiac actions. Therefore, it was demonstrated that alinidine has anti-muscarinic properties.

Additional Indexing Words:
Clonidine  Alinidine  Cross-perfused dog atrial preparation
Vagal stimulation  Carbachol

Clonidine is a widely used antihypertensive agent which has been reported to have a central nervous mechanism of action. It is also reported that intravenous administration of clonidine in anesthetized animals induces a short-lasting hypertensive effect. This hypertensive response has
been considered to result from stimulation of peripheral vascular alpha-adrenoceptors by clonidine.\(^1\)–\(^3\) In 1970, Scriabine et al\(^4\) reported that clonidine has a powerful peripheral negative chronotropic action when administered into the sinus node artery of in situ dogs. However, they did not show whether intravenous clonidine produced a direct or indirect negative chronotropic action. Primm et al\(^5\) reported that clonidine could inhibit the release of norepinephrine in the SA node area using direct perfusion of the sinus node artery of the dog heart. Recently, we reported that a large dose of clonidine depressed positive chronotropic and inotropic responses in an isolated and cross-perfused canine atrial preparation.\(^6\) In 1981, Chiba et al\(^7\) also reported that a large dose of clonidine depressed positive chronotropic and inotropic responses to norepinephrine and cardiac sympathetic nerve stimulation in isolated dog atria. This study attempted to investigate the cardiovascular responses of intact dogs and simultaneously the chronotropic and inotropic responses of isolated atria to intravenous clonidine and alinidine using a cross-perfused atrial preparation.\(^8\),\(^9\) Finally, we examined the antimuscarinic actions of alinidine and clonidine because clonidine is a metabolite of alinidine and alinidine has anti-muscarinic properties.

**Methods**

Twenty-six mongrel dogs weighing 7 to 15 kg were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). After intravenous administration of sodium heparin (500 units/kg), the right atrium was quickly excised and plunged into cold Tyrode’s solution. The sinus node artery was cannulated with a tapered polyethylene tube via the right coronary artery of the isolated right atrium. The isolated preparation was perfused with arterial blood conducted from the carotid artery of the donor dog with a peristaltic pump (Harvard Apparatus, model 1210). The perfusion pressure was maintained constantly at 100 mmHg by pneumatic resistance in parallel with the perfusion circuit. The flow rate to the isolated atrium was 6–9 ml/min. The atrium was suspended in a glass container filled with blood at a constant temperature of 37°C. The atrium was usually stretched to a resting tension of 2 g. The isometric developed tension was measured with a force-displacement transducer (Nihon Kohden, AP 620G). Two bipolar silver electrodes were placed in contact with the epicardial surface of the atrium. The first pair of electrodes was placed on the caval margin of the atrium to stimulate intramural vagal nerve fibers. The second pair of electrodes was placed on the atrial free wall to record the atrial electrogram. The atrial rate was derived from
the electrogram with a cardiotachometer (Nihon Kohden, AT 600G). The isometric developed tension and atrial rate of the isolated preparation were recorded on a thermowriting rectigraph (Nihon Kohden, WT 685T). Intramural vagal nerve fibers were stimulated electrically with a stimulator (Nihon Kohden, SEN 7103) at frequencies of 5, 10 and 30 Hz. To stimulate the intramural vagal nerves, we set the duration of the electrical pulses at less than 0.3 msec, and adjusted the voltage so that it was subthreshold for the pacemaker cells and atrial muscle fibers.10) The mean voltage was 4 V. These negative chrono- and inotropic effects of vagal nerve stimulation were readily blocked by 10 µg of atropine. The effects of clonidine and alinidine on the cardiac responses to cholinergic agents were studied in the same preparations because the antimuscarinic action of alinidine has been reported in the dog heart.5,11,12)

Donor dogs weighing from 12–22 kg were also anesthetized with sodium pentobarbital (30 mg/kg, i.v.), and artificially ventilated with room air by means of a Harvard respirator. Sodium heparin (500 units/kg, i.v.) was administered intravenously at the beginning of the perfusion and 200 units/kg were added at 1 hour intervals. The heart rate was derived from the electrocardiogram (standard lead II) and femoral arterial blood pressure were measured and recorded on a rectilinear pen recorder (Sanei).

The drugs used in this study were clonidine hydrochloride (Boehringer Ingelheim), alinidine hydrochloride (Boehringer Ingelheim), adenosine (Tokyo Kasei) and carbamylcholine chloride (carbachol, Aldrich).

Data reflect the maximum change in each response. Data are expressed as % changes in each parameter and are shown as mean±SEM. The data were analyzed by Student’s t-test for paired values.

**Results**

**Cardiovascular effects of intravenous administration of clonidine to the donor dog in cross-perfused atrial preparations:**

When clonidine was administered into the jugular vein of the donor dog in a dose range of 0.1 to 300 µg, the heart rate of the intact dog decreased. Biphasic pressure responses also appeared, consisting of a transient increase followed by a long-lasting decrease in systemic blood pressure. However, only a large dose of intravenous clonidine produced simultaneous negative chrono- and inotropic effects in the isolated atrium. The control mean arterial blood pressure (MABP) was 88±11.2 mmHg (mean±SEM, n=6), and the control heart rate (HR) was 137±11.0 beats/min in donor dogs. The control atrial rate (AR) was 95±5.2 beats/min, while the control developed ten-
Fig. 1. Effects of clonidine and alinidine on mean arterial blood pressure (MABP) and heart rate (HR) of donor dogs, and on atrial rate (AR) and atrial developed tension (DT) of isolated atria. Drugs were administered into the jugular vein of the donor (support) dog.

