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Similarity and Dissimilarity in Mode and Mechanism of Action between YT-146, a Selective Adenosine Receptor A_2 Agonist, and Adenosine in Isolated Canine Hearts

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YONEYAMA, F., AIHARA, K., KOGI, K., SATOH, K. and TAIRA, N. *Similarity and Dissimilarity in Mode and Mechanism of Action between YT-146, a Selective Adenosine Receptor A_2 Agonist, and Adenosine in Isolated Canine Hearts.* Tohoku J. Exp. Med., 1999, **188** (1), 31-45 —— To elucidate the differences in mode and mechanism of action between YT-146, a highly selective adenosine A_2 receptor agonist, and adenosine, we compared their effects on coronary circulation and myocardium and modifications of these effects by glibenclamide, a blocker of ATP-sensitive potassium (K) channels, in three kinds of isolated, blood-perfused canine heart preparations. YT-146 and adenosine were injected i.a. In all preparations both YT-146 and adenosine increased coronary blood flow and in this respect YT-146 was about 5 times as potent as adenosine. The increase in blood flow caused by adenosine was transient, whereas that produced by YT-146 was biphasic; the transient increase was followed by a sustained one. In isolated, blood-perfused sinoatrial (SA) node preparations, YT-146 failed to affect sinus rate, whereas adenosine reduced sinus rate by about 38% at its maximum effect. In isolated, blood-perfused atrioventricular (AV) node preparations, when injected into the artery supplying the AV node, YT-146 exerted no effect on AV conduction time, whereas adenosine prolonged AV conduction time by about 17% at the maximum effect. In isolated, blood-perfused papillary muscle preparations, the force of contraction was affected by neither YT-146 nor adenosine. In the same preparations the effect of YT-146 in increasing coronary blood flow was antagonized by glibenclamide in such a manner that the maximum increase was suppressed, but that of adenosine was not. Reactive hyperemia induced by ischemia for 30 seconds was not affected by glibenclamide. These results suggest that although both YT-146 and adenosine produce an increase in coronary blood flow via adenosine A_2 receptors, the opening of ATP- or glibenclamide-sensitive K channels is involved in the action of the former, but scarcely in the action of the latter. The opening of ATP- or glibenclamide-sensitive K-channels is less likely involved in reactive hyperemia. ———— adenosine A_2 receptor agonist; ATP-sensitive K-channel; coronary blood flow; YT-146

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YT-146 (2-[1-octynyl]-adenosine), a novel vasodilator, was characterized by a binding study as being highly selective for adenosine A_2 receptors (Abiru et al. 1990, 1991). In accordance with the results of the binding study, YT-146 injected i.v. into anesthetized spontaneously hypertensive rats (SHR) lowered blood pressure without affecting heart rate (Abiru et al. 1990, 1991). Oral administration of YT-146 also exerted a potent and long-lasting antihypertensive effect without negative chronotropic effect in conscious SHR (Abiru et al. 1990, 1991; Kogi et al. 1991). YT-146 was about 16 and 13 times more potent than adenosine in relaxing isolated porcine coronary arteries and in increasing dog coronary blood flow in anesthetized dogs, whereas YT-146 was nearly equipotent to adenosine in causing a negative inotropic effect in isolated guinea-pig right atria and was less potent than adenosine in producing atrioventricular (AV) conduction block in guinea pigs (Kogi et al. 1991). At present the vasodilator mechanism mediated via adenosine A_2 receptors is understood as involving the activation of adenylate cyclase (Collis and Brown 1983; Kusachi et al. 1983; Li and Fredholm 1985). This conceptual frame work dose not involve the opening of ATP-sensitive potassium (K) channels which have drown increasing attention as a target of not only K-channel openers but vasoactive biological substances like calcitonin gene-related peptide (Nelson et al. 1990).

In our previous study on spinally anesthetized dogs (Yoneyama et al. 1992) we found that the vasodepressor effect of YT-146 was reduced by not only theophylline, a non-selective adenosine receptor antagonist (Williams 1989) but glibenclamide, a blocker of ATP-sensitive K-channels (Sturgess et al. 1985; Schmid-Antomarchi et al. 1987a, b).

Based on these findings we suggested that the vasodilator mechanism of action of YT-146 mediated via adenosine A_2 receptors involves the opening of ATP- or glibenclamide-sensitive K-channels (Yoneyama et al. 1992). However, details of the antagonism by glibenclamide of YT-146 have remained to be elucidated, because the antagonism was observed with a single very high dose of glibenclamide which dogs only just survived. In view of these, in the present study we firstly investigated how far the coronary vasodilator effect (the effect in increasing coronary blood flow) and the cardiac effects of YT-146 are separated and secondly how the former effect is modified by glibenclamide using various kinds of isolated, blood-perfused canine heart preparations. As in the previous study (Yoneyama et al. 1992) we used adenosine as a reference drug.

Materials and Methods

Preparations

The heart preparations described below were obtained from mongrel dogs of either sex, weighing 7 to 13 kg, anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and given calcium heparin (500 U/kg, i.v.). All the preparations were placed in funnel-shaped glass water-jackets warmed at about 38°C and cross-circulated

with support dogs through the cannulated carotid artery. Cross-circulation at a constant pressure was achieved by the use of a peristaltic pump (model 1215, Harvard Apparatus, South Natick, MA, USA) and a pump-speed controller (SCS-22, Data Graph, Tokyo).

