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Comparative Profile of Vasodilation by CVT-3146, a Novel A$_{2A}$ Receptor Agonist, and Adenosine in Conscious Dogs

Gong Zhao, Axel Linke, Xiaobin Xu, Manuel Ochoa, Francis Belloni, Luiz Belardinelli, and Thomas H. Hintze

CV Therapeutics, Inc., Palo Alto, CA 94304, (G. Z. and L. B.) and Department of Physiology, New York Medical College, Valhalla, NY 10595 (A. L., X. X., M. O., F. B. and T. H.)

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Correspondence to: Gong Zhao, MD, PhD

Phone: (650)384-8673

Fax: (650)475-0392

E-mail: gong.zhao@cvt.com

Luiz Belardinelli, MD

Phone: (650)384-8526

Fax: (650)475-0450

E-mail: luiz.belardinelli@cvt.com

CV Therapeutics, Inc.,

3172 Porter Drive,

Palo Alto, CA 94304

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Abbreviations: LDCR: late diastolic coronary resistance, LVR: Lower body vascular resistance, MVR: Mesenteric vascular resistance, RVR: Renal vascular resistance, TPR: Total peripheral resistance

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Abstract

This study was to determine the magnitude of vasodilation by CVT-3146 in different vascular beds, and to compare it with that by adenosine in conscious dogs. Intravenous bolus injections of CVT-3146 (0.1 to 2.5µg/kg) or adenosine (10 to 250µg/kg) caused a dose-dependent increase in the coronary blood flow (CBF) and a dose-dependent decrease in the late diastolic coronary resistance (LDCR). Although the maximal increase in CBF response to the two drugs was not significantly different, the ED_{50} of CVT-3146 and adenosine were 0.45±0.07µg/kg and 47±7.77µg/kg, respectively. The highest dose of CVT-3146 caused a much longer coronary vasodilation than the highest dose of adenosine. There were no significant differences in increases in cardiac output induced by higher doses of CVT-3146 or adenosine. Most importantly, CVT-3146 resulted in a smaller decrease in total peripheral resistance (TPR) compared to that seen with adenosine. In addition, CVT-3146 yielded a smaller increase in the lower body flow (LBF) than adenosine. Adenosine also caused dose-dependent renal vasoconstriction, whereas CVT-3146 did not affect the renal blood flow. The administration of CVT-3146 or adenosine caused a dose-dependent vasodilation in the mesentery, which was not significantly different from each other. In summary, CVT-3146 is a 100-fold more potent coronary vasodilator than adenosine. CVT-3146 causes smaller decreases in TPR and smaller increases in LBF than those induced by adenosine, indicating that it is more selective for coronary than peripheral vasodilation. Furthermore, CVT-3146 did not cause renal vasoconstriction. These features make CVT-3146 a better candidate for pharmacologic stress testing.

Exercise is a common method of physiologic stress testing and is widely used in the diagnosis of coronary artery disease with radionuclide agents. However, for those who are unable to exercise adequately, an alternative procedure is needed. Pharmacologic stress testing with radionuclide agents is an option, which has been used for more than 20 years in the diagnosis of coronary artery disease. Two kinds of agents—coronary vasodilators (adenosine and dipyridamole) and ß-adrenergic receptor agonists (dobutamine)—are used in pharmacologic stress testing. These agents exert their actions through different mechanisms. Dobutamine increases contractility and heart rate by stimulating ß-adrenergic receptors in the heart, thereby resulting in a significant increase in CBF. Adenosine increases CBF via direct vasodilation, and dipyridamole increases the circulating concentration of adenosine by blocking its reuptake and metabolism (Mahmarian and Verani, 1994; McGuinness and Talbert, 1994; Allison et al., 1996; Leppo, 1996; Marwick, 1997; Cerqueira, 2000).

Four types of adenosine receptors (AdoR) —A_1, A_{2A}, A_{2B}, and A_3—have been identified. It has been well established that the chronotropic, dromotropic, inotropic (in atria), and anti-ß-adrenergic effects of adenosine are mediated by A_1 AdoR (Belardinelli, 1995; Shryock and Belardinelli, 1997). The vasodilator effect of adenosine is thought to be mediated primarily, but not exclusively, by A_{2A} AdoR. However, other types of AdoR (e.g. A_{2B} AdoR) appear to play a significant role in the vasodilation induced by adenosine (McGuinness and Talbert, 1994; Morrison et al., 2002; Talukder et al., 2002).

The coronary vasodilator effect of adenosine is the basis for the use of this nucleoside with radionuclide imaging in the heart to detect underperfused areas of myocardium. However, pharmacologic stress induced by adenosine is associated with a high prevalence of side effects, including dyspnea, chest pain, and atrioventricular nodal block (Mahmarian and Verani, 1994; McGuinness and Talbert, 1994; Marwick, 1997; Cerqueira, 2000). In addition, the hypotension

resulting from the adenosine-induced vasodilation in the peripheral circulation is another undesirable effect. Overall, these side effects limit the usefulness of adenosine in pharmacologic stress testing. Because a number of side effects induced by adenosine appear to be mediated by AdoR types other than the A_{2A} receptor, it is likely that selective agonists of A_{2A} receptors may be more vascular specific and cause fewer undesirable effects than adenosine.

