Cardiovascular Effects of a New Positive Inotropic Agent, 
(−)-(R)-1-(p-Hydroxyphenyl)-2-[(3,4-Dimethoxyphenethyl)Amino]-Ethanol (TA-064) in the Anesthetized Dog and Isolated Guinea Pig Heart

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Abstract—The positive inotropic effect of TA-064, (−)-(R)-1-(p-hydroxyphenyl)-2-[(3,4-dimethoxyphenethyl)amino]ethanol, was studied in the anesthetized dog and isolated guinea pig heart. An intravenous administration of TA-064 dose-dependently increased the cardiac contractile force with little effect on blood pressure in dogs. The positive inotropic activity of TA-064 was 1/100 that of isoproterenol and similar to that of dobutamine. This effect of TA-064 was stereospecific, and it was blocked by practolol. Thus TA-064 has beta_1-adrenoceptor agonistic action. The positive inotropic effect of TA-064 was more pronounced than the positive chronotropic effect, compared with those of isoproterenol. Similar effect of TA-064 was observed in the reserpinized dog and in the isolated perfused heart of the guinea pig as well. TA-064 administered intraduodenally at a dose of 0.1 mg/kg increased contractile force by 120% of the control, and the effect lasted for more than 150 min. TA-064 given in the femoral artery demonstrated a very weak vasodilating effect on the artery. TA-064 is an orally active, positive inotropic agent. The selective positive inotropic action of TA-064 may result from its beta_1-adrenoceptor agonistic property.

Some catecholamines such as dopamine and dobutamine (1) have been used as inotropy supporting agents. These agents, however, are clinically available only in the injectable form. Recently, orally active positive inotropic compounds have been developed which have beta-sympathomimetic (2–7) or phosphodiesterase inhibiting action (8–11). These agents have been reported to have some separation between positive inotropic and positive chronotropic actions. TA-064, (−)-(R)-1-(p-hydroxyphenyl)-2-[(3,4-dimethoxyphenethyl)amino]ethanol, is a newly synthesized compound (Fig. 1).

![Fig. 1. Chemical structure of TA-064. *: asymmetric carbon atom.](attachment:image.png)
Femoral blood flow: Male mongrel dogs weighing 12 to 17 kg were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) with subsequent intravenous infusion of 4 mg/kg per hour. After intubation into the trachea, ventilation was maintained with a respirator (Takashima, Model 100). Femoral blood flow was measured by the flow probe (2.5 to 3 mm, inner diameter) of an electromagnetic flowmeter (Nihon Kohden, MF-27).

For administration of drugs, a 22G needle with polyethylene tubing was inserted into the femoral artery, distal to the flow probe. The drug whose dose causes no effect on systemic blood pressure was administered in a volume of 0.2 to 0.3 ml/10 sec. Thus an increase and a decrease in flow were considered as vasodilation and vasoconstriction, respectively. Propranolol was administered at doses of 0.2 mg/kg, i.v., followed by 0.1 mg/kg, i.a. Phentolamine was administered at doses of 0.5 mg/kg, i.v., followed by 0.5 mg/kg, i.a.

Drugs: TA-064 (free base), the racemate (hydrochloride) of TA-064, the (+)-isomer (hydrochloride) of TA-064 and dobutamine hydrochloride were synthesized by our Organic Chemistry Research Laboratory. Other drugs were obtained from the following sources: reserpine (Daiich), propranolol hydrochloride (Sumitomo), practolol (I.C.I.), phentolamine mesylate (Ciba-Geigy), dl-isoproterenol hydrochloride (Kaken) and l-phenylephrine hydrochloride (Kowa).

Statistical analysis: All data were expressed as means±S.E. Statistical analysis was carried out using Student's t-test and the discriminant function.