Sinoatrial node preparation. The sinoatrial (SA) node preparation (Kubota and Hashimoto 1973) was essentially the right atrium and perfused through the cannulated right coronary artery (RCA), with atrial blood at a constant pressure of about 100 mmHg. Sinus rate was measured with a cardiotachometer (1321, San-ei Instrument, Tokyo) triggered by bipolar electrograms obtained from the SA node area.

Atrioventricular node preparation. The atrioventricular (AV) node preparation (Hashimoto et al. 1972) consisted of the right atrium and ventricular septum. The preparation was perfused through cannulae placed in the RCA, the posterior septal artery (PSA), and the anterior septal artery (ASA), with arterial blood at a constant pressure of about 120 mmHg. The right atrium was paced with rectangular pulses of about twice the threshold voltage and 1 millisecond duration at a rate of 150 stimuli/minute through bipolar stimulating electrodes sutured on to the endocardium of the crista terminalis. Atrial bipolar electrograms were obtained from an area near the coronary sinus, and ventricular bipolar electrograms were obtained from an area between the AV ring and the anterior papillary muscle of the right ventricle. Both electrograms monitored on the screen of an oscilloscope (VC-9, Nihon Kohden, Tokyo) were fed to an AV interval meter (HN-110, Data Graph, Tokyo), which measured AV conduction time at a resolution of 1 millisecond.

Papillary muscle preparation. The papillary muscle preparation (Endoh and Hashimoto 1970) was essentially the anterior papillary muscle of the right ventricle taken together with the ventricular septum. The preparation was fixed to a plastic plaque with a hole to which the papillary muscle was fitted at the base. The preparation was perfused through the cannulated ASA with arterial blood at a constant pressure of about 100 mmHg. The papillary muscle was preloaded with a weight at which maximum tension developed. The papillary muscle was paced with rectangular pulses of about 1.5 times the threshold voltage and 5-millisecond duration at a rate of 120 stimuli/minute through bipolar electrodes placed at its base. Tension developed by the papillary muscle was measured isometrically with a strain-gauge transducer (45 196A, San-ei Instruments, Tokyo).

Blood flow through each nutrient artery was measured with an electromagnetic flowmeter (MFV-2000, Nihon Kohden, Tokyo). All measured variables were recorded on chart with a rectilinear pen-recorder (8S, San-ei Instrument, Tokyo).

Mongrel dogs of either sex, weighing 13 to 38 kg, were used as support dogs. They were anesthetized with sodium pentobarbital initially at a dose of 30 mg/kg

i.v. and later at supplemental doses when necessary. They were also given calcium heparin initially at a dose of 500 U/kg i.v., which was followed by doses of 100 U/kg i.v. at hourly intervals.

Drugs

YT-146 (Toa Eiyo, Fukushima) was dissolved in 10% ethanol at a concentration of 1 μmol/ml and diluted with 0.9% NaCl to the desired concentrations. Glibenclamide (Yamanouchi, Tokyo) was dissolved in 100% dimethylformamide (DMF) at a necessary concentration. Adenosine (Sigma, St. Louis, MO, USA) was dissolved in and diluted with 0.9% NaCl to the desired concentrations. YT-146 and adenosine solutions were injected into the nutrient artery in each preparation in a volume of 30 ml in 4 seconds or 100 ml (100 nmol of YT-146 and 1000 nmol of adenosine) in 10 seconds with a microsyringe. Drug doses were increased at a factor of about 3, and a drug injection was made after the effects of the previous one had worn off.

Experimental protocol for isolated, blood-perfused SA node preparations and AV node preparations

Six SA node preparations received both YT-146 and adenosine. The order of administration of the two drugs was randomized. Likewise six AV node preparations were given both YT-146 and adenosine. The order of their administration was also randomized.

Experimental protocol of investigation of antagonism between glibenclamide and YT-146 or adenosine in isolated, blood-perfused papillary muscle preparations

Six pairs of isolated, blood-perfused papillary muscle preparations and support dogs were used for YT-146 and another six pairs for adenosine. After an equilibration period control dose-response curves for changes in blood flow through the ASA and developed tension of the papillary muscle were determined for YT-146 or adenosine. Then, glibenclamide was given at a dose of 0.1 μmol/kg i.v., into a support dog. 15 minutes later determination of dose-response curves for the same drugs as in control was started. Similar experiments were repeated about 15 minutes after the dose of glibenclamide had been increased cumulatively to 0.3 μmol/kg i.v.; the second dose was 0.2 μmol/kg i.v. The effects of a further higher dose, a cumulative dose of 1 μmol/kg i.v., of glibenclamide were also investigated. Possible modification of reactive hyperemia by glibenclamide was also investigated in 6 of the 12 preparations in the following way. Reactive hyperemia was induced by 30-second occlusion of blood-conducting circuit to the ASA. Such a procedure was done before glibenclamide for control and about 15 minutes after glibenclamide at a cumulative dose of 1 μmol/kg i.v. to a support dog. Support dogs received about 300 ml of 5% glucose solution soon after they had been given 0.1, 0.3 or 1 μmol/kg i.v. of glibenclamide

so that possible hypoglycemia was avoided.