The ultra-short duration of coronary vasodilation by adenosine also makes it a less-than-an-ideal-agent for pharmacologic stress testing, as it is inconvenient to infuse adenosine intravenously for 4-6 min during pharmacologic stress testing. Several potent, high-affinity, and selective A_{2A} AdoR agonists have been developed (Alberti, 1997; Belardinelli et al., 1998; Glover et al., 1996; Shryock et al., 1998). However, the clinical use of these compounds may be limited because of their high affinity for the receptor, which yields an excessively prolonged duration of action and may cause significant hypotension. A novel, selective, moderate-affinity, A_{2A} AdoR agonist—the 2-(N-pyrazolyl) adenosine derivative, CVT-3146—has been developed (Gao et al., 2001a). Our previous results have indicated that CVT-3146 causes a longer and more potent coronary vasodilation in conscious dogs than adenosine, but is not associated with the magnitude of hypotension seen with adenosine (Trochu et al., 2003).

The aims of this study were to compare the vasodilator effects of CVT-3146 and adenosine on coronary and peripheral circulations and their effects on cardiac output in conscious dogs.

METHODS

The experiments were performed in 13 chronically instrumented conscious dogs (weighing from 21 to 33 kg, either sex). The protocols were approved by the Institutional Animal Care and Use Committee of New York Medical College and conform to the "Guiding Principles for the Use and Care of Laboratory Animals," from the National Institutes of Health

Surgical Procedures

The dogs were sedated with Acepromazine (0.3mg/kg, im) and anesthetized with pentobarbital sodium (25 mg/kg, iv).

Effects of CVT-3146 or Adenosine on the Coronary Blood Flow and the Cardiac Output (n=7): The chest was scrubbed for sterile surgery and a thoracotomy was made in the fifth intercostal space under artificial ventilation. A Tygon catheter (Cardiovascular Instruments, Wakefield, MA) was inserted into the descending thoracic aorta for the measurement of blood pressure. A Doppler ultrasonic flow transducer (Craig Hartley, Houston, TX) was placed around the left circumflex coronary artery for the measurement of CBF. An electromagnetic flow transducer (45 or 50 mm, Carolina Medical Electronic Inc., King, NC) was placed around the aorta for the measurement of aortic blood flow as cardiac output (CO).. The chest was closed in layers. The catheter and wires were tunneled subcutaneously and exited the skin at the back of the dog's neck.

Effects of CVT-3146 or Adenosine on the Blood Flow in the Lower Body, the Mesentery, and the Kidney (n=6): The dogs were fasted for at least 24 hours prior to the surgery. An endotracheal tube was inserted into the trachea, and the surgery was carried out under spontaneous ventilation. A midline laparotomy was made using sterile surgical techniques. A

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catheter was inserted into the distal abdominal aorta for the measurement of blood pressure, and

an electromagnetic flow transducer (25 mm, Carolina Medical Electronic Inc., King, NC) was

placed around the abdominal aorta just above the iliac bifurcation for the measurement of LBF.

Doppler ultrasonic flow transducers were placed around the superior mesenteric artery and left

renal artery for measurements of the mesenteric blood flow (MBF) and renal blood flow (RBF),

respectively. The abdomen was closed in layers. The catheter and wires were run

subcutaneously and exited from the back of the dog's neck. The dogs were allowed to recover

from the surgery for 10-14 days and were trained to lie on the laboratory table quietly.

Recording From Chronically Instrumented Dogs

Arterial pressure was measured by connecting the previously implanted catheter to a strain-gauge

transducer (Statham P23 ID, Newark, NJ), and mean arterial pressure (MAP) was derived using

a 2Hz low-pass filter. Heart rate was monitored from the pressure pulse interval using a

cardiotachometer (Beckeman Instruments, Newark, NJ).

Effects of CVT-3146 or Adenosine on the Coronary Blood Flow and the Cardiac Output:

The coronary flow velocity was measured from the ultrasonic flow transducer using a pulsed

Doppler flow meter (System 6, Triton Technology, San Diego, CA). The mean coronary flow

velocity was derived using a 2Hz low-pass filter. CBF (ml/min) was calculated using the

formula provided by the vendor. CO (ml/min) was measured from the electromagnetic flow

transducer using an electromagnetic flow meter (Carolina Medical Electronic Inc., King, NC).

Late diastolic coronary resistance (LDCR) and total peripheral resistance (TPR) were calculated

as diastolic arterial pressure divided by late diastolic coronary blood flow and as MAP divided

by CO, respectively. Mean and phasic signals were recorded at the same time. And a faster speed

(25mm/sec) was run to allow to calculate LDCR if needed. LDCR was chosen as the index of

coronary vascular resistance since it is independent of the compressive effect of ventricular contraction on coronary microvessels and was calculated as the quotient of late diastolic arterial blood pressure and CBF (Liang and Stone, 1982; Hintze and Vatner, 1984).

