Cardiotonic and Coronary Vasodilatatory Effects of Amrinone in the Canine Heart-Lung Preparation with a Support Dog as Compared with Those of Dobutamine

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Abstract—Analysis of the relative magnitude of the positive inotropic and chronotropic effects and the coronary vasodilating effects of amrinone conducted in the canine heart-lung preparation with a support dog in comparison with those of dobutamine demonstrated that amrinone was a preferential coronary vasodilator rather than a selective positive inotropic agent. An in vitro experiment in the canine papillary muscle suggested the initiation of the slow response action potential as a mechanism of the positive inotropic effect of amrinone.

Amrinone is a novel orally effective nonglycoside cardiotonic agent. Several recent reports indicate that the actions of this substance are exerted preferentially on the myocardial contractility; the chronotropic and vasodilatatory effects on the coronary and other peripheral vascular beds are thought to be rather limited both in vivo and in vitro (1, 2), although there are papers maintaining that the substance is a preferential vasodilator (3–6). However, quantitative comparison of the effects of amrinone on the heart and the coronary vasculature has not been adequately conducted yet.

The present experiments using the canine heart-lung preparation with a support dog were, therefore, undertaken to quantitatively analyze the effects of bolus intravenous administration of amrinone on the heart and coronary vasculature in comparison with those of dobutamine. Dobutamine is a potent positive inotropic agent with relatively slight effects on the heart rate as compared with isoproterenol, epinephrine and dopamine (7, 8). The ability of this compound to induce the slow response action potentials and the associated contraction in the partially depolarized papillary muscle from the same species was also examined in order to elucidate the possible mechanisms of the positive inotropic effect.

Materials and Methods
The canine heart-lung preparation supported with a support dog (HLP c donor)

The mongrel dogs of either sex weighing between 9 and 13 kg were anesthetized with an intraperitoneal injection of 35 mg/kg of sodium pentobarbital. The HLP c donor was prepared according to the method reported in our previous papers (9, 10). Briefly, the coronary sinus outflow led out by a Morawitz cannula and measured by means of a cannulating type probe (2 mm, i.d.) of an electromagnetic flowmeter (Statham SP 2201) was sent to the femoral vein of a donor dog via a small venous reservoir, and the fresh arterial blood from the femoral artery of the donor dog was returned to a venous reservoir of the HLP. Systemic cardiac output was measured with another electromagnetic flowmeter (Statham SP 2201) with a cannulating type probe (6 mm, i.d.) placed after a Starling’s resistance of the extracorporeal...
circuit of HLP. The right atrial pressure and the heart rate were measured as reported previously. The difference of the oxygen content between the arterial and the coronary sinus blood was measured with an A-Vox system (A-Vox system), and the myocardial oxygen consumption was calculated as reported in our previous paper (10).

The donor dog, weighing between 20 and 30 kg, was anesthetized by intravenous administration of α-chloralose (45 mg/kg) and urethane (450 mg/kg) after premedication with morphine (1.5 mg/kg, s.c.). The isolated canine papillary muscle preparation

Under sodium thiopental anesthesia, hearts were removed from the animals via the left thoracotomy and immediately immersed in physiological saline solution. The papillary muscles were dissected out in Krebs-Henseleit’s solution which had the following composition (in mM): NaCl, 118; KCl, 4.7; NaHCO3, 25; KH2PO4, 1.2; MgSO4, 1.2; CaCl2, 2.5; and glucose, 11.

i) Recording of the developed tension: Papillary muscles were trimmed into preparations of approximately 1 mm in diameter and 6 mm in length and suspended vertically in a 20 ml organ bath containing Krebs-Henseleit solution aerated with 95% O2 + 5% CO2 and kept at a temperature of 37°C. The initial tension was set at about 0.2 g, and the preparation was stimulated electrically at 1 Hz with square wave pulses of 2 msec duration by means of an electronic stimulator (Nihon Kohden MSE-3). Developed tension was recorded on a linearly recording ink writing oscillograph with a force-displacement transducer (Nihon Kohden SB-1T) coupled with a carrier amplifier.