Statistical analysis

Basal values were expressed in terms of mean±S.E.M. All changes in blood flow and developed tension were expressed as absolute values. Differences in basal values were analyzed by the use of analysis of variance among preparations which received YT-146 and those which received adenosine under four conditions; before and after cumulative doses of glibenclamide at 0.1, 0.3 and 1 μmol/kg i.v. to support dogs. A p value smaller than 0.05 was considered to be significant.

Results

Effects on sinus rate and blood flow through the RCA

In six SA node preparations, the basal sinus rate was 97±4 beats/minute and the basal blood flow through the RCA was 4.2±1.3 ml/minute at a constant pressure of 100 mmHg. Single injections of adenosine (0.3–1000 nmol) into the RCA produced a dose-dependent decrease in sinus rate down to maximally 59 beats/minute; a decrease by 38 beats/minute (about 38% of the basal value). One of such experiments is shown in Fig. 1 and the dose-response curve for decrease in sinus rate is presented in Fig. 2. The dose of adenosine that produced a 15% decrease in sinus rate (nearly half-maximum decrease in sinus rate obtainable with most calcium antagonists [Taira 1987]) was 17.6 nmol (12.1–25.5 nmol). In contrast, single injections of YT-146 (0.03–30 nmol) into the RCA scarecely

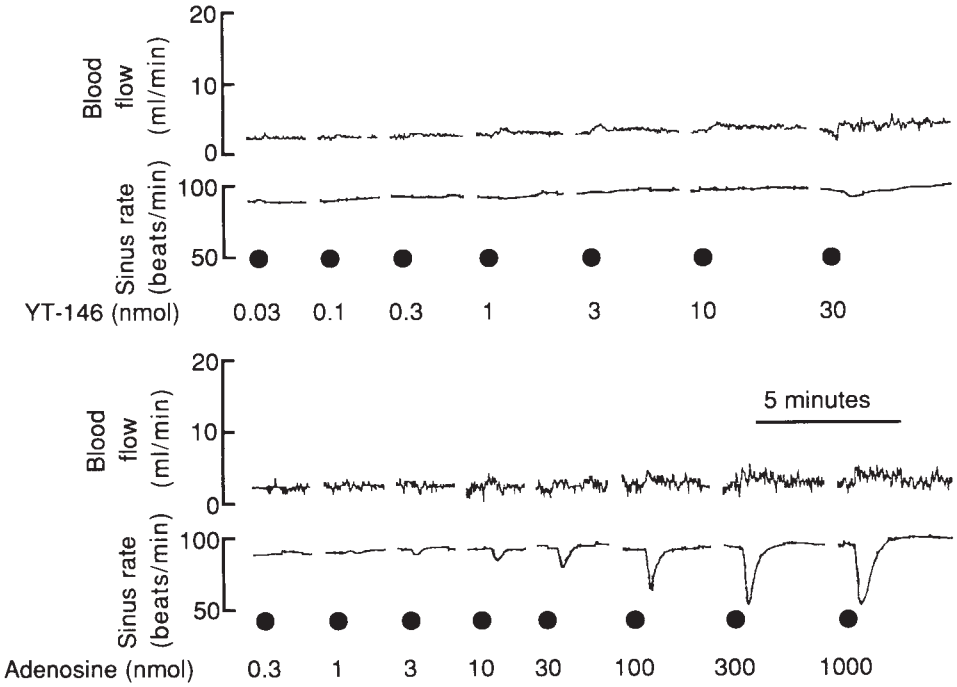

Fig. 1. Effects of YT-146 (upper panel) and adenosine (lower panel) on sinus rate in an isolated blood-perfused sinoatrial node preparation of the dog. YT-146 and adenosine were injected into the right coronary artery.

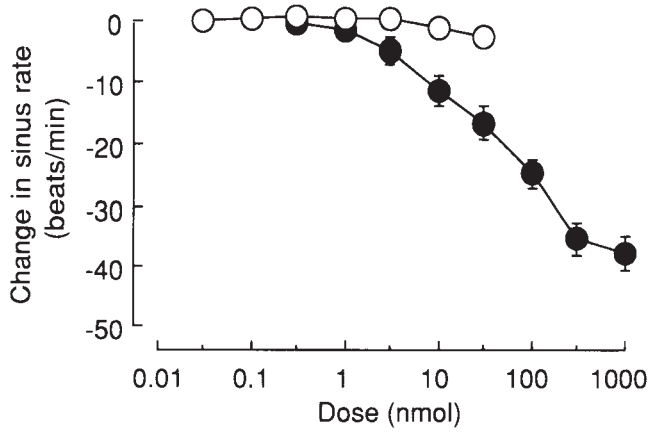

Fig. 2. Dose-response curves for change in sinus rate to YT-146 (○) and adenosine (●) injected into the right coronary artery of isolated, blood-perfused sinoatrial node preparations of the dog. Data points represent means ± S.E.M. of six preparations.

affected sinus rate (Figs. 1 and 2). Blood flow through the RCA was increased by YT-146 and adenosine.