Results

Effects on contractile force, heart rate and blood pressure: Figures 2 and 3 show the effects of TA-064, isoproterenol and dobutamine on the left ventricular contractile force, heart rate and systemic blood pressure in anesthetized open chest dogs. As shown in Fig. 2, TA-064 at a dose of 2 μg/kg, i.v., increased cardiac contractile force without affecting significantly systemic blood pressure. The positive inotropic action of TA-064 lasted for more than 20 min and was significantly longer than those of isoproterenol
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Fig. 2. Experimental records for the effects of TA-064, isoproterenol and dobutamine on the contractile force of the left ventricle, heart rate and blood pressure in a thoracotomized dog. Doses were selected to increase contractile force to a similar degree. B.P.=systemic blood pressure, H.R.=heart rate, C.F.=left ventricular contractile force. Drugs were administered intravenously.

Fig. 3. Effects of TA-064 (○), isoproterenol (●) and dobutamine (△) on systemic blood pressure, heart rate and contractile force of the left ventricle in thoracotomized dogs. Broken lines represent the secondary changes in blood pressure. Each symbol shows the mean of seven experiments for isoproterenol and TA-064 and five to seven experiments for dobutamine. Some of the standard errors are within the symbols. Control values (mean±S.E.) are as follows: systolic blood pressure, 146±8.6 mmHg; diastolic blood pressure, 110±5.7 mmHg; and heart rate, 158±7.8 beats/min.

and dobutamine. Dose-response curves (Fig. 3) indicate that the inotropic activity of TA-064 is quite similar to that of dobutamine, but is approximately one hundredth that of isoproterenol. Figure 3 shows that TA-064 increases contractile force by approximately 50% at 2 μg/kg, i.v., and by approximately 100% at 4 μg/kg, i.v. TA-064 and dobutamine increased heart rate dose-dependently to a lesser extent than isoproterenol. As shown in Fig. 4A and 4B, a line was obtained by the discriminant function. This means that the
positive inotropic effects of TA-064 and dobutamine were more pronounced than the positive chronotropic effects, when compared with isoproterenol.

TA-064 produced an increase in systolic and diastolic blood pressure. At higher doses, TA-064 caused an increase, followed by a small decrease in diastolic pressure (Fig. 3). Isoproterenol decreased blood pressure, and dobutamine first increased and then decreased blood pressure.

Intraduodenal administration of TA-064 also increased the cardiac contractile force in a dose-related manner; contractile force increased by 30, 50 and 120% approximately 30 min after the administration at 30, 50 and 100 μg/kg, respectively (Fig. 5). The effect lasted for more than 60 min at 30 μg/kg, i.d., approximately 120 min at 50 μg/kg, i.d., and for more than 150 min at 100 μg/kg, i.d. Mean blood pressure was not changed by TA-064 at these doses. Heart rate increased at the highest dose, while it did not significantly increase at lower doses. The results suggest
that TA-064 is well absorbed from the digestive tract to produce the positive inotropic effect.

Positive inotropic action of stereoisomers of TA-064 and the influence of pretreatment with practolol: We examined the effects of the racemate and the (+)-isomer of TA-064 on cardiac contractile force in anesthetized open chest dogs and compared the results with that of TA-064 (Fig. 6). All of the isomers of TA-064 increased cardiac contractile force dose-dependently, TA-064 being approximately twice as potent as the racemate and over 100 times as potent as the (+)-isomer. The slope of the dose-response curve of TA-064 was identical with those of isoproterenol and the racemate, while that of the (+)-isomer was less steep. However, the dose-response curves for all the compounds were similarly shifted to the right by pretreatment with practolol (1 mg/kg, i.v.). The results demonstrate that the mechanism involved in the positive inotropic action of TA-064, the racemate and the (+)-isomer is stimulation of beta-/adrenoceptors in intact animals. The positive chronotropic action of these three compounds was inhibited by pretreatment with practolol to a similar degree.

