Pharmacological Profile of the 2-Alkynyladenosine Derivative 2-Octynyladenosine (YT-146) in the Cardiovascular System

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ABSTRACT — We investigated the cardiovascular effects of 2-octynyladenosine (YT-146), an adenosine A_2 agonist, in various mammalian preparations in comparison with adenosine and 2-chloroadenosine. YT-146, when intravenously administered, caused a dose-dependent decrease of blood pressure in anesthetized normotensive rats (with ED_{30} values of 0.4 μg/kg), and YT-146 was 250 times more potent than adenosine. Whereas adenosine and 2-chloroadenosine decreased heart rate at approximately equihypotensive doses, YT-146 had no negative chronotropic effects at hypotensive doses. Orally given YT-146 (0.1 – 1 mg/kg) produced a potent and long-lasting antihypertensive effect in spontaneously hypertensive rats. YT-146 was 15.9 and 12.5 times more potent than adenosine in producing relaxation of isolated porcine coronary arteries and in increasing dog coronary blood flow, respectively. Although YT-146 was equipotent to adenosine in causing a negative inotropic effect in isolated guinea pig atria, it was less potent than adenosine in producing atrioventricular conduction block in guinea pigs. On the other hand, 2-chloroadenosine was 9.1, 1.8 and 2.4 times more potent than adenosine in lowering blood pressure, relaxing isolated porcine coronary arteries and increasing dog coronary blood flow, respectively. 2-Chloroadenosine was the most potent in producing cardiodepression, i.e., negative inotropy and atrioventricular conduction block in guinea pigs. From these results, we concluded that YT-146 is a potent coronary vasodilator and also a potent, orally active and long-acting hypotensive agent having less cardiac depressant activity.

Adenosine receptors mediate a great variety of biological responses in non-excitable and excitable tissues (1). Two subtypes of adenosine receptors have been identified, i.e., A_1 and A_2 adenosine receptors, which mediate inhibition and stimulation of adenylate cyclase, respectively (2, 3). In the cardiovascular system, it has been established that the hypotensive action of adenosine occurs via two different mechanisms, i.e., A_2 receptor-mediated vasodilation (4–6) and A_1 receptor-mediated cardiac depression (7–9). Adenosine derivatives synthesized so far retain the two mechanisms of action of adenosine. The cardiodepressant action is by no means acceptable in the treatment of hypertension. Thus, adenosine derivatives with non-vasoselective properties developed so far were not suitable for use as antihypertensive drugs. In attempts to develop vasoselective or A_2 selective adeno-

sine derivatives, chemical modifications of adenosine in the N^6, 2 or 5'-position have already been documented (10–12). However, the clinical evaluation of their therapeutic potential has not been established. Recently, Matsuda et al. (13, 14) synthesized novel 2-alkynyladenosine derivatives as C_2-substituted adenosine derivatives, each of which has a substituent introduced by a carbon-carbon bond at the 2-position of adenosine. Previously, we reported that 2-alkynyladenosine derivatives were potent and selective A_2 adenosine receptor agonists (15).

In the present study, we investigated the cardiovascular effects of 2-alkynyladenosine derivatives, especially those of 2-octynyladenosine (YT-146), in several species of animals, and characterized YT-146 as not only a potent coronary vasodilator but also an orally active and long-acting antihypertensive agent.

MATERIALS AND METHODS

Isolated coronary artery preparations

Porcine hearts obtained from registered local slaughter houses were placed in ice-cold physiological salt solution and transported to our laboratory. The composition of the physiological salt solution was as follows: 125 mM NaCl, 4.7 mM KCl, 1.7 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$ and 10 mM glucose. Branch samples of the left anterior descending coronary artery were cut from both large (about 3 mm in outside diameter) and small (about 1 mm in outside diameter) segments of the same artery and were carefully cleaned of adhering tissues. These coronary arteries were cut into helical strips (large segments, 25 mm × 2 mm; small segments, 15 mm × 1 mm) and mounted in 10-ml organ baths containing oxygenated (95% O_2 + 5% CO_2) physiological salt solution at 32°C and equilibrated for 1 hr under resting tensions of 1 g for large segments and 0.3 g for small segments. Tonic contractions were obtained with 40 mM KCl. Afterwards, test compounds were added cumulatively to the organ baths to induce relaxation. Changes in

tension were measured through an FD pick-up (Nihon Kohden, TB-612T) and a carrier amplifier (Nihon Kohden, AP-621G). At the end of each series of experiments, papaverine (3×10^{-4} M) was added to attain the maximum relaxation; the papaverine-induced relaxation was taken as 100%.