Effects on AV nodal and intraventricular conduction and blood flow through the PSA and the ASA

In six AV node preparations, the basal AV conduction time was 126±8 milliseconds at a pacing rate of 150 stimuli/minute. In these preparations the basal blood flow through the PSA which supplies the AV node was 4.8±0.5 ml/minute and the corresponding value of blood flow through the ASA which supplies the His-Purkinje-ventricular system was 6.0±1.0 ml/minute at a constant perfusion pressure of 120 mmHg.

When adenosine (0.3–1000 nmol) was injected into the PSA, AV conduction time was increased in a dose-dependent manner maximally by about 22 milliseconds (about 17% of the basal value) at 1000 nmol. One of experiments is shown in Fig. 3 and the dose-response curve for increase in AV conduction time is presented in Fig. 4. The dose of adenosine that produced a 15% increase in AV conduction time (nearly half-maximum increase in AV conduction time obtainable with most calcium antagonists [Taira 1987]) was 639 nmol (204–1994 nmol). However, single injections of YT-146 into PSA failed to affect AV conduction time. With the doses examined of adenosine (0.3–1000 nmol) and YT-146 (0.03–30 nmol) blood flow through the PSA increased (Fig. 3). However, the increases in PSA flow produced by the two drugs were different in pattern; with YT-146 the transient increase in flow was followed by a sustained one but with adenosine PSA flow increased only transiently. The dose that produced a 100% increase in PSA flow was 2.8 nmol (1.4–5.6 nmol) for YT-146 and 12.0 nmol (4.9–29.6 nmol) for adenosine, respectively. Thus, YT-146 was about 4 times as potent as adenosine.

When YT-146 (0.03–30 nmol) was injected into the ASA, AV conduction time

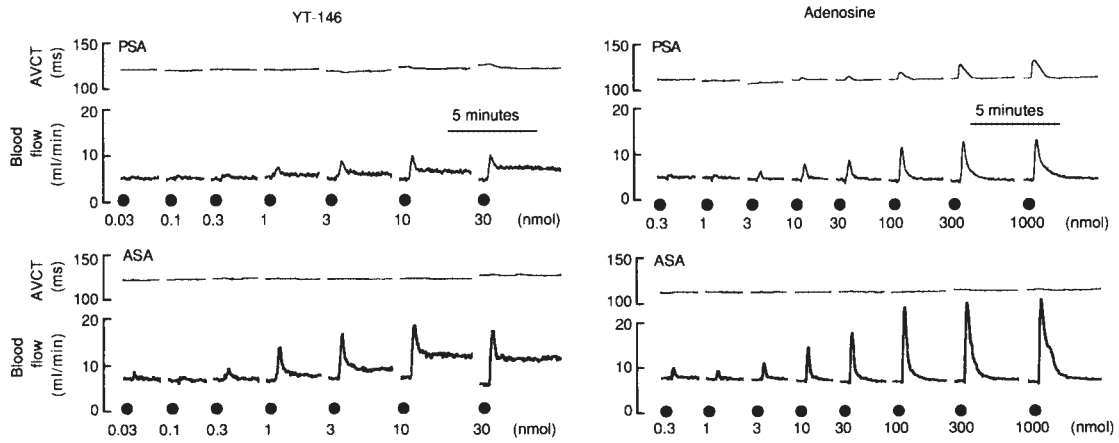

Fig. 3. Effects of YT-146 (left panels) and adenosine (right panels) injected into the posterior septal artery (PSA) (upper pair) and the anterior septal artery (ASA) (lower pair) on atrioventricular (AV) conduction time (AVCT) and blood flow through the respective arteries in an isolated, blood-perfused AV node preparation of the dog.

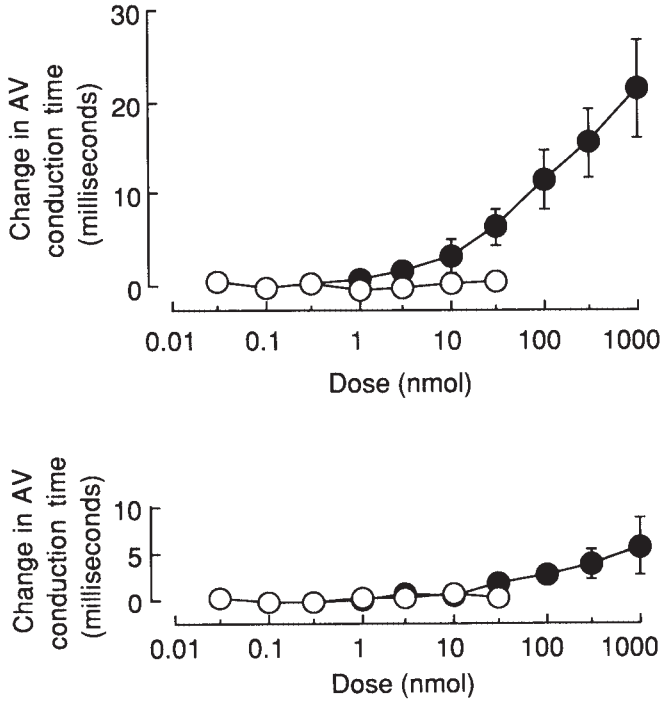

Fig. 4. Upper panel: dose-response curve for change in AV conduction time (AVCT) to YT-146 (○) and adenosine (●) injected into the PSA in the same isolated, blood-perfused AV node preparations of the dog. Data points represent means ± S.E.M. of six preparations. Lower panel: dose-response curve for increase in AV conduction time (AVCT) to YT-146 (○) and adenosine (●) injected into the ASA in the same six preparations that are presented in the upper panel. Otherwise, the same as in the upper panel.

also remained unaltered. However, adenosine (0.3–1000 nmol) injected into the ASA, was effective, although very slightly, in prolonging AV conduction time; by about 7 milliseconds at the maximum dose (1000 nmol) (Figs. 3 and 4).