Reserpinized dogs: In order to examine whether the positive inotropic action of TA-064 is due to release of catecholamine, we studied the cardiovascular effect of TA-064 in reserpinized dogs. The cardiovascular response to isoproterenol in the reserpinized dog was almost identical with that in the non-reserpinized normal dog (cf. Fig. 3), although the basal blood pressure and heart rate were significantly (P<0.05) lowered by the pretreatment with reserpine. As shown in Fig. 7, TA-064 exerted similar increases in cardiac contractile force, heart rate and blood pressure to those in the non-reserpinized normal dog.

Isolated perfused heart: To examine the direct action of TA-064 on the heart, we studied the effect of TA-064 on the isolated perfused heart in guinea pigs (Fig. 8). As shown in Fig. 8A, TA-064 and isoproterenol increased contractile force and heart rate dose-dependently. The relationship between positive inotropic and positive chronotropic actions is illustrated in Fig. 8B, showing that TA-064 selectively produced inotropic

![Fig. 6. Effects of stereoisomers of TA-064 on contractile force of the left ventricle before and after treatment with practolol in thoracotomized dogs. ○: TA-064 (n=7), Δ: the racemate (n=7). □: the (+)-isomer (n=4), ●: isoproterenol (n=7). Broken lines show dose-response curves after treatment with practolol at the dose of 1 mg/kg, i.v. Control values: before practolol, 25.9±5.41 g (n=7) and after practolol, 19.5±3.21 g (n=7).](image)

![Fig. 7. Cardiovascular responses to TA-064 and isoproterenol in reserpinized dogs. ○: TA-064, ●: isoproterenol. Each point represents the mean of five experiments with S.E. Some of the standard errors are within the symbols. Control values: systolic blood pressure, 118±6.4 mmHg; diastolic blood pressure, 86±4.6 mmHg; and heart rate, 113±8.8 beats/min.](image)
action compared with isoproterenol in the isolated heart.

Femoral blood flow: The effect of intraarterial injection of TA-064 on the femoral vascular bed in anesthetized dogs was examined (Fig. 9). Isoproterenol increased femoral blood flow at very low doses, while TA-064 increased it only at very high doses. At a dose of 8 μg/kg, i.a., an increase in the femoral blood flow was followed by a slight change in systemic blood pressure. The result that TA-064 dilates the femoral artery very weakly and its effect being 10000 times less potent than that of isoproterenol are consistent with the finding that TA-064 has only slight effect on systemic blood pressure (cf. Figs. 2 and 3). On the other hand, the intraarterial administration of dobutamine at higher doses decreased femoral blood flow sometimes followed by an increase. Although both TA-064 and dobutamine have a weak effect on systemic blood pressure, the directions of the vascular response to the drugs were different.

Pretreatment with propranolol caused a small decrease in femoral blood flow concomitant with reductions in systemic blood pressure (−15.5±4.6 mmHg) and heart rate. As shown in Fig. 9, propranolol caused a rightward shift of the dose-response curve of isoproterenol by approximately 300 times, while it shifted that of TA-064 by only 4 times. On the other hand, propranolol potentiated the dobutamine-induced reduction in femoral blood flow.

To elucidate the differences in the action between TA-064 and dobutamine more clearly, the effect of phentolamine on the vascular responses to both TA-064 and dobutamine was examined in the same femoral artery preparation (Fig. 10). Treat-
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ment with phentolamine reduced blood pressure by 23.2 ± 6.3 mmHg. As shown in Fig. 10, vasoconstriction evoked by phenylephrine and dobutamine was reversed by the administration of phentolamine, respectively. Phentolamine was administered at a dose of 0.5 mg/kg, i.a. Each point represents the mean of five experiments with S.E. Control values: before medication, 80.0 ± 12.6 ml/min and after phentolamine, 72.0 ± 8.9 ml/min.