Effects of CVT-3146 or Adenosine on the Blood Flow in the Lower Body, the Mesentery, and the Kidney: LBF was measured from the electromagnetic flow transducer using an electromagnetic flow meter (Carolina Medical Electronic Inc., King, NC). The mesenteric and renal flow velocity was measured from the flow transducers using a pulsed Doppler flow meter (System 6, Triton Technology, San Diego, CA). Mean blood flow velocities were derived using a 2Hz low-pass filter. MBF and RBF were calculated using the formula provided by the vendor. Mean resistances in the vascular beds were calculated as MAP divided by the mean blood flow.

Experimental Protocols

On the day of the experiment, the dog was brought to the laboratory and put on an experimental table. The previously implanted devices were attached to the recording equipment. A catheter was inserted into a peripheral vein on the leg and attached to an infusion line so that the drugs could be administered without disturbing the dog. The experiment began after the baseline hemodynamics and blood flow were stable.

Effects of CVT-3146 or Adenosine on the Coronary Blood Flow and the Cardiac Output (n=7): A dose-response curve of CBF or CO to CVT-3146 at doses of 0.1, 0.25, 0.5, 1.0, and 2.5μg/kg was obtained following intravenous bolus injections. The dose-response curve to adenosine at doses of 10, 25, 50, 100, and 250μg/kg was obtained as well. Hemodynamics and CBF were allowed to return to the baseline before the next dose was administered. The interval between each dose was 5 to 15 min, depending on the duration of action of the agents.

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Effects of CVT-3146 or Adenosine on the Blood Flow in the Lower Body, the Mesentery and the Kidney (n=6): A dose-response curve of LBF, MBF, or RBF to CVT-3146 at doses of 0.1, 0.25, 0.5, 1.0, and 2.5µg/kg was obtained following intravenous bolus injections. The dose-response curve to adenosine (10, 25, 50, 100, and 250µg/kg) was obtained following intravenous bolus injections as well.

Materials

Adenosine was purchased from Sigma Chemical Company (St. Louis, MO), and CVT-3146 was synthesized by CV Therapeutics, Inc., (Palo Alto, CA).

Data Analysis

All data are presented as mean±SEM. The statistical significance of differences was determined using a paired t-test for the response to each injection of the drugs. The significant differences in the responses between CVT-3146 and adenosine were determined using an one-way ANOVA followed by Tukey's Test. Significant changes were considered to be P<0.05. A computer-based software package (SigamaStat 2.03) was used for statistical analysis.

RESULTS

Effects of CVT-3146 or Adenosine on the Coronary Blood Flow and the Cardiac Output

Intravenous injections of CVT-3146 or adenosine resulted in a dose-dependent increase in CBF and a small increase in CO as shown in Figure 1. Following injections of CVT-3146 at doses of 0.1, 0.25, 0.5, 1.0, and 2.5µg/kg, CBF increased by 35±6, 80±12, 151±22, 173±12, and 205±23%, respectively, from 40±4 ml/min (all P<0.05). A significant increase in CBF was also observed following injections of adenosine at doses of 10, 25, 50, 100, and 250µg/kg (58±13, 94±19, 128±7, 158±11, and 163±16%, respectively, all P<0.05) from 41±5ml/min. The maximal CBF to CVT-3146 or adenosine was not significantly different (Figure 1), whereas the ED_{50} values (potency) of CVT-3146 and adenosine necessary to increase CBF were significantly different (0.45±0.07µg/kg for CVT-3146 vs. 47±7.77µg/kg for adenosine, P<0.05).

The second difference in CBF response to CVT-3146 and adenosine was in the duration of coronary vasodilation. As shown in Figure 2, the duration of coronary vasodilation with adenosine (250µg/kg) was markedly shorter than that induced by CVT-3146 (2.5µg/kg). There was an increase in CBF following the injection of adenosine at a dose of 250µg/kg, but CBF returned to the baseline within 1 min. Following the injection of CVT-3146 at a dose of 2.5µg/kg, CBF remained at 2-fold above the baseline for more than 2 min. The duration of CBF 2-fold above the baseline for CVT-3146 (2.5µg/kg) and adenosine (250µg/kg) was 130±19 sec. and 16±3 sec. (P<0.05), respectively. There were dose-dependent decreases in LDCR following injection of CVT-3146 or adenosine (Figure 1), and these were not significantly different from each other (P>0.05).

CVT-3146 and adenosine caused significant increases in CO. CVT-3146 at doses of 0.1 and 0.25µg/kg resulted in a smaller increase in CO when compared to adenosine at doses of 10

and 25μg/kg (2.2±0.96 vs. 13±1% and 5.0±1.4 vs. 16±3%, both P<0.05). There was no

significant difference in the increase in CO induced by higher doses of CVT-3146 or adenosine

(P>0.05). Most importantly, CVT-3146 resulted in a markedly smaller decrease in TPR

compared to that induced by adenosine, even though CVT-3146 and adenosine caused a similar

peak increase in CBF and a comparable decrease in LDCR. Figure 3 shows the ratio between the

decrease in LDCR and the decrease in TPR for CVT-3146 and adenosine (0.5 vs. 50μg/kg and

1.0 vs. 100μg/kg, respectively, both P<0.05). The ratio of LDCR:TPR for CVT-3146 was

significantly greater than that for adenosine.