Effects on blood flow through the ASA and developed tension of the papillary muscle and their modification by glibenclamide

Table 1 shows that basal values (obtained before the injection of YT-146 or adenosine) of blood flow through the ASA and developed tension of the papillary muscle in papillary muscle preparations paced at a rate of 120 stimuli/minute before (control) and after cumulative administrations of 0.1, 0.3 and 1 mmol/kg, i.v. of glibenclamide to support dogs. There were no significant differences in corresponding values of the two groups under the four conditions each.

Under control conditions single injections of YT-146 (0.03 to 100 nmol) into the ASA produced a dose-dependent increase in blood flow through this artery, but reduced developed tension only slightly at the highest dose. One of such experiments is shown in the top panel of Fig. 5 and the dose-response curves for increase in blood flow and change in developed tension are presented in Fig. 6. The dose of YT-146 that produced a 100% increase in ASA flow was 1.4 nmol (1.0–2.2 nmol). As was the case with PSA flow, the increase in ASA flow produced by YT-146 was biphasic; the initial transient increase was followed by a sustained one. After administration of glibenclamide 0.1, 0.3 and 1 μmol/kg, i.v. to support dogs, the effect of YT-146 in increasing blood flow was reduced in such a manner that the maximum increase was suppressed (Figs. 5 and 6). After 1 μmol/kg, i.v. glibenclamide the maximum increase became about one-third of the control value.

Under control conditions adenosine (0.3 to 1000 nmol) injected into the ASA produced a transient but dose-dependent increase in ASA flow, but exerted virtually no effect on developed tension. One of such experiments is shown in Fig. 7, and the dose-response curves for increase in ASA flow and change in developed tension are presented in Fig. 6. The dose of adenosine that produced a 100% increase in ASA flow was 8.2 nmol (3.8–17.7 nmol). Thus, adenosine was

TABLE 1. *Basal values of blood flow through the anterior septal artery and developed tension of the papillary muscle before (control) and after cumulative doses of glibenclamide given i.v. to support dogs*

	YT-146 ($n=6$)		Adenosine ($n=6$)	
	Blood flow (ml/min)	Developed tension (g)	Blood flow (ml/min)	Developed tension (g)
Control	6.1±0.6	5.7±0.7	7.2±1.2	4.1±0.4
Glibenclamide 0.1 μmol/kg	5.3±0.5	5.8±1.5	6.6±0.8	4.1±0.6
Glibenclamide 0.3 μmol/kg	5.3±0.4	5.4±2.0	5.4±0.5	3.8±0.6
Glibenclamide 1 μmol/kg	4.8±0.5	5.4±2.2	5.5±0.9	3.5±0.7

All values are expressed in terms of mean ± S.E.M.