After an equilibration time of about 30 to 40 min in normal Krebs-Henseleit solution, the preparations were transferred to a K⁺-rich Krebs-Henseleit’s solution (K⁺-rich solution) containing NaCl, 98; KCl, 21; NaHCO3, 25; KH2PO4, 1.2; MgSO4, 1.2; CaCl2, 2.5; and glucose, 11 (mM). The solution contained additionally 3 μM tetrodotoxin, 10 μM (±)-propranolol, 10 μM metiamide, and 10 μM mepyramine. In the K⁺-rich solution, the papillary muscle was stimulated at 0.2 Hz with square wave pulses of 2 msec duration and voltages 4 to 5 times higher than the threshold.

ii) Electrophysiological studies: Papillary muscles were suspended horizontally in a 2.5 ml organ bath containing the Krebs-Henseleit’s solution and equilibrated for a certain period. During this period, preparations were stimulated at 1 Hz using field stimulation electrodes and an electronic stimulator (Nihon Kohden SEN 6100) triggered by a Digitimer (Devices 3290). After this period, the preparations were partially depolarized in K⁺-rich solution, and the stimulation rate was switched to a low frequency of 0.2 Hz. Transmembrane potential was recorded using glass capillary microelectrodes filled with 3 M KCl (5–20 M ohm) with the aid of a cathode follower amplifier (Nihon Kohden, MZ-4), oscilloscope (Nihon Kohden VC-7A), and a continuously recording camera (Nihon Kohden RLC-6101). The maximal rate of rise of the phase 0 depolarization was measured using a home-made differentiator with a time constant of 50 μsec.

Drugs used were (±)-dobutamine (Shionogi), (–)-isoproterenol (Nikken), tetrodotoxin (Sankyo), (±)-propranolol (ICI Pharma), phentolamine (Ciba-Geigy), mepyramine (K & K) and metiamide (Smith, Kline & French). Amrinone was generously supplied by Yamanouchi Pharmaceutical Co., Ltd. Diltiazem was generously supplied by Tanabe Seiyaku Co., Ltd.

Statistical analysis was performed with Student’s paired t-test.

Results

Effects of amrinone and dobutamine on the canine heart-lung preparation with a support dog (HLP c donor)

Figure 1 is a typical tracing showing the effects of amrinone and dobutamine on the canine HLP c donor. Bolus administration of amrinone, in doses from 1 to 10 mg, resulted in a marked and long-lasting increase in coronary blood flow associated with a decrease in the right atrial pressure (RAP) and an increase in heart rate (HR), although the decrease in RAP and the increase in HR was rather small even at the highest dose. Dobutamine (10 to 100 μg) produced a definite fall of the RAP and a steep rise of HR.
However, the increase in coronary blood flow was small as compared with that produced by amrinone.

Figures 2 and 3 depict the dose-response relation of the effects of amrinone and dobutamine on the systemic cardiac output (SOP), RAP, HR, coronary blood flow and myocardial oxygen consumption ($O_2C$). As these figures show, dobutamine was around 1000 times more potent than amrinone as regards the positive inotropic and chronotropic effects, and amrinone was a coronary vasodilator rather than a positive inotropic and chronotropic agent. In Fig. 4 are depicted the relative magnitude of the positive inotropic and chronotropic effects as evidenced by a decrease in RAP ($\Delta$RAP) and an increase in HR ($\Delta$HR). The ratios $\Delta$RAP/$\Delta$HR were 2.26 for amrinone and 0.81 for dobutamine. Thus, as compared with dobutamine, amrinone was a more selective positive inotropic agent. Reflecting the trivial increase in heart rate, only a minimal increase in myocardial $O_2C$ was noted with amrinone. The ratios of the changes in the RAP ($\Delta$RAP) vs. the changes in the myocardial $O_2C$ ($\Delta$O$_2$C) were 1.51 for amrinone and 0.76 for dobutamine as shown in Fig. 5.

To analyze the effects of these substances on the coronary vasculature, the percent increase in the coronary blood flow was plotted against the percent increase in the myocardial $O_2C$ (Fig. 6). For comparison, the regression line representing the effects of isoproterenol, taken from our previous paper (9), is also depicted in this figure. As is evident from this figure, amrinone produced disproportionately larger increases in the coronary blood flow, indicating that the agent exerted a direct dilatatory effect on the coronary vasculature. In contrast, increases in the coronary blood flow produced by
Fig. 2. The dose-response relation of the effects of amrinone and dobutamine on the systemic cardiac output (SOP), right atrial pressure (RAP) and heart rate (HR) in the canine HLP c donor. Numbers in parentheses are the numbers of experiments.

Fig. 3. The dose-response relation of the effects of amrinone and dobutamine on the coronary blood flow (Cor. F) and myocardial oxygen consumption ($O_2$C) in the canine HLP c donor. Numbers in parentheses are the numbers of experiments.
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Dobutamine were associated with increases in the myocardial O$_2$C to a similar extent, the gradient of the regression line being slightly larger than 1.