**Effects of CVT-3146 or Adenosine on the Blood Flow in the Lower Body, the Mesentery
and the Kidney**

Following injections of adenosine at doses of 10, 25, 50, 100, and 250μg/kg, LBF increased by

26±7, 45±14, 51±9, 73±17, and 74±11%, respectively, from 0.68±0.05 L/min (all P<0.05); LVR

decreased by 22±3, 30±6, 36±4, 42±6, and 46±5%, respectively, from 165±12 mmHg/L/min.

CVT-3146 at doses of 0.1 and 0.25μg/kg did not affect LBF (1.0±1.0 and 5.2±1.9%,

respectively, both P>0.05) and LVR (-1.8±1.0 and –6.2±3.1%, respectively, both P>0.05). The

administration of CVT-3146 at doses of 0.5, 1.0, and 2.5μg/kg increased LBF by 17±3, 21±2,

and 33±6% (P<0.05), respectively, from 0.70±0.05L/min, and LVR decreased by 14±2, 20±3,

and 22±4% (all P<0.05), respectively, from 158±13mmHg/L/min. The vasodilation by CVT-

3146 in the lower body was significantly smaller than that induced by adenosine (Figures 4 and

5).

As shown in Figures 4 and 5, the administration of CVT-3146 or adenosine caused

significant increases in MBF and a decrease in mesenteric vascular resistance (MVR). In the

mesentery, there was no significant difference in vasodilation induced by CVT-3146 or

adenosine. MBF increased by 18±4, 28±8, 48±8, 69±6, and 88±14% from 216±31mL/min (all

P<0.05) following respective injections of CVT-3146 at doses of 0.1, 0.25, 0.5, 1.0, and

2.5µg/kg; MVR decreased by 15±3, 18±5, 32±4, 40±3, and 48±3%, respectively, from

0.58±0.10mmHg/mL/min (all P<0.05). Adenosine at doses of 10, 25, 50, 100, and 250µg/kg

increased MBF by 36±8, 46±10, 66±9, 72±10, and 84±5%, respectively, from 211±26ml/min (all

P<0.05); MVR decreased by 26±5, 29±5, 41±4, 43±5, and 49±2%, respectively, from

0.57±0.09mmHg/mL/min (all P<0.05) following injections of adenosine. There were no

significant differences in the increase in MBF or the decrease in MVR in response to CVT-3146

administration as compared to adenosine.

As shown in Figure 6, adenosine resulted in a dose-dependent decrease in RBF and a

dose-dependent increase in RVR. Adenosine at doses of 10, 25, 50, 100, and 250µg/kg

decreased RBF by 46±7, 54±5, 71±6, 80±5, and 85±4%, respectively, from 246±27ml/min (all

P<0.05) and increased RVR by 109±30, 125±26, 309±80, 545±174, and 683±197%,

respectively, from 0.49±0.09 mmHg/mL/min (all P<0.05). In contrast, CVT-3146 had no effect

on RBF or RVR (Figure 6). At the highest dose of CVT-3146 (2.5µg/kg), a small but significant

decrease in RBF (11±4%, P<0.05 compared to the baseline) was observed.

Effects of CVT-3146 or Adenosine on Blood Pressure and Heart Rate

Table 1 shows changes in MAP and HR in response to CVT-3146 and adenosine in all 13 dogs.

There was a dose-dependent decrease in MAP following injections of adenosine. After

injections of adenosine at doses of 10, 25, 50, 100, and 250µg/kg, MAP decreased by 9±2, 12±2,

18±3, 21±3, and 35±5 mmHg, respectively, from 103±3mmHg (all P<0.05). CVT-3146 at doses

of 0.1, 0.25, 0.5, 1.0, and 2.5 µg/kg resulted in significantly smaller decreases in MAP at 3±1,

4 ± 1, 7 ± 1, 8 ± 1, and 14 ± 2 mmHg, respectively, from 102 ± 4 mmHg, (all $P<0.05$) as compared to

adenosine at doses of 10, 25, 50, 100, and 250µg/kg. There was a significant increase in HR

following injections of CVT-3146 or adenosine; however, as shown in Figure 7, the patterns of

tachycardia induced by CVT-3146 and adenosine were significantly different. Adenosine caused

a short duration of tachycardia, whereas CVT-3146 resulted in a longer-lasting tachycardia.

DISCUSSION

The coronary vasodilator effect of adenosine is the basis for its use in pharmacologic stress testing. Unfortunately, pharmacologic stress induced by adenosine is often associated with a high incidence of side effects including dyspnea, chest pain, and atrioventricular nodal block (Mahmarian and Verani, 1994; McGuinness and Talbert, 1994; Marwick 1997). The atrioventricular nodal block is mediated by A_1 AdoR. Some evidence suggests that A_{2B} AdoR may play a role in increasing airway resistance (Fozard and Hannon, 1999). This implies that the A_{2B} AdoR might be responsible for the dyspnea that occurs in pharmacologic stress testing. Because a number of side effects induced by adenosine appear to be mediated by AdoR subtypes other than the A_{2A} receptor, it is presumed that selective agonists of A_{2A} receptors would cause fewer undesirable effects.