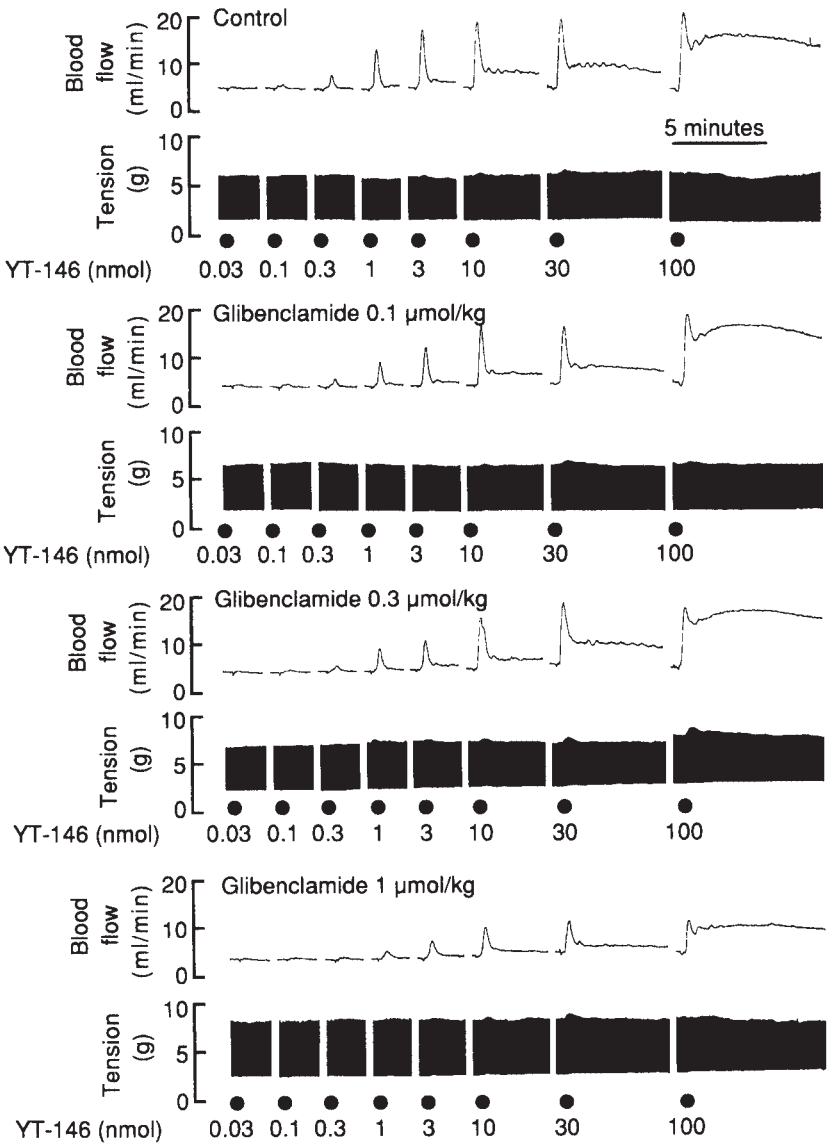

Fig. 5. Effects of YT-146 on blood flow through the anterior septal artery (upper traces of each panel) and developed tension (lower traces of each panel) of the papillary muscle of an isolated blood-perfused papillary muscle preparation of the dog, before (control; top panel) and after cumulative doses of 0.1 μmol/kg, i.v. (middle upper panel), 0.3 μmol/kg, i.v. (middle lower panel) and 1 μmol/kg, i.v. (bottom panel) of glibenclamide given to a support dog.

about 1/6 as potent as YT-146. After 0.1 and 0.3 μmol/kg, i.v. of glibenclamide the effect of adenosine in increasing blood flow was not affected (Figs. 6 and 7). After 1 μmol/kg, i.v. of glibenclamide the increase in blood flow was reduced slightly but not significantly.

Effects of glibenclamide on reactive hyperemia in the vascular bed of the ASA

Six of 12 papillary muscle preparations described in the preceding section underwent ischemia caused by 30-second occlusion of the ASA before and after glibenclamide (1 μmol/kg, i.v.). Reactive hyperemia in response to ischemia was not affected by glibenclamide (Fig. 8).

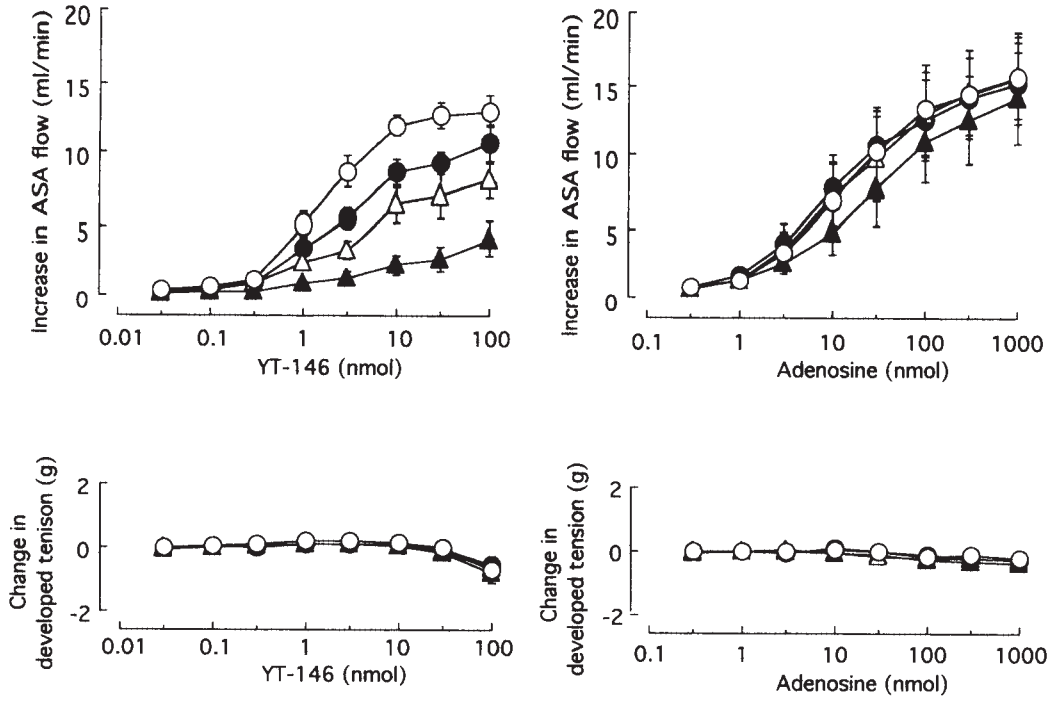

Fig. 6. Dose-response curves for increase in blood flow through the anterior septal artery (ASA) (upper panels) and change in developed tension of the papillary muscle (lower penels) produced by intra-arterial YT-146 (left panels) and adenosine (right panels) in isolated, blood-perfused papillary muscle preparations of dogs, before (control, ○) and after cumulative doses of 0.1 μmol/kg, i.v. (●), 0.3 μmol/kg, i.v. (△) and 1 μmol/kg, i.v. (▲) of glibenclamide given to support dogs. Data points represent means ± S.E.M. of six preparations for YT-146 and another six for adenosine.