Measurements of coronary blood flow

Mongrel dogs of either sex weighing 8 to 15 kg were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). The trachea was intubated, and the animals were artificially ventilated with room air. The chest was opened by an incision at the 5th left intercostal space, and the heart was suspended in the pericardial cradle. After heparinization (1000 units/kg, i.v.), the left anterior descending coronary artery was isolated from the surrounding tissues and cannulated antegradely with polyethylene tubing. The blood to be supplied to the myocardium was led from the left femoral artery via an extracorporeal circuit in which a cannulating-type flow probe of an electromagnetic blood flowmeter (Nihon Kohden, MFV-2100) was placed. Arterial blood pressure was recorded via polyethylene cannula inserted into the right carotid artery with a pressure transducer (Nihon Kohden, TP-200T). Heart rate was measured with a heart rate counter (Nihon Kohden, AT-601G) triggered by the pulse of arterial blood pressure. An intracoronary injection of test compounds was conducted through a rubber tube interposed in the extracorporeal circuit in a volume of 0.01 ml over a period of 3 sec. As the first of each series of experiments, reactive hyperemia was caused by reperfusion after occluding for 15 sec. Increases in coronary blood flow in response to the test compounds were expressed as percentages of the reactive hyperemia. The average value of reactive hyperemia of 16 dogs given test compounds was 51.5 ± 5.1 ml/min.

Measurements of arterial blood pressure

Direct measurements: Male normotensive Wistar-Kyoto rats, weighing 230 to 250 g (8 or

9 weeks-old) were used. Rats were fasted for 15 hr before experiments. A polyethylene catheter filled with heparinized saline was inserted into the right carotid artery under anesthesia with urethane (1.5 g/kg, i.p.). Arterial blood pressure was measured through the catheter connected with a pressure transducer (Nihon Kohden, TP-200T). Heart rate was recorded by a heart rate counter (Nihon Kohden, AT-601G) triggered by the pulse of arterial blood pressure. The test compounds were injected intravenously via a cannula inserted into the femoral vein.

Plethysmographical method: Male spontaneously hypertensive rats (SHR) weighing 300 to 400 g (15 to 20 weeks-old) showing a systolic blood pressure around 200 mmHg were used. Rats were fasted overnight before experiments. Systolic blood pressure and heart rate were measured by the tail cuff plethysmographical method under mild restraint using a programmable sphygmomanometer (Riken Kaihatsu, PS-100). Before blood pressure measurement, rats were placed individually in boxes warmed at $37-38°C$ for 15 min. Changes in systolic blood pressure were measured 0.5, 1, 2, 3, 5 and 7 hr after oral administration of test compounds which were suspended in 0.5% carboxymethylcellulose (CMC).

Isolated atrial preparations

In this study, isolated spontaneously beating atrial preparations were prepared according to Nakashima et al. (16). Male Hartley guinea pigs (Shizuoka Animal Center) weighing 300 to 400 g were killed, and their hearts were removed and placed in Locke-Ringer solution of the following composition: 154 mM NaCl, 5.6 mM KCl, 2.2 mM $CaCl_2$, 13.9 mM $NaHCO_3$ and 5.6 mM glucose. The isolated spontaneously beating atria were dissected from the hearts, and they were mounted in 30-ml organ baths containing oxygenated (95% O_2 + 5% CO_2) Locke-Ringer solution (32°C) under 0.5-g resting tension. The spontaneous contractions of the guinea pig atria were measured with FD pick-ups (Nihon Kohden, TB-612T) and car-

rier amplifiers (Nihon Kohden, AP-621G). Each preparation was used for only one test compound. Test compounds were added cumulatively to the bathing solution of 30 ml.

Measurements of atrioventricular conduction

Male Hartley guinea pigs (Shizuoka Animal Center) weighing 250 to 350 g were anesthetized with urethane (1.0 – 1.1 g/kg, i.p.). The anesthetized animals were prepared for measurements of PQ interval and atrioventricular conduction block (A-V block) from the limb lead II electrocardiogram (ECG) recorded at a chart speed of 25 mm/sec. Concomitantly with ECG, blood pressure and heart rate were also monitored. The test compounds were injected intravenously via a cannula inserted into the right jugular vein.

Preparations of washed platelets

Japanese white rabbits of either sex, weighing 2.2 to 3.0 kg, were anesthetized with sodium pentobarbital (30 mg/kg, i.m.). Blood was collected from the carotid artery with one-seventh volume of acid-citrate-dextrose. Platelet-rich plasma was prepared by centrifuging the blood at $140 \times g$ for 15 min at room temperature. Platelets were separated from the plasma and washed twice in wash solution, followed by centrifuging at $1,300 \times g$ for 7 min at room temperature. The composition of the first wash solution was as follows: 130 mM NaCl, 4.7 mM KCl, 4.0 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 11.5 mM dextrose, 10 mM HEPES, 0.2 mM EGTA and 0.1% bovine serum albumin, pH 6.5. The second wash solution (pH 7.35) was the same as the first one except it did not contain EGTA. Platelet pellets were finally suspended in the second wash solution containing 0.1% human fibrinogen; and then $CaCl_2$ and $MgCl_2$ were added to the final concentration of 1.8 and 1.2 mM, respectively. Aggregation studies were performed with 5.8×10^8 platelets/ml at 37°C. Platelet aggregation was measured by an aggregometer (NBS, PAT-2M) with stirring at a constant speed of 1,100 rpm. To 380 μl of the platelet suspension, 10 μl of either a test

compound or the solvent as the control was added. Three minutes later, 10 μl of a platelet aggregating substance (10 μM of adenosine 5'-diphosphate, sodium (ADP) or 1 μg/ml of collagen, as the final concentration) was added to induce aggregation.