CVT-3146 is a novel A_{2A} AdoR agonist. Our previous results have indicated that, in conscious dogs, CVT-3146 is a more potent coronary vasodilator than adenosine (Trochu et al., 2003). The present study demonstrates that the administration of CVT-3146 or adenosine results in a dose-dependent increase in CBF and a dose-dependent decrease in LDCR. Although the maximal increases in CBF were not significantly different, the ED_{50} values of CVT-3146 and adenosine necessary to increase CBF were $0.45\pm0.07\mu g/kg$ and $47\pm7.77\mu g/kg$, respectively ($p<0.05$), indicating that, as a coronary vasodilator, CVT-3146 is 100-fold more potent than adenosine.

CVT-3146 or adenosine also caused dose-dependent decreases in TPR, however, the decrease in TPR following the administration of CVT-3156 was significantly smaller than that induced by adenosine (Figure 1). The smaller decrease in TPR by CVT-3146 may account for the smaller hypotension caused by CVT-3146 as compared to adenosine (Table 1). Furthermore,

the ratios between decreases in LDCR and TPR observed with CVT-3146 were markedly greater than those for adenosine (Figure 3), confirming that CVT-3146 is a more selective coronary vasodilator than adenosine.

The duration of the coronary vasodilation induced by pharmacologic stress testing agents is an important determinant of their usefulness. Because adenosine has an ultra-short duration of action, it is usually administered intravenously for 4-6 minutes to achieve the desired coronary vasodilation during stress testing (Mahmarian and Verani, 1994; McGuinness and Talbert, 1994; Allison et al., 1996; Leppo, 1996; Marwick, 1997; Cerqueira, 2000). Several potent, high-affinitive, selective A_{2A} AdoR agonists, such as CGS-21680 and WRC-0470, have been developed (Glover et al., 1996; Alberti, 1997; Belardinelli et al., 1998; Shryock et al., 1998; Gao et al., 2001a). Our previous results have shown that these two agents have excessively prolonged coronary vasodilator effects in the isolated rat heart (Gao et al., 2001a), which may limit the clinical usefulness of these agents in pharmacologic stress testing because of their potential to cause significant hypotension. While the duration of coronary vasodilation caused by CVT-3146 is longer than that seen with adenosine (Figure 2), there was no significant difference in the magnitude of the peak increase in CBF produced by the two agents. The duration of CBF 2-fold above the baseline was 130±19 sec. for CVT-3146 (2.5 µg/kg) and 16±3 sec. for adenosine (250 µg/kg) (P<0.05). These results clearly indicate that CVT-3146 yields a longer duration of coronary vasodilation than adenosine. Furthermore, CVT-3146 may be administered by a single injection during radionuclide myocardial perfusion imaging, thereby simplifying the procedure. Our recent study in humans has revealed that CVT-3146 can cause a dose-dependent coronary vasodilation following a bolus intravenous injection. The profile of coronary vasodilation by CVT-3146 in humans is very similar to that found in conscious dogs in the present study (Kerensky et al., 2002). This provides another piece of evidence to support that CVT-3146 could

be administered by a single injection. Increased potency and longer duration of the action make

CVT-3146 a much better candidate for pharmacologic stress testing than adenosine.

In addition to coronary vasodilation, the present results indicate that the administration of

CVT-3146 or adenosine causes vasodilation in the mesentery and the lower body. There was no

significant difference in the vasodilation response to CVT-3146 in the mesentery as compared to

adenosine. However, CVT-3146 caused a smaller increase in LBF than adenosine (Figures 4 and

5). The increase in LBF seen with adenosine was ~2-fold greater as compared to that induced by

CVT-3146 and may account for marked hypotension and reduced TPR by adenosine, even

though both drugs caused equivalent increases in CBF. The mechanism for the differential

vasodilation in the lower body induced by CVT-3146 and adenosine is likely related to the

distribution and/or density of AdoR. It has been demonstrated that there are A_1, A_{2A}, and A_{2B}

AdoR on vascular smooth muscle and endothelial cells in human skeletal muscle (Lynge and

Hellsten, 2000). Adenosine can activate all three types of receptors, thereby causing a greater

increase in LBF. CVT-3146 can only activate A_{2A} AdoR, thus resulting in a smaller increase in

LBF. This, in turn, reduces the magnitude of hypotension following injection of CVT-3146 as

compared to adenosine, which would be advantageous in pharmacologic stress testing.

Another important finding of the present study is that adenosine caused dose-dependent

renal vasoconstriction, while CVT-3146 had little effect on the renal circulation (Figures 6).

This is in keeping with previous studies (McCoy et al., 1993; Shryock and Belardinelli, 1997;

Pelueger et al, 1999), in which the administration of adenosine yielded afferent arteriolar

constriction, an effect mediated by A_1 AdoR. Our findings are consistent with the idea that

CVT-3146 does not activate A_1 AdoR (Gao et al., 2001a), and the lack of renal vasoconstriction

by CVT-3146 is yet another benefit of using this agent in pharmacologic stress testing. The

administration of adenosine over 4-6 min may yield prolonged vasoconstriction in the kidney,

which is a potentially undesirable effect. More importantly, renal vasoconstriction by adenosine is invisible and difficult to recognize, and may impair renal function. Elderly patients and those with coronary arterial disease whose renal function is already diminished may be at the greatest risk for adenosine-induced renal vasoconstriction. Because CVT-3146 has little or no effect on renal blood flow, it is unlikely to induce renal dysfunction when used in pharmacologic stress testing.