Discussion

In isolated, blood-perfused SA node preparations, YT-146 scarcely affected sinus rate, whereas adenosine decreased sinus rate as in previous studies (Chiba 1974; Chiba and Himori 1975). In isolated, blood-perfused AV node preparations when injected into the artery supplying the AV node, adenosine increased AV conduction time as in a previous study (Narimatsu and Taira 1977), whereas YT-146 was entirely ineffective. In isolated, blood-perfused papillary muscle preparations both YT-146 and adenosine had virtually no effect on the force of contraction (developed tension) of the papillary muscle (ventricular myocardium). In all preparations YT-146 and adenosine increased coronary blood flow. It is known that adenosine exerts negative chronotropic (Evans et al. 1982) and dromotropic (Clemo and Belardinelli 1986) effects and a negative inotropic effect on atrial muscle (Evans et al. 1982) by stimulating adenosine A_1 receptors and vasodilator effects by stimulating adenosine A_2 receptors. However, adenosine exerts no direct negative inotropic effect on ventricular muscle (Drury and Szent-Gyorgyi 1929; Lammerant and Becsei 1973; Chiba and Himori 1975), although there exist adenosine A_1 receptors (Henrich et al. 1987). YT-146 has been characterized as being highly selective for adenosine A_2 receptors by a

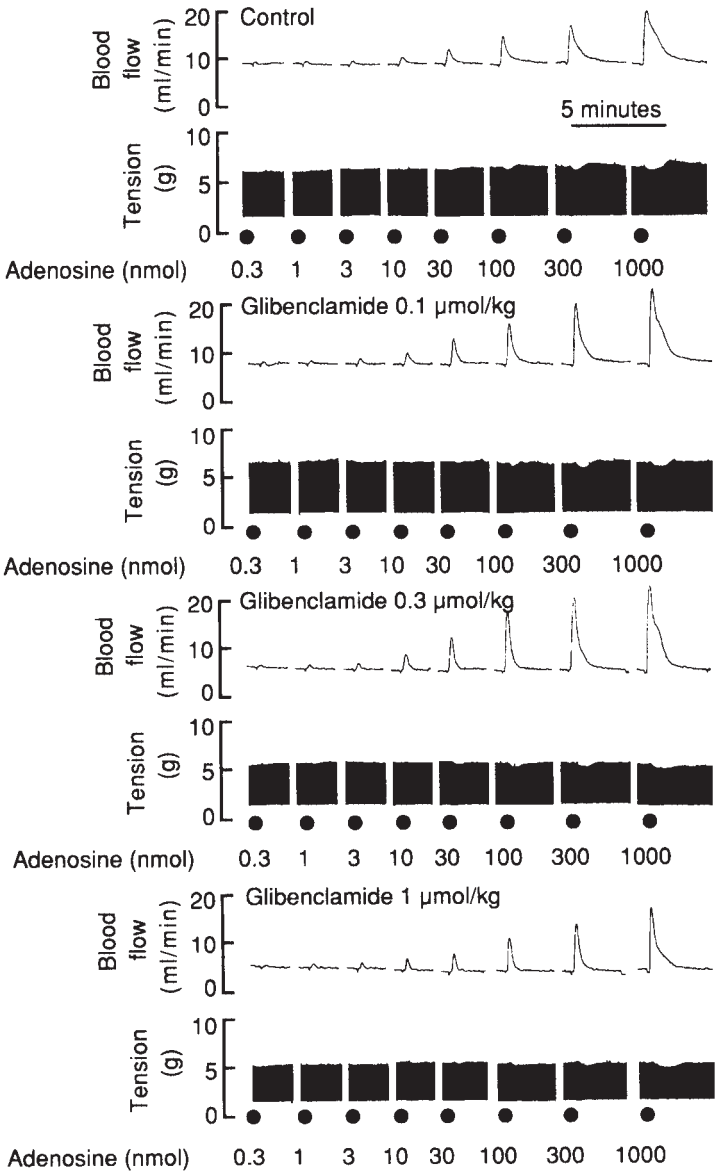

Fig. 7. Effects of adenosine on blood flow through the anterior septal artery (upper traces of each panel) and developed tension (lower traces of each panel) of the papillary muscle of an isolated blood-perfused papillary muscle preparation of the dog, before (control; top panel) and after cumulative doses of 0.1 μmol/kg, i.v. (middle upper panel), 0.3 μmol/kg, i.v. (middle lower panel) and 1 μmol/kg, i.v. (bottom panel) of glibenclamide given to a support dog.

binding study (Abiru et al. 1990, 1991). When these are taken together, the present results are readily explainable and consistent with previous results that YT-146 caused vasodepression without producing a nagative chronotropic effect in SHR (Abiru et al. 1990, 1991; Kogi et al. 1991).

