Cardiovascular Responses to a Newly Developed Cardiotonic Agent, ZSY-39 [4-methyl-5-(4-pyridinyl)-thiazole-2-carboxyamide] in Dog Cross-Circulated Atrial and Ventricular Preparations

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SUMMARY

The cardiovascular effects of ZSY-39 [4-methyl-5-(4-pyridinyl)-thiazole-2-carboxyamide] were investigated in isolated and blood-perfused atrial and ventricular muscles perfused with donor's arterial blood. When ZSY-39 was given i.v. to the intact donor dog, hypotension with a slight tachycardia was induced at a dose range of 30–1,000 µg/kg. At the same time, slight positive chronotropic and inotropic responses appeared in isolated, perfused atria at i.v. doses of 300 and 1,000 µg/kg ZSY-39, indicating a relatively dominant inotropic action.

Direct injection of ZSY-39 into the cannulated sinus node artery of the isolated atrium produced positive chronotropic and inotropic responses in a dose-related manner (1 to 300 µg). ZSY-39 also induced a dose-dependent increase in developed tension in the isolated ventricle. The positive chronotropic and inotropic effects of ZSY-39 were not modified by an adequate dose of propranolol which completely blocked norepinephrine-induced positive chronotropic and inotropic responses. From these results, it is concluded that ZSY-39 has mild cardiotonic properties, showing relatively selective positive inotropic activity.

Additional Indexing Words:
Isolated dog atrium Isolated dog ventricle Sinus rate Developed tension Propranolol

ZSY-39 [4-methyl-5-(4-pyridinyl)-thiazole-2-carboxyamide] is a newly synthesized compound which has potent phosphodiesterase inhibitory properties. Recently, several new cardiotonics which are not sympathomimetics have been developed and investigated, as reviewed by Farah et al19 in 1984.
There are numerous cardiotonic compounds in development such as amrinone, milrinone, cilostamide, sulmazole, buquineran and trapidil\textsuperscript{[3]–[6]} which have phosphodiesterase inhibitory properties.

In the present study, we made an attempt to investigate the effects of ZSY-39 on pacemaker activity and contractility, using isolated, blood-perfused canine atrial and ventricular preparations which were developed by Chiba et al.\textsuperscript{[7], [8]}

**Methods**

Sixteen mongrel dogs of either sex, weighing from 8 to 19 kg were anesthetized with sodium pentobarbital, 30 mg/kg, i.v. After intravenous administration of sodium heparin (200 units/kg), the right atrium was quickly excised and plunged into cold saline at approximately 4 to 10°C. The sinus node artery was cannulated via the right coronary artery of the isolated right atrium and perfused with fresh arterial blood obtained from the carotid artery of the donor dog by aid of a peristaltic pump (Harvard Apparatus, model 1210). The perfusion pressure was consistently kept at 100 mmHg by use of a pneumatic resistance in parallel with the perfusion circuit. The flow rate was 2 to 6 ml/min. The atrium was suspended in the bath filled with blood at a constant temperature of 37°C. The atrial rate was measured with a tachometer which was triggered by atrial electrograms. The isometric developed tension was measured with a force displacement transducer (Grass FTO3B).

The effects of ZSY-39 were investigated in 3 isolated ventricular preparations. The left ventricle was quickly excised and immersed in saline at 4 to 10°C. The muscle was perfused through the cannulated anterior descending branch of the left coronary artery. The perfused blood was introduced from the carotid artery of the donor dog as in the atrial preparation. The flow rate was 4 to 13 ml/min. The ventricular muscle was electrically driven with rectangular pulses using an electronic stimulator (Nihon Kohden MSE). The stimulus strength was about twice the threshold voltage (5 msec duration and 2–5 volts). Details of the preparations have been de-

\begin{center}
\textbf{Fig. 1.} Chemical structure of ZSY-39 [4-methyl-5-(4-pyridinyl)-thiazole-2-carboxyamide].
\end{center}
scribed in previous papers.\textsuperscript{7,8})

The donor dogs, weighing 12 to 25 kg, were also anesthetized with an intravenous injection of 30 mg/kg of sodium pentobarbital and artificially ventilated with room air using a Harvard respirator. Sodium heparin, 500 units/kg, was administered intravenously at the beginning of the perfusion and 200 units/kg added at 1 hour intervals.

The drugs utilized in this study were ZSY-39 [4-methyl-5-(4-pyridinyl)-thiazole-2-carboxamide] (synthesized by Zenyaku Kogyo Co. Ltd., Tokyo, Fig. 1), dl-norepinephrine hydrochloride (Sankyo Co.) and dl-propranolol hydrochloride (Sumitomo Chemicals). ZSY-39 was dissolved in 0.2 N hydrochloride, and then diluted with saline. Each drug was administered into the cannulated sinus node artery of the isolated atrium or into the cannulated anterior descending branch of the left coronary artery of the isolated left ventricular muscle over a period of 4 sec.

\textbf{Results}

1. Effects of ZSY-39 on intact dogs and on isolated atria

When ZSY-39 was injected intravenously into the jugular vein of the support dog in a dose range of 10 to 1,000 $\mu$g/kg, a triphasic pressure response was frequently induced as shown in Fig. 2, i.e., an initial brief hypotensive, secondary hypertensive and finally long-lasting hypotensive response were observed. The threshold dose was approximately 30 $\mu$g/kg. The secondary

\begin{figure}[h]
\centering
\includegraphics[width=0.6\textwidth]{figure2.png}
\caption{Cardiovascular effects of 300 $\mu$g/kg and 1 mg/kg of intravenous ZSY-39 to a donor dog on heart rate and arterial blood pressure in an anesthetized donor dog and on atrial rate and developed tension in an isolated atrium.}
\end{figure}
hypertensive effect of ZSY-39 was sometimes not clear because blood pressure remained below the control level. At the same time, a slight tachycardia was usually observed in the support dogs.