Discussion

TA-064 is a phenylethanolamine derivative having a β-hydroxymoiety in its side chain, but unlike isoproterenol and dobutamine, it has no catecholamine moiety. An intravenous bolus injection of TA-064 caused a dose-dependent increase in the cardiac contractile force; its potency was one hundredth that of isoproterenol and was the same as that of dobutamine, but its effect was actually longer-lasting than those of the catecholamines. The positive inotropic action of TA-064 was stereospecific, and the (-)-isomer, i.e. TA-064, was the most active. The inotropic action was inhibited by prcatolol, a beta1-blocker, showing that it is due to stimulation of beta1-adrenoceptors. TA-064, dobutamine and isoproterenol increased heart rate dose-dependently. When compared with the positive inotropic action, the positive chronotropic action of TA-064 and dobutamine was significantly weaker than that of isoproterenol.

The fact that TA-064 produced the cardiovascular effect after its intraduodenal administration suggests that TA-064 is well absorbed from the digestive tract.

In the reserpinized dog, TA-064 also produced the positive inotropic and positive chronotropic actions, relative potencies to isoproterenol being almost identical with those in intact dogs. Therefore, the positive inotropic and positive chronotropic actions of TA-064 observed in intact dogs are not due to release of endogenous catecholamines. Moreover, the inotropic action of TA-064, being relatively selective, was observed also in the reserpinized dog.

As demonstrated, an intraarterial injection of TA-064 produced a very weak vasodilating effect on the femoral artery only at high doses, which corresponded to the intravenous doses; the relative potency of TA-064 was approximately 1/10000 compared with that of isoproterenol. After treatment with propranolol, the dose-response curve for TA-064 was shifted to the right by only 4 times, while that for isoproterenol was shifted by 300 times. These results suggest that TA-064 has a weak beta2-adrenoceptor agonistic action that contributes partly to the TA-064-induced vasodilation. In contrast to dobutamine, vasodilation induced by TA-064 was not potentiated by phentolamine, indicating that TA-064 has practically no alpha-agonistic action. Therefore, a slight increase in blood pressure observed after the intravenous administration of TA-064 is probably due to its positive inotropic action.

On the other hand, the intraarterial injection of dobutamine produced a decreased femoral blood flow that was intensified by propranolol, whereas the decrease in femoral blood flow was reversed by phentolamine, suggesting that dobutamine-induced vasodilation was mediated by beta2-adrenoceptors. In the presence of phentolamine,
the vasodilator effect of dobutamine was stronger than that of TA-064. Tuttle and Mills (1) also reported that the mild effect of dobutamine on blood pressure is ascribed to a balance between alpha- and beta2-agonistic effects.

It has been reported that the apparent inotropic selectivity of dobutamine in vivo is partly due to its peripheral vascular effect (12–14). In conscious instrumented dogs, TA-064 had a high inotropic selectivity (15, 16). In the present study, however, TA-064 showed a significant inotropic selectivity in the isolated preparation as well as in the anesthetized preparations, suggesting that the inotropic action of TA-064 is a direct one, although the autonomic nervous system may partly affect the inotropic selectivity in intact dogs.

As mentioned above, TA-064 has a selective beta1-agonistic action. Lands (17, 18) proposed that there are subtypes of beta-adrenoceptors, i.e., beta1- and beta2-adrenoceptors. This hypothesis has been supported by the introduction of many selective agonists and antagonists. It has long been recognized, however, that positive inotropic and positive chronotropic actions of catecholamines do not necessarily run parallel to each other (19). Carlsson (20) demonstrated the possibility that both beta1- and beta2-adrenoceptors are involved in chronotropic responses in the cat heart. In addition, many selective beta2-agonists have more pronounced effects on heart rate than on contractility of the heart (21–23). These results led to the hypothesis of a coexistence of beta1- and beta2-adrenoceptors in atrial tissue, including the sinus node (3, 21, 24–26). Therefore, it is possible that the inotropic selectivity of TA-064 results from its selective beta1-agonistic action. However, since TA-064 has no catechol moiety, its precise pharmacological action other than beta1-agonistic action must be clarified in various organs.

The present study showed that TA-064 is an orally active agent having inotropic action. The inotropic selectivity of TA-064 has also been observed in humans (27), which reflects the pharmacological properties of TA-064 shown in the present study. TA-064 may be useful as an orally and intravenously active, positive inotropic agent.

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