The effect of YT-146 in increasing coronary blood flow was antagonized by glibenclamide administered to support dogs with arterial blood of which papillary muscle preparations were perfused. Thus, the present results are on the line of the previous ones that the vasodepressor effect of YT-146 was antagonized by glibenclamide in spinally anesthetized dogs whose blood pressure was maintained

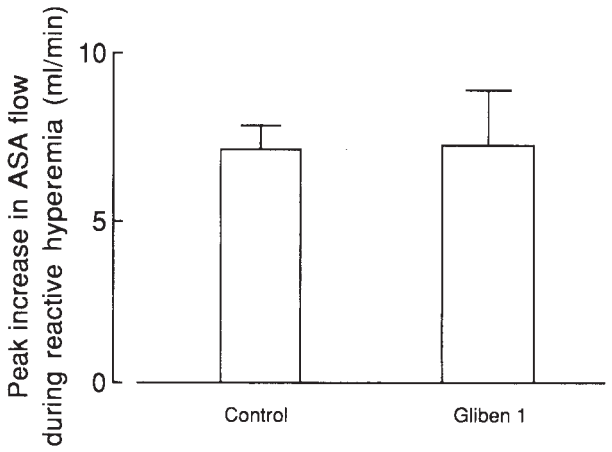

Fig. 8. Peak increase in blood flow through the anterior septal artery (ASA) during reactive hyperemia after 30-second interruption of ASA flow in isolated, blood-perfused papillary muscle preparations of dogs, before (control; left column) and after 1 μmol/kg, i.v. (Gliben 1, right column) of glibenclamide given to support dogs. Data columns represent means $\pm$ S.E.M. of six preparations.

being elevated by i.v. infusion of noradrenaline (Yoneyama et al. 1992). The two results, however, differed in details. In the present study the coronary vasodilator effect of YT-146 was more succeptible to the antagonizing effect of glibenclamide than the vasodepressor effect in the previous study (Yoneyama et al. 1992). Consequently in the present study three doses of glibenclamide were available. The antagonism by glibenclamide thus revealed was of insurmountable type; the maximum vasodilator effect of YT-146 was suppressed by increasing doses of glibenclamide, and after 1 μmol/kg, i.v. the maximum effect became nearly one-third of the control.

At present glibenclamide is understood as a blocker of ATP-sensitive potassium (K)-channels (Sturgess et al. 1985; Schmid-Antomarchi et al. 1987a, b). Thus, the antagonism by glibenclamide of the vasodilator effect of YT-146 suggests that the coronary vasodilatation produced by YT-146 involves the opening of ATP-sensitive K-channel in resistance coronary vessels. Here arises the question why there occurred no antagonism by glibenclamide of the coronary vasodilator effect of adenosine in the present study. If adenosine produces coronary vasodilatation by stimulation of adenosine A_2 receptors, the coronary vasodilator effect of adenosine should be antagonized by glibenclamide. However, in the previous study (Yoneyama et al. 1992), too, the vasodepressor effect of single bolus i.v. injection of adenosine was not antagonized by glibenclamide. In the previous article (Yoneyama et al. 1992), to explain the differential antagonism by glibenclamide of the vasodepressor effects of YT-146 and adenosine we have put forward a hypothesis as follows: Two mechanisms are operative in producing vasodilatation following stimulation of adenosine A_2 receptors; one is the activation of adenylate cyclase (Collis and Brown 1983; Kusachi et al. 1983; Li and Fredholm 1985) and the other is the opening ATP-sensitive K-channels, but the

latter mechanism comes into play slowly. As a result the activation of adenylate cyclase which is independent of the opening ATP-sensitive K-channels plays a central role in the transient vasodilator or vasodepressor effect of adenosine. Like the vasodepressor effect (Yoneyama et al. 1992), the coronary vasodilator effect of adenosine was transient, whereas that of YT-146 was long-lasting. Hence, the opening of ATP-sensitive K-channels likely plays a more dominant role in coronary vasodilatation produced by YT-146 than in that caused by a single bolus adenosine. Alternatively, there may be two subtypes of adenosine A_2 receptors; one is involved in the activation of adenylate cyclase and the other in the opening of ATP-sensitive K-channels.

In the present study reactive hyperemia in response to 30-second interruption of the blood supply to the ASA was not affected by the maximum dose (1 μmol/kg, i.v.) of glibenclamide. The present results are thus discrepant from the findings that coronary vasodilatation after 30-second ischemia in guinea-pig Langendorff hearts perfused with physiological salt solution was abolished by 2 μM glibenclamide (Daut et al. 1990). Several reasons for this discrepancy can be reckoned: Species difference, guinea pigs vs. dogs; difference in perfusate, physiological salt solution vs. blood. The nominal concentrations of glibenclamide used in the two studies were close; 2 μM vs. 1 μmol/kg. However, taking into consideration that sulforylureas bind to plasma proteins by 90–99% and their volume of distribution was about 0.2 liters/kg (Kahn and Shechter 1990), the effective concentration of glibenclamide in the present study was about 0.05 μM. This value is definitely lower than 2 μM and may be responsible for the discrepancy. Alternatively, the opening of ATP-sensitive K-channels in smooth muscle of coronary resistance vessels may not be responsible for reactive hyperemia. Until recently adenosine has been thought to be the major causative substance of reactive hyperemia (Rubio et al. 1969). If so, the resistance of reactive hyperemia to the antagonizing effect of glibenclamide seen in the present study is understandable.

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