Reagents

YT-146 was synthesized in our laboratory and the chemical structure is shown in Fig. 1. The sources of the other reagents were as follows: adenosine and 2-chloroadenosine (Sigma Chemical Company), ADP (P-L Biochemicals), collagen (Hormon-Chemie Munchen GMBH), urethane (Junsei Chemical), sodium pentobarbital (Shinnihonyakugyou) and isosorbide dinitrate (Toho Kasei Kogyo). All the other chemicals and solvents were purchased from Wako Pure Chemical Industries, Ltd. Adenosine and 2-chloroadenosine were dissolved in distilled water or saline. YT-146 was dissolved in dimethylsulfoxide (DMSO) or ethanol (Et-OH) as a stock solution, and appropriate dilution with distilled water or saline was carried out to prepare the solution for the following experiments: isolated coronary artery preparations, less than 0.19% Et-OH (final concentrations); isolated atrial preparations, less than 0.57% Et-OH (final concentrations); measurements of coronary blood flow, 0.3% DMSO-saline; direct measurements of arterial blood pressure, 0.1% DMSO-saline; measurements of A-V block, less than 3.4% DMSO-saline; preparations of washed platelets, 0.25% DMSO (final concentration). Isosorbide dinitrate was dissolved in

Fig. 1. Chemical structure of 2-octynyladenosine (YT-146).

distilled water. YT-146 was also suspended in 0.5% CMC for oral administration.

Statistical analysis

Experimental results were presented as the mean $\pm$ S.E., and the unpaired t-test was used to evaluate statistical significance of the difference. P-values of less than 0.05 were considered to indicate significant differences. As the doses or concentrations to produce a certain change in cardiovascular variables, the following values were chosen: the ED_{30} (μg /kg, i.v.) that decreased diastolic blood pressure in normotensive rats by 30% of its basal value, as an indicator of hypotensive action; the EC_{30} (M) that produced 30% relaxation in porcine small coronary arteries contracted by 40 mM KCl; the ED_{50} (μg/kg, i.a.) that produced an increase in coronary blood flow in dogs by 50% of reactive hyperemia caused by reperfusion after occlusion for 15 sec, as an indicator of the drug's effect on coronary blood flow; the IC_{30} (M) that decreased spontaneous contraction of guinea pig atria by 30% of its basal value, as an indicator of negative inotropic action; the IC_{50} (M) that produced 50% inhibition of ADP-induced platelets aggregation. The minimum effective dose (μg/kg, i.v.) that produced atrial arrest or second-degree A-V block in guinea pigs was also chosen as an indicator of the drug's abilility to case A-V block.

RESULTS

Effects on KCl-induced contraction in isolated coronary artery preparations

The inhibitory effects of YT-146, adenosine, 2-chloroadenosine and isosorbide dinitrate on the KCl-induced contractions of porcine coronary arteries are shown in Fig. 2. These test compounds produced a concentration-dependent relaxation. However, YT-146 showed a point of inflection in the concentration-response curve at the dose of 10 ^{6} M. YT-146, adenosine and 2-chloroadenosine caused greater relaxation of small vessels than large vessels. There were significant differences in the
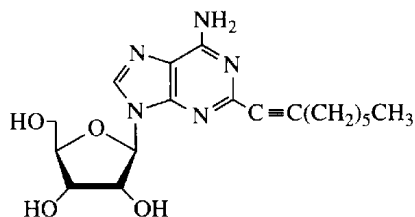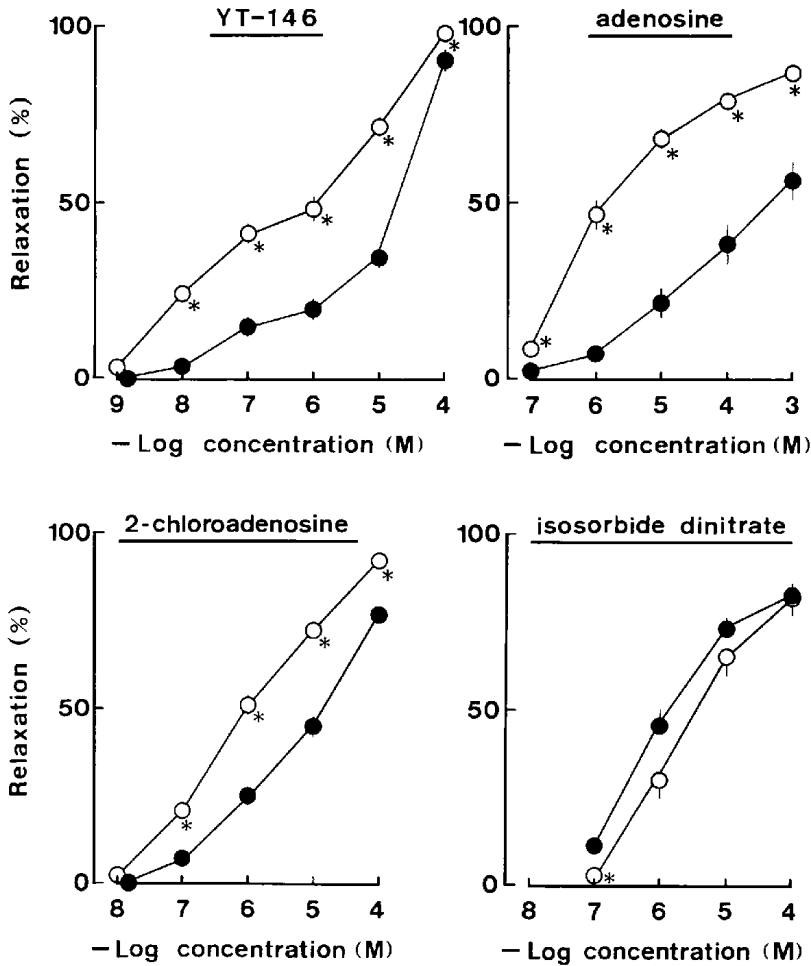
Fig. 2. Concentration-relaxant effect curves for YT-146, 2-chloroadenosine, adenosine and isosorbide dinitrate on KCl-induced contractions in porcine isolated small (○) and large (●) vessels. Each value represents the mean ± S.E. from 5 to 6 experiments. *P < 0.05 vs. corresponding values in large vessels. The maximum relaxation of papaverine (3 × 10^{-4} M) was taken as 100%.