Some studies showed that adenosine could increase sympathetic nerve activity in humans, thereby causing direct tachycardia (Biaggioni et al, 1991; Lucarini et al, 1992; Engelstein et al, 1994). The present results have also shown that there is a significant increase in heart rate or tachycardia following injections of CVT-3146 or adenosine. However, as shown in Figure 7, the patterns of the tachycardia induced by CVT-3146 and adenosine were significantly different. This suggests that the mechanism(s) responsible for tachycardia caused by CVT-3146 and adenosine may be different. Adenosine-induced tachycardia in conscious dogs was of short duration and mostly due to the baroreflex effect caused by hypotension. This reflex tachycardia overcomes bradycardia, which is the direct depressant effect of adenosine on HR and is mediated by A_1 AdoR (Gao et al., 2001b). Our previous work showed that vagal tone might play an important role in adenosine-induced tachycardia in normal conscious dogs, because the blockade of muscarinic receptors or bilateral vagotomy could unmask adenosine-induced bradycardia in normal, conscious dogs (Belloni and Hintze, 1987; Belloni et al., 1989; Hintze et al., 1985). Moreover, adenosine resulted in bradycardia in conscious dogs after the development of pacing-induced heart failure, in which vagal tone is reduced (Belloni et al., 1992). In contrast, our current results show that CVT-3146-induced tachycardia lasts much longer than that caused by adenosine, especially at higher doses (1.0 and 2.5μg/kg). The tachycardia lasted up to 4-5 min following the injection of a 2.5μg/kg dose of CVT-3146 (Figure 7). Whether CVT-3146-

induced tachycardia observed in the present studies is due to the increased sympathetic nerve activity remains to be determined.

It has been reported that the increased endogenous adenosine induced by dipyridamole could cause hyperventilation in conscious humans (Engelstein et al, 1994), which is mediated by peripheral chmoreceptor activation since peripheral chemoreceptor suppression (with hyperoxia) abolished hyperventilation induced by dipyridamole. Adenosine-induced hyperventilation can result in changes in CBF and HR indirectly. We did not measure ventilation in our present studies quantitatively, although hyperventilation was observed in some dogs during the administration of CVT-3146 or adenosine. This is one of limitations of our studies.

In summary, our study indicates that CVT-3146 is a 100-fold more potent coronary vasodilator than adenosine, producing a longer duration of coronary vasodilation that may be useful in pharmacologic stress testing. CVT-3146 also causes a smaller decrease in total peripheral resistance and a smaller increase in LBF than that induced by adenosine, suggesting CVT-3146 is more selective for coronary than for peripheral vasodilation. This study also demonstrates that CVT-3146 causes no renal vasoconstriction. These features make CVT-3146 a promising candidate for pharmacologic stress testing with myocardial perfusion imaging using radionuclides for the diagnosis of coronary artery disease.

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Selective A_{2A} adenosine receptor agonist as a coronary vasodilator in conscious dogs:

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Figure Legends

Figure 1. Graph shows dose-response curves of changes in coronary blood flow (CBF), cardiac output (CO), late diastolic coronary resistance (LDCR), and total peripheral resistance (TPR) following injections of CVT-3146 or adenosine. Administration of CVT-3146 or adenosine resulted in a dose-dependent increase in CBF and CO.; LDCR and TPR also decreased following injections of CVT-3146 and adenosine. Although CVT-3146 caused a markedly smaller decrease in TPR compared to adenosine, both CVT-3146 and adenosine caused similar coronary vasodilation. Values are mean±SEM, n=7, #$P<0.05$, compared to adenosine.

Figure 2. Time course of the increase in the coronary blood flow (CBF) following injections of CVT-3146 (2.5 µg/kg) and adenosine (250 µg/kg). CVT-3146 caused a longer-lasting increase in CBF than adenosine, although there was no significant difference in the maximal increase in CBF in response to the two agents. Values are mean±SEM, n=7.

Figure 3. Graph shows the ratio between the decrease in late diastolic coronary resistance (LDCR) and the decrease in total peripheral resistance (TPR) induced by CVT-3146 and adenosine. The ratio for CVT-3146 was much greater than that for adenosine, suggesting that CVT-3146 is a more selective for coronary vasodilation than peripheral vasodilator. Values are mean±SEM, n=7, #$P<0.05$, compared to adenosine.

Figure 4. Graph summarizes the vasodilation by CVT-3146 (A) and adenosine (B) in different vascular beds in conscious dogs. There were no significant differences in the maximal increase in CBF and in mesenteric blood flow (MBF). However, CVT-3146 resulted in a smaller increase

in the lower body flow (LBF). Values are mean±SEM, n=6 (MBF and LBF), n=7 (CBF), P<0.05, compared to the response to adenosine

Figure 5. Changes in vascular resistance in different vascular beds following injections of CVT-3146 (A) or adenosine (B). CVT-3146 or adenosine caused a similar decrease in LDCR and mesenteric vascular resistance (MVR). However, CVT-3146 resulted a smaller decrease in the vascular resistance in the lower body (LVR). Values are mean±SEM, n=6 (MVR and LVR), n=7 (LDCR), P<0.05, compared to the response to adenosine.