MABP was 1.4±0.2 g in isolated atria (n=6). Intravenous administration of 0.1–3 μg/kg clonidine caused no significant influence on either the AR or DT of the isolated atrium (Fig. 1). However, at larger doses (10–300 μg/kg, i.v.), clonidine produced a slight decrease in both AR and DT in the isolated atrium. The threshold dose of clonidine for inducing negative chrono- and inotropic effects was approximately 10 μg/kg in the isolated atrium. The decreases in AR and DT were dose-related. On the other hand, intravenous alinidine produced a simultaneous depressor response, a decrease in heart rate in the donor dog and decreases in AR and DT of the isolated atrium.
Effects of clonidine and alinidine on the cardiac responses to cholinergic interventions and to adenosine:

Effects of clonidine and alinidine on the negative chrono- and inotropic responses to intramural vagal nerve stimulation, and direct injections of carbachol and adenosine were also investigated in isolated and blood-perfused dog right atrial preparations. The cardiac responses to cholinergic interventions and adenosine were produced consecutively in the same preparation. In this study, intramural vagal nerve stimulation at a frequency of 5, 10 or 30 Hz decreased both the AR and DT in a frequency-dependent manner (Figs. 2 and 3). An injection of carbachol into the sinus node artery of the isolated atrium at doses of 0.03 and 0.1 μg also caused dose-dependent decreases in atrial rate and developed tension. An injection of adenosine (30 μg) into the sinus node artery readily evoked negative chrono- and inotropic responses, as reported previously.

The cardiac responses to vagal nerve stimulation and to carbachol were significantly inhibited by atropine in a dose-dependent manner. However, cardiac responses to adenosine were not reduced by the same dose of atropine. Doses of 100 and 300 μg of clonidine (Fig. 2) and alinidine (Fig. 3) were used to determine the effects of cholinergic interventions and adenosine-induced responses. The negative chrono- and inotropic effects of either vagal nerve

![Fig. 2. Effects of intraarterial clonidine (100 and 300 μg) on negative chrono- and inotropic responses to vagal stimulation, carbachol (Cch) and adenosine in isolated dog atrial preparations. AR = atrial rate; DT = developed tension.](image-url)
stimulation and/or injections of carbachol or adenosine were not affected significantly by clonidine treatment (Fig. 2). By contrast, the negative chrono- and inotropic effects of cholinergic interventions were significantly reduced by treatment with 100 μg of alinidine; 300 μg of alinidine produced a greater inhibition of these cholinergic responses. The negative chrono- and inotropic responses to adenosine were not affected by alinidine.

**DISCUSSION**

It has been reported that intravenous clonidine produced an initial increase in arterial blood pressure, followed by both long-lasting hypotension and a decreased heart rate in anesthetized dog. When clonidine is administered peripherally in anesthetized animals, it produces a short-lasting hypertensive response which has been considered to result from a direct stimulation of peripheral alpha-adrenoceptors.\(^1\)-\(^3\),\(^14\),\(^15\) We replicated this sequence of responses in anesthetized dogs. Within a dose range of 3 μg/kg, clonidine caused a depressor response with an initial pressor response and bradycardia in anesthetized dogs without any modification of atrial rate and contractile force of isolated atria, confirming that an adequate dose of clonidine (0.3—3 μg/kg, i.v.) causes hypotension and bradycardia without any direct cardiac influence. With larger doses (10—300 μg/kg), clonidine caused direct negative chronotropic and inotropic effects.

By contrast, alinidine did not produce a pressor effect, which indicates
an absence of alpha-adrenoceptor agonist effect. Although alinidine also induced bradycardia and hypotension in intact dogs, direct cardiac depressant actions were consistently observed; intravenous administration of alinidine to intact donor dogs produced negative chronotropic and inotropic responses in isolated atria perfused with arterial blood from the intact animals. Thus, it is clear that a relatively small dose of clonidine has cardiovascular effects without direct cardiac actions, and that alinidine produces cardiovascular responses with a direct cardiodepressant component. Recently, Ogiwara et al\(^1\) showed that alinidine induced dose-related negative chronotropic and inotropic effects when injected into the sinus node artery of the isolated atrial preparations. Although alinidine depresses the SA node by directly suppressing SA nodal automaticity,\(^1^{6-20}\) we could confirm that any dose of alinidine which influenced the cardiovascular system readily induced direct cardiac depressant actions different from those of clonidine-induced actions.

In a previous paper,\(^6\) it was demonstrated that alinidine had attenuated negative chronotropic and inotropic responses to both exogenous choline esters and endogenous ACh in the dog heart. In particular, the negative chronotropic and inotropic responses to vagal nerve stimulation and injected ACh were depressed by alinidine treatment. The present study revealed that clonidine did not significantly inhibit these negative cardiac responses. This suggests that the two drugs have at least partially independent actions.

Alinidine is an N-allyl derivative of clonidine. However, it may differ from clonidine in both its cardiovascular effects and its antimuscarinic properties. In 1981 Chiba et al\(^7\) reported that clonidine did not significantly depress the chronotropic responses to norepinephrine or sympathetic nerve stimulation. However, Rodgers et al (1980)\(^21\) reported that clonidine non-competitively antagonized the positive chronotropic effect of isoproterenol, 4-methylhistamine and glucagon. These reports suggested that a large dose of clonidine has nonspecific cardiac depressant properties. The present study, though, confirmed that 1) a metabolite of alinidine, clonidine, has no distinct direct cardiac action except at extremely large doses, and 2) that, unlike alinidine, clonidine has no anti-muscarinic action in the dog heart.

**References**

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