concentration-response curves between small and large vessels in all test compounds. YT-146 was the most potent compound with EC$_{30}$ (M) values of 2.7 × 10^{-8} for relaxation in small vessels. 2-Chloroadenosine and adenosine were next to YT-146 in order of potency; they had EC$_{30}$ (M) values of 2.4 × 10^{-7} and 4.3 × 10^{-7}, respectively. On the other hand, isosorbide dinitrate caused greater relaxation of large vessels than small vessels.

Effects on coronary blood flow in anesthetized dogs

Intracoronary injection of YT-146 (0.001 – 0.3 μg/kg) and 2-chloroadenosine (0.01 – 1.0 μg/kg) produced a dose-dependent increase in coronary blood flow, which reached a peak within 30 sec and decayed thereafter (Fig. 3). The durations of action of YT-146 and 2-chloroadenosine were also dose-dependent. On the other hand, intracoronary injection of adenosine (0.003 – 3 μg/kg) caused an increase
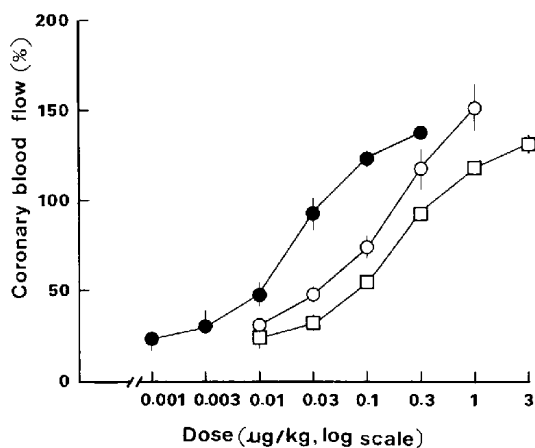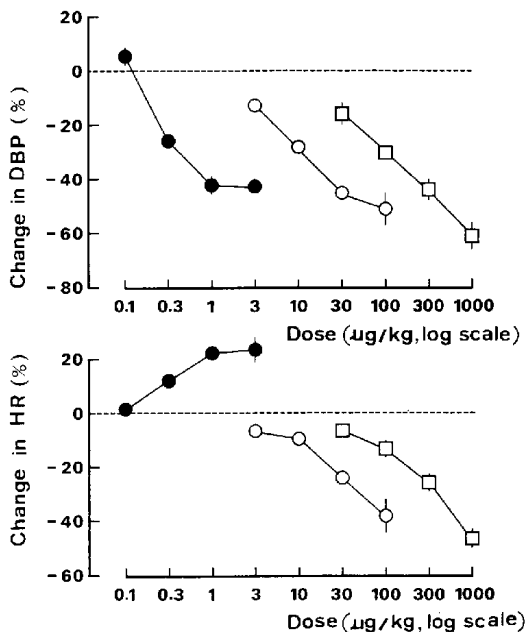
Fig. 3. Dose-response curves to YT-146 (●), 2-chloro-adenosine (○) and adenosine (□) for increase in coronary blood flow in anesthetized dogs. Test compounds were administered by the intracoronary route. At the beginning of each series of experiments, reactive hyperemia was caused by reperfusion after 15-sec occlusion; the blood flow responses to the three agents were expressed as percentages of reactive hyperemia. Each value represents the mean ± S.E. from 5 to 6 experiments.

Fig. 4. Effects of YT-146 (●), 2-chloroadenosine (○) and adenosine (□) on diastolic blood pressure (DBP) and heart rate (HR) in anesthetized normotensive rats. Test compounds were administered intravenously. Each value represents the mean ± S.E. from 5 to 6 experiments.

in coronary blood flow, which was more rapid in onset and much shorter in duration than those produced by YT-146 or 2-chloroadenosine. None of the compounds affected the systemic blood pressure or heart rate (not shown) by the intracoronary injection of the above doses. YT-146 was the most potent compound, and 2-chloroadenosine and adenosine were next to YT-146 in potency. The doses of YT-146, 2-chloroadenosine and adenosine required to induce an increase in coronary blood flow by 50% of the reactive hyperemia (ED_{50}) were estimated to be 0.008, 0.042 and 0.1 μg/kg, respectively.