When ZSY-39 was administered intravenously to the support dog, the same arterial blood concentration of ZSY-39 was administered to the isolated atrium where it exerted its action on atrial pacemaker activity and contractility. At 300 μg/kg of ZSY-39, slight positive chronotropic and inotropic effects on isolated atria were observed. At 1 mg/kg of ZSY-39, they were much more pronounced as illustrated in Fig. 2. Summarized data following intravenous injection of ZSY-39 are shown in Fig. 3.

2. Effects of intraarterial injection of ZSY-39 on atrial rate and development of atrial and ventricular tension

When ZSY-39 was administered into the cannulated sinus node artery of the isolated atrium, positive chronotropic and inotropic effects were induced in a dose-related manner. A typical tracing of increasing doses of ZSY-39 is shown in Fig. 4. Summarized data are shown in Fig. 5.

In 3 experiments, ZSY-39 was administered to isolated and blood-perfused left ventricular muscle paced at 2 Hz. ZSY-39 also induced a positive inotropic effect in a dose-related manner as shown in Fig. 6. The threshold dose for inducing a positive inotropic effect was approximately 3 μg.
Fig. 4. Chronotropic and inotropic responses to selective injection of increasing doses of ZSY-39 into the cannulated sinus node artery of an isolated canine right atrial preparation.

Fig. 5. Summarized data of positive chronotropic and inotropic effects of increasing doses of ZSY-39. Open circles represent mean values, and vertical bars show standard errors. Numbers in parentheses represent number of experiments.

Fig. 6. Effects of increasing doses of ZSY-39 on an isolated and perfused canine ventricular preparation.
The percent increases in ventricular developed tension were $5.0 \pm 2.0$ (mean ± SEM, n=3), $10.2 \pm 5.9$, $32.7 \pm 18.9$, $52.0 \pm 31.4$ and $93.7 \pm 47.4$ at 3, 10, 30, 100 and 300 $\mu$g, respectively.

3. Effects of propranolol on ZSY-39-induced positive chronotropic and inotropic responses

When norepinephrine was injected into the sinus node artery of the isolated atrium, dose-dependent positive chronotropic and inotropic effects were induced. After treatment with 3 $\mu$g of propranolol, positive chronotropic and inotropic effects were significantly reduced.

![Figure 7](image-url)  
Fig. 7. Effects of 3 $\mu$g of propranolol on responses to 0.1 $\mu$g of norepinephrine (NE) and 100 $\mu$g of ZSY-39 in an isolated atrial preparation.

![Figure 8](image-url)  
Fig. 8. Summarized data of effects of propranolol on norepinephrine (NE)- and ZSY-39-induced positive chronotropic and inotropic responses in 5 isolated atrial preparations. Vertical bars represent standard errors.
tropic and inotropic responses to 0.1 μg of norepinephrine were significantly inhibited, but those to 100 μg of ZSY-39 were not significantly suppressed as shown in Fig. 7. Summarized data of the effects of propranolol are shown in Fig. 8.

**DISCUSSION**

It is well recognized that substances exerting phosphodiesterase inhibitory action have cardiac stimulating properties, suggesting that an intracellular accumulation of cyclic AMP causes positive chronotropic and inotropic actions. Aminophylline, a phosphodiesterase inhibitor in common clinical use, induced slight positive chronotropic and inotropic effects when administered directly into the sinus node artery. However, another potent phosphodiesterase inhibitor, papaverine, has not only positive but also negative chronotropic and inotropic properties in isolated atrial preparations. It is known that catecholamines induce positive chronotropic and inotropic effects via an increase in intracellular cyclic AMP by stimulating adenylate cyclase. It has been reported that dopamine induced positive chronotropic and inotropic responses after intraarterial injection. However, a dopamine derivative, N-glycylglycylleucyl-dopamine, caused a dominant inotropic effect in the isolated dog atrial preparation, suggesting different actions of cyclic AMP on pacemaker activity and contractility. One of the potent stimulators of adenylate cyclase, glucagon, induced a dominant chronotropic effect with a small inotropic one. Moreover, dibutyryl cyclic AMP produced positive chronotropic and inotropic effects only at extremely large doses in the isolated dog atrium. Thus, complicated mechanisms might exist for the production of positive chronotropic and inotropic responses to increases in intracellular cyclic AMP.

In the present experiments, ZSY-39 produced a depressor response with a slight tachycardia in intact dogs, showing responses similar to intravenous aminophylline. In isolated atria, ZSY-39 induced positive chronotropic and inotropic effects, and it induced neither negative chronotropic nor inotropic effects, differing from papaverine in this respect. The positive chronotropic and inotropic responses to ZSY-39 were not blocked by propranolol in doses which significantly blocked norepinephrine-induced positive chronotropic and inotropic effects. Therefore, ZSY-39-induced effects were not mediated by an adrenergic beta-receptor mechanism. ZSY-39-induced positive chronotropic and to a greater extent, inotropic effects were greater than aminophylline-induced ones, indicating the dominant inotropic activity of this compound. Therefore, ZSY-39 may exert its action on the myocardium in
a way similar to that of a mild cardiotonic.

REFERENCES