Studies using the canine papillary muscle

i) Restitution of contraction in the par-
tially depolarized preparation: After an equilibration period of 30 to 40 min in the normal Krebs-Henseleit's solution (stimulation rate=1 Hz), the papillary muscles were immersed in the K+-rich solution (22 mM K+). In this condition, the mechanical responses quickly disappeared. Then, the stimulation rate was lowered to 0.2 Hz and the stimulation intensity was increased several times. After confirming that no mechanical responses were observable in the newly established condition, amrinone was added in a cumulative fashion in the presence of selected pharmacologic antagonists (3 μM tetrodotoxin, 10 μM propranolol, 10 μM phenotolamine, 10 μM mepyramine and 10 μM metiamide). This resulted in a restitution of contraction, and the developed contractile tension was dependent on the concentrations of amrinone (0.53–5.3 μM) as shown in Fig. 7. With 1 mg/kg, the developed tension almost reached the level observed in the normal Krebs-Henseleit's solution. These contractile responses were inhibited by 0.3 μg/ml of diltiazem, a calcium antagonist (data not shown).

ii) Electrophysiological studies: To search into the mechanism of the above phenomenon, electrophysiological experiments were conducted under the same condition with the microelectrode technique. As shown in Fig. 8, amrinone (1.6 μM) induced "slow" response action potentials in the partially depolarized papillary muscle preparations of the dog, and the action potential was completely inhibited by application of diltiazem (0.67 μM).

Discussion

In the present study conducted in the
canine heart-lung preparation with a support
dog, it was clearly demonstrated that the
prominent effect of amrinone was on the
coronary blood flow rather than on the
cardiac function, and the increase in the
coronary blood flow was not associated with
any appreciable increase in myocardial
oxygen consumption. Thus it may be con-
cluded that amrinone is a coronary vasodilator
rather than a positive inotropic and chrono-
tropic agent. In contrast, dobutamine
produced a marked positive inotropic and
chronotropic effect and an increase in
coronary blood flow, which was associated
with a considerable increase in the myo-
cardial oxygen consumption, indicating that
the substance did not exert a direct
dilatatory effect on the coronary vasculature.
It was found that amrinone was a rather
selective positive inotropic agent. However,
it was not so potent as dobutamine in this
respect. As possible mechanisms of the
positive inotropic effects of amrinone,
activation of the slow calcium channels may
be invoked. Adams et al. (11) demonstrated
that amrinone consistently restored the
membrane excitability and contractile function
in K⁺-depolarized atrial and ventricular
myocardium of the guinea pig. In the present
study conducted with the dog papillary
muscle preparations similar effects were
observed; amrinone elicited the slow
response action potential and associated
contractions that could be blocked by
diltiazem. Since the effect was observed in
the presence of blockers of the adrenoceptors
and the histaminergic receptors, it is without
doubt that the effect was independent of
activation of adrenoceptor or histaminergic
receptors. Nor was it associated with the
neuronal mechanism, for it was observed in
the presence of tetrodotoxin. This is in
agreement with the previous report of Kondo
et al. (12). Using the papillary muscle pre-
paration of the guinea pig, they showed that
the positive inotropic effect of amrinone was
not affected with β-blockers or TTX, but
was blocked by verapamil or lowering of the
calcium concentration of the bathing medium.
They further demonstrated the augmentation
of the slow response action potential in the
presence of 30 mM K⁺ and the slow inward
current observed in the voltage clamp
experiments. As a mechanism of augmen-
tation of the slow response action potential,
the accumulation of cyclic AMP due to an
inhibition of phosphodiesterase is conceiv-
able, for Endoh et al. (13) demonstrated in
right ventricular trabeculae of the dog a rise
of the cyclic AMP level after amrinone. They
also demonstrated in in vitro experiments the
inhibition of phosphodiesterase by this com-

![Fig. 8. The slow response action potential induced by amrinone in the partially depolarized canine papillary muscle preparations (B) and the effect of diltiazem (C). (A) represents a normal action potential.]
pound. Involvement of cyclic AMP in the inotropic action of amrinone was also demonstrated by Honerjäger et al. (14). The vasodilatation of the coronary artery may also be explained by a similar mechanism as postulated by Meisheri et al. (15). The reason why amrinone is more potent as a coronary vasodilator is not clear at present. However, a similar tendency was noted of the effects of dibutylryl cyclic AMP in our previous work with the dog heart-lung preparation.

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