Effects on systemic blood pressure

Direct measurement: The average values of the systolic and diastolic blood pressure of 17 normotensive rats anesthetized with urethane were 105 ± 5 and 58 ± 4 mmHg, respectively. Intravenous administration of YT-146 (0.1 – 3 μg/kg) caused a dose-dependent decrease in

diastolic blood pressure with a slight increase in heart rate (Fig. 4). The hypotensive action of YT-146 attained reached its peak within 2 min after administration. The duration of the action was also dose-dependent, being longer than 30 min with 3 μg/kg. On the other hand, adenosine (30 – 1000 μg/kg) transiently decreased systolic and diastolic blood pressure; the effect was more rapid in onset and much shorter in duration than that of YT-146. Adenosine decreased the heart rate in a dose-dependent manner. 2-Chloroadenosine (3 – 100 μg/kg) also decreased systemic blood pressure, and it decreased the heart rate in a dose-dependent manner (Fig. 4).

Plethysmographicalmeasurement: Effects of YT-146 on systolic blood pressure and heart rate in SHR are shown in Fig. 5. Single oral administration of YT-146 (0.1 – 1.0 mg/kg) produced a dose-dependent decrease in systo-
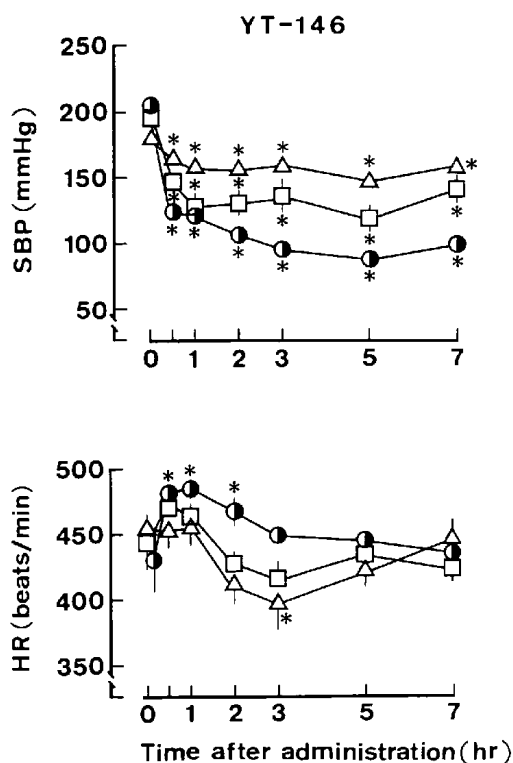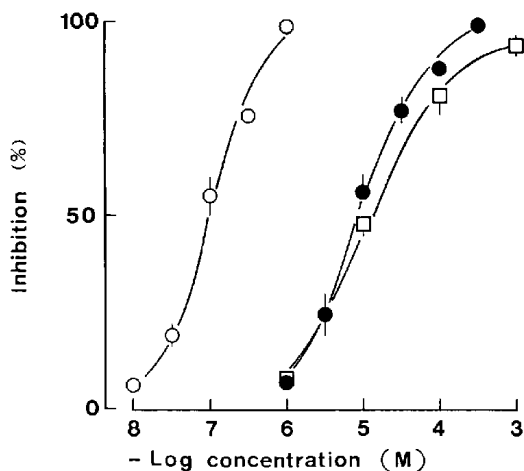
YT-146

Fig. 5. Effects of YT-146 on systolic blood pressure (SBP) and heart rate (HR) in conscious SHRs. Test compound was administered orally. Each value represents the mean ± S.E. of 5 experiments. *Statistically significant difference compared with the value obtained at 0 min (P < 0.05). △: 0.1 mg/kg, □: 0.3 mg/kg, ◑: 1.0 mg/kg.

Fig. 6. Concentration-negative inotropic effect curves for YT-146 (●), 2-chloroadenosine (○) and adenosine (□) in spontaneously beating atria of guinea pigs. Each value represents the mean ± S.E. of 6 experiments.

lic blood pressure. Peak effects were attained at 5 hr after administration in all doses. Approximately a 100 mmHg decrease in systolic blood pressure was obtained at a dose of 1.0 mg/kg of YT-146. Tachycardia developed at a dose of 1 mg/kg and was sustained for up to 2 hr after administration. A lower dose (0.1 mg/kg) of YT-146, however, showed a slight decrease in heart rate at the point of 3 hr after administration.

Effects on developed tension of spontaneously beating atrial muscle

YT-146, 2-chloroadenosine and adenosine

produced a negative inotropic effect in spontaneously beating atria of guinea pigs (Fig. 6). 2-Chloroadenosine was the most potent compound with IC_{30} (M) values of 5.1×10^{-8}. YT-146 and adenosine were next to 2-chloroadenosine in order of potency; they had IC_{30} (M) values of 3.9×10^{-6} and 5.8×10^{-6}, respectively. The action of these test compounds were reversible by washing the drugs from the organ baths.