Figure 6. Adenosine caused a dose-dependent decrease in renal blood flow (RBF) and an increase in renal vascular resistance (RVR), whereas there were no significant changes in RBF (except for at the dose of 2.5 µg/kg) and RVR following injections of CVT-3146. Values are mean±SEM, n=6, #P<0.05, compared to the response to adenosine.
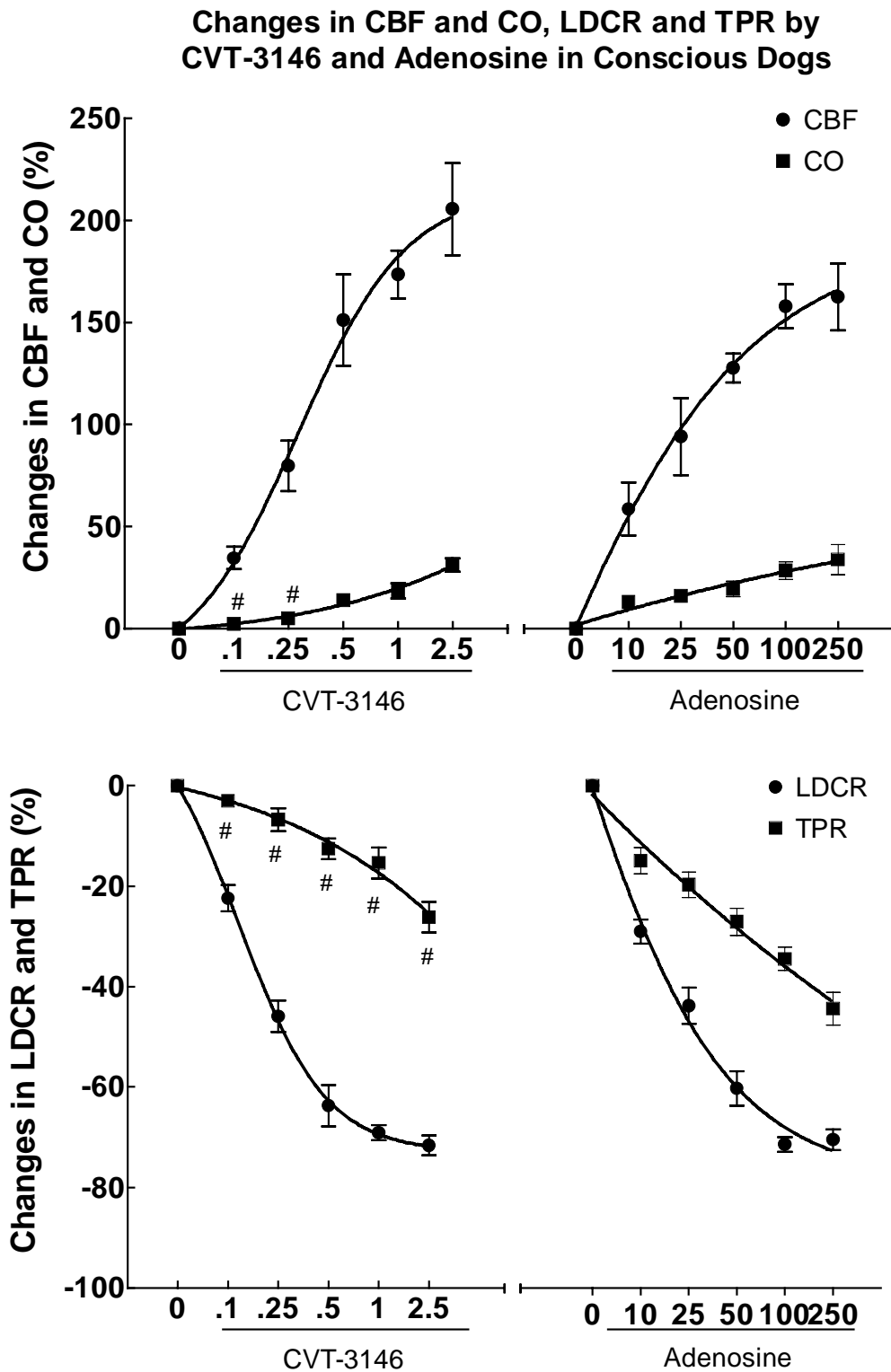
Figure 7. Graph shows the time course of the increase in heart rate (HR) following injections of CVT-3146 (2.5µg/kg) and adenosine (250µg/kg). The pattern of the tachycardia induced by CVT-3146 and adenosine was different. Adenosine caused a shorter-lasting tachycardia, whereas CVT-3146 resulted in a longer-lasting increase in HR. Values are mean±SEM, n=7.
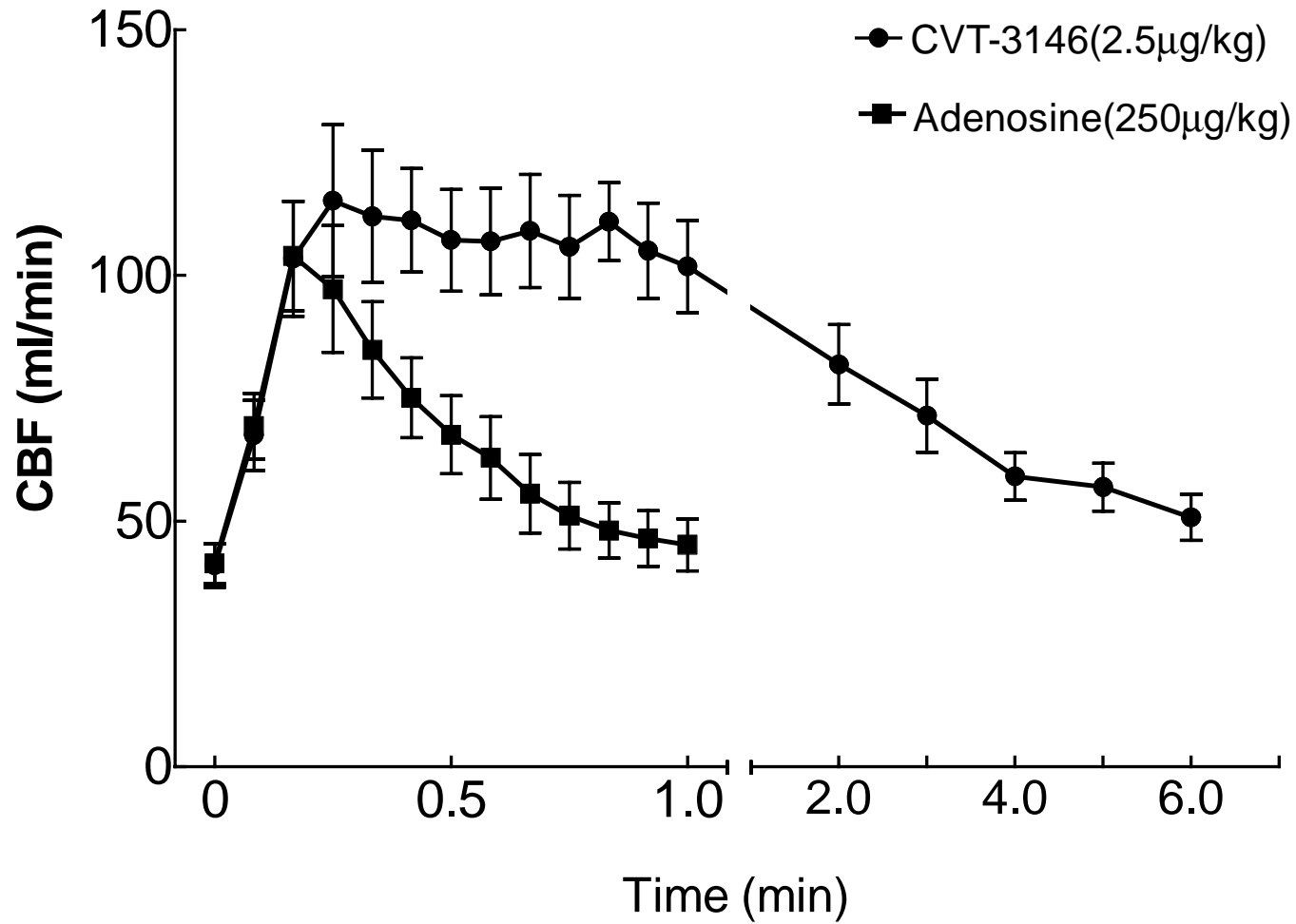
Table 1. Changes in Arterial Blood Pressure and Heart Rate Following Injections of CVT-3146 and Adenosine in Conscious Dogs