Effects on atrioventricular conduction

The average values of mean blood pressure of 18 guinea pigs anesthetized with urethane was 59.2 ± 2 mmHg. Intravenous administration of 100 μg/kg adenosine caused a significant decrease in systemic blood pressure in guinea pigs. At that time, PQ-duration was slightly prolonged. However, adenosine at 300 μg/kg produced transient sinus bradycardia, prolongation of PQ-duration (values at just before the A-V block) and second-degree A-V block. On the other hand, intravenous administration of YT-146 at doses of 30 and 100 μg/kg caused a significant decrease in systemic blood pressure without A-V block. Only one of the six guinea pigs developed A-V

block after intravenous administration of YT-146 at 300 μg/kg. 2-Chloroadenosine was the most potent in producing A-V block; in all of six guinea pigs, severe A-V block occurred at the dose of 10 μg/kg (Fig. 7). When adenosine (300 μg/kg) and 2-chloroadenosine (10 μg /kg) were administered, all of six guinea pigs developed second-degree A-V block, respectively. Therefore, the blood pressure could not be measured. The suppressant effects of these three test compounds on A-V conduction developed in a few seconds and wore off within 1 to 2 min.

Effects on platelet aggregation

YT-146 and 2-chloroadenosine inhibited platelet aggregation induced by ADP or collagen in a concentration-dependent manner at concentration ranges of $3 \times 10^{-8} - 10^{-5}$ and $10^{-7} - 10^{-5}$ M, respectively. Adenosine also inhibited platelet aggregation at doses of $3 \times 10^{-7} - 10^{-4}$ M (Fig. 8). The IC_{50} (M) values

of YT-146, 2-chloroadenosine and adenosine for the inhibition of ADP-induced aggregation were 2.2×10^{-7}, 1.0×10^{-6} and 3.2×10^{-6}, respectively.

Selectivity for lowering blood pressure vs. cardiac depression

Comparison of cardiovascular variables of YT-146, adenosine and 2-chloroadenosine are presented in Table 1. In order to describe the profile of the cardiovascular actions of YT-146 in comparison with those of adenosine and 2-chloroadenosine, the relative potencies of YT-146 and 2-chloroadenosine to adenosine were determined by dividing the doses or concentrations of adenosine that produced a certain change in cardiovascular variables (described in the section of data analysis) by those of YT-146 or 2-chloroadenosine that produced a similar change in the same variables. The relative potencies of YT-146 or 2-chloroadenosine to adenosine thus determined are presented in
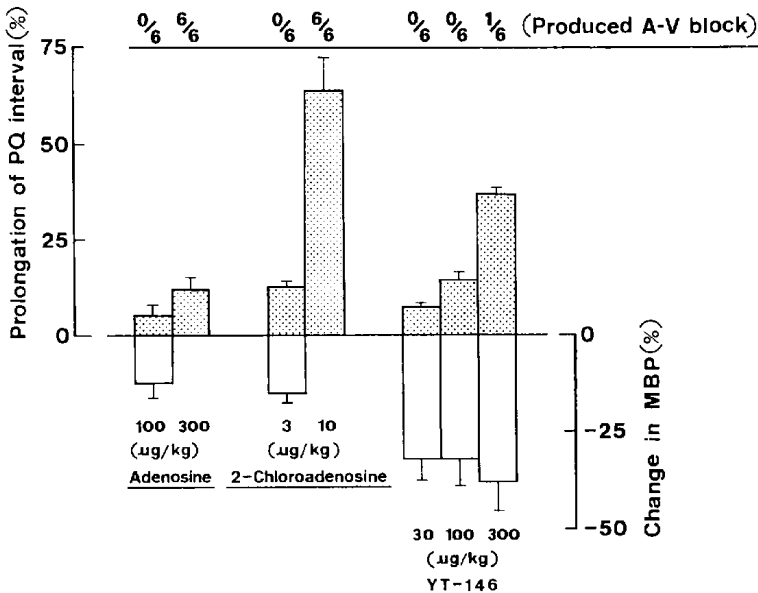
Fig. 7. Comparative effects of YT-146, 2-chloroadenosine and adenosine on PQ interval, incidence of A-V block and mean blood pressure (MBP) in anesthetized guinea pigs. Prolongation of PQ interval refers to the PQ interval just before occurrence of the A-V block and is expressed as a percentage of the control value. Incidence of A-V block was expressed as the number of animals in which A-V block occurred/number of animals examined. Test compounds were administered intravenously. Each value represents the mean ± S.E. from 5 to 6 experiments.

**ADP-induced aggregation**   **Collagen-induced aggregation**
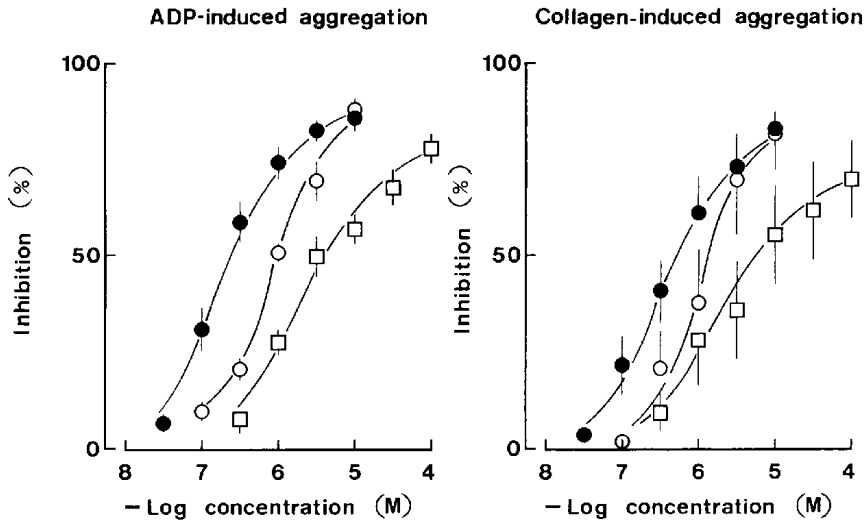
Fig. 8. Concentration-inhibitory effect curves for YT-146 (●), 2-chloroadenosine (○) and adenosine (□) on ADP- and collagen-induced aggregation in washed rabbit platelets. Each value represents the mean ± S.E. from 6 to 9 experiments.

Table 1. Comparison of cardiovascular variables of YT-146, adenosine and 2-chloroadenosine

Compounds and values Parameters	Adnosine Values	2-Chloroadenosine Values	YT-146 Values
Hypotensive action ED_{30} (μg/kg, i.v.)	100	11	0.4
Coronary relaxation EC_{30} (M)	4.3×10^{-7}	2.4×10^{-7}	2.7×10^{-8}
Increasing coronary blood flow ED_{50} (μg/kg, i.a.)	0.1	0.042	0.008
Negative inotropic action IC_{30} (M)	5.8×10^{-6}	5.1×10^{-8}	3.9×10^{-6}
Appearance of A-V block Minimal dose (μg/kg, i.v.)	300	10	300
Inhibition of platelet aggregation IC_{50} (M)	3.2×10^{-6}	1.0×10^{-6}	2.2×10^{-7}

Fig. 9 (figured as selectivity spectra on hexagonal plan in a logarithmic scale). YT-146 was the most potent in lowering blood pressure, causing coronary relaxation, increasing coronary blood flow and inhibiting platelet aggregation. However, YT-146 was only equipotent to adenosine in producing a negative inotropic action and A-V block. On the other hand, 2-chloroadenosine was more potent in cardiovascular actions than adenosine. Especially, 2-chloroadenosine was the most potent in causing cardiac depression, i.e., negative inotropy and A-V block.
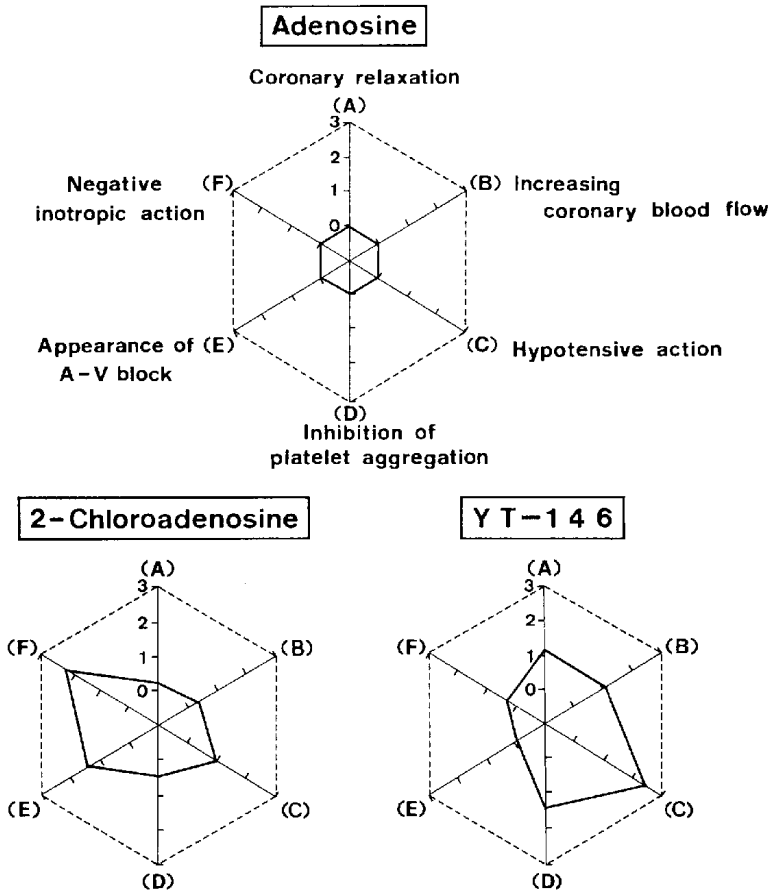
Fig. 9. Spectrum of selectivity of YT-146 for cardiovascular variables in comparison with those of adenosine and 2-chloroadenosine. Values for test compounds are adopted from Table 1. The potencies of adenosine in cardiovascular variables are taken as 1, and relative potencies are given in terms of the logarithm.

DISCUSSION

We investigated whether the 2-alkynyladenosine derivatives (13, 14), including YT-146, have a potent coronary dilator actions and orally active antihypertensive actions without cardiac depression. Adenosine receptors have been classified into A_1 and A_2 receptors (2, 3, 17). A number of adenosine agonists with high A_1 selectivity (3, 18) have been reported to preferentially elicit bradycardia, while there are only a few agonists that have been reported to have a potent and selective affinity for A_2 receptors (19–21). Although 5'-N-ethylcarboxamidoadenosine has generally

been used as a ligand for A_2 receptors in binding experiments, it has selectivity for neither A_1 or A_2 adenosine receptors. We previously assessed the affinities of 2-alkynyladenosine derivatives for A_1 and A_2 adenosine receptors in rat brain by measuring their ability to displace [^{3}H]N^6-cyclohexyladenosine and [^{3}H]5'-N-ethylcarboxamidoadenosine which are relatively selective for A_1 and A_2 adenosine receptors, respectively (15). In that study (15), we observed that 2-alkynyladenosine derivatives were potent and selective A_2 agonists.

In the present study, we investigated the effects of YT-146 on coronary blood flow whose increase is thought to be mediated by

A_2 receptors. YT-146 and 2-chloroadenosine were 12.5 and 2.4 times more potent in increasing coronary blood flow than adenosine, respectively. In addition, the durations of action of YT-146 and 2-chloroadenosine were dose-dependent. Moreover, in isolated porcine coronary arteries, YT-146 produced a concentration-dependent relaxation, and YT-146 was more potent than 2-chloroadenosine or adenosine. Interestingly, YT-146 showed a point of inflection in the concentration-response curve at the dose of 10^{-6} M. The mechanism through which this phenomena occurs has yet to be sufficiently clarified, and its elucidation largely depends upon future multilateral studies. In eliciting relaxation, all three compounds were more potent in small arteries than in large coronary arteries. On the other hand, isosorbide dinitrate caused greater relaxation in large vessels than in small ones. Winbury and his associates (22, 23) and Fam and McGregor (24) have explained differences in the antianginal action between nitrate and non-nitrate vasodilators based on the results obtained in *in situ* studies that nitrate vasodilators preferentially dilate large coronary arteries, whereas some non-nitrate vasodilators act preferentially on small coronary arteries. The results of our present study with adenosine and 2-chloroadenosine were in agreement with the results of previously reported in vitro studies of coronary vasodilators (25–27).

Intravenous administration of YT-146, 2-chloroadenosine and adenosine caused a dose-dependent decrease in diastolic blood pressure with the dose range of 0.1 to 1000 μg/kg. YT-146 was the most potent compound with ED_{30} values of 0.4 μg/kg, i.v. 2-Chloroadenosine and adenosine were next in order of potency with ED_{30} values of 11 and 100 μg/kg, i.v., respectively. The hypotensive effects of YT-146 was approximately 250 times more potent than adenosine. Whereas adenosine and 2-chloroadenosine decreased heart rate at doses that decreased blood pressure, YT-146 had no negative chronotropic effect at hypotensive doses. Instead, intravenous administration of YT-146 produced tachycardia, presumably as a

result of the reflex in response to the decrease in blood pressure in normotensive rats. YT-146 also showed a potent and long-lasting antihypertensive effect in SHR, when administered by the p.o. route. The tachycardia was observed at a high dose (1 mg/kg) of YT-146, and a slight decrease in heart rate was also observed at a low dose (0.1 mg/kg).

Recently, Belardinelli et al. (28) reported that the atrioventricular conduction delay and block caused by adenosine is solely due to a prolongation of the atria-to-His bundle (A-H) interval. 2-Chloroadenosine is well-known as an agent that potently produces not only coronary vasodilation but also negative inotropy (29). Adenosine is also known to inhibit the sinus rate and to cause A-V block in humans and guinea pigs (30). For that reason, the use of adenosine agonists as antihypertensive agents has been limited. Therefore, selective A_2 receptor agonists would be more advantageous as potential antihypertensive agents than non-selective adenosine agonists. Intravenous administration of adenosine at a dose of 100 μg/kg caused a slight and transient decrease in systemic blood pressure; and with a 3-fold increase in dosage (300 μg/kg), it produced a second-degree AV-block in all of six guinea pigs. 2-Chloroadenosine at the dose of 3 μg/kg, i.v. also produced a significantly greater decrease in blood pressure without A-V block, but its higher dosage (10 μg/kg, i.v.) produced a second-degree A-V block. On the other hand, YT-146 at doses of 30 and 100 μg/kg elicited a marked decrease in blood pressure without A-V block, although 300 μg/kg produced A-V block in one of the six guinea pigs.

From the spectrum of selectivity of YT-146 for vasodepressor vs. cardiac effects, it is expected that when administered intravenously in doses to produce lowering of the blood pressure, YT-146 will exert less cardiodepressant effects. In anesthetized guinea pigs, YT-146 in doses that produced more than 30% lowering of blood pressure did not cause A-V block. Moreover, in the normotensive rats, YT-146 at a dose that produced about 40%

lowering of diastolic blood pressure did not elicit a decrease in heart rate.

In conclusion, YT-146 is a potent coronary vasodilator and it has a potent and orally active long-lasting antihypertensive effect with less cardiac depressant activity.

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