	MAP (mmHg)		HR (beats/min)	
	Baseline	Response	Baseline	Response
CVT-3146 (μg/kg)				
0.10	103±4	100±4*#	90±3	98±4*
0.25	102±4	97±4*#	88±3	100±4*#
0.50	102±4	95±5*#	87±3	111±7*
1.00	102±4	95±5*#	88±3	123±7*
2.50	103±3	89±4*#	87±3	139±8*
Adenosine (μg/kg)				
10	104±3	96±4*	89±3	103±4*
25	102±3	92±4*	87±3	113±7*
50	103±4	86±4*	86±3	121±9*
100	104±4	84±4*	89±3	140±9*
250	105±3	75±5*	89±3	152±10*

MAP: mean arterial blood pressure, HR: heart rate. Mean±SEM, n=13, *P<0.05, compared with the baseline, #P<0.05, compared with the response to adenosine.

**Changes in CBF and CO, LDCR and TPR by
CVT-3146 and Adenosine in Conscious Dogs**

JPET #53306

Figure 1

Time Course of Changes in CBF by CVT-3146 and Adenosine in Conscious Dogs

Legend:
- ● CVT-3146(2.5μg/kg)
- ■ Adenosine(250μg/kg)

Y-axis: CBF (ml/min)
X-axis: Time (min)

JPET #53306   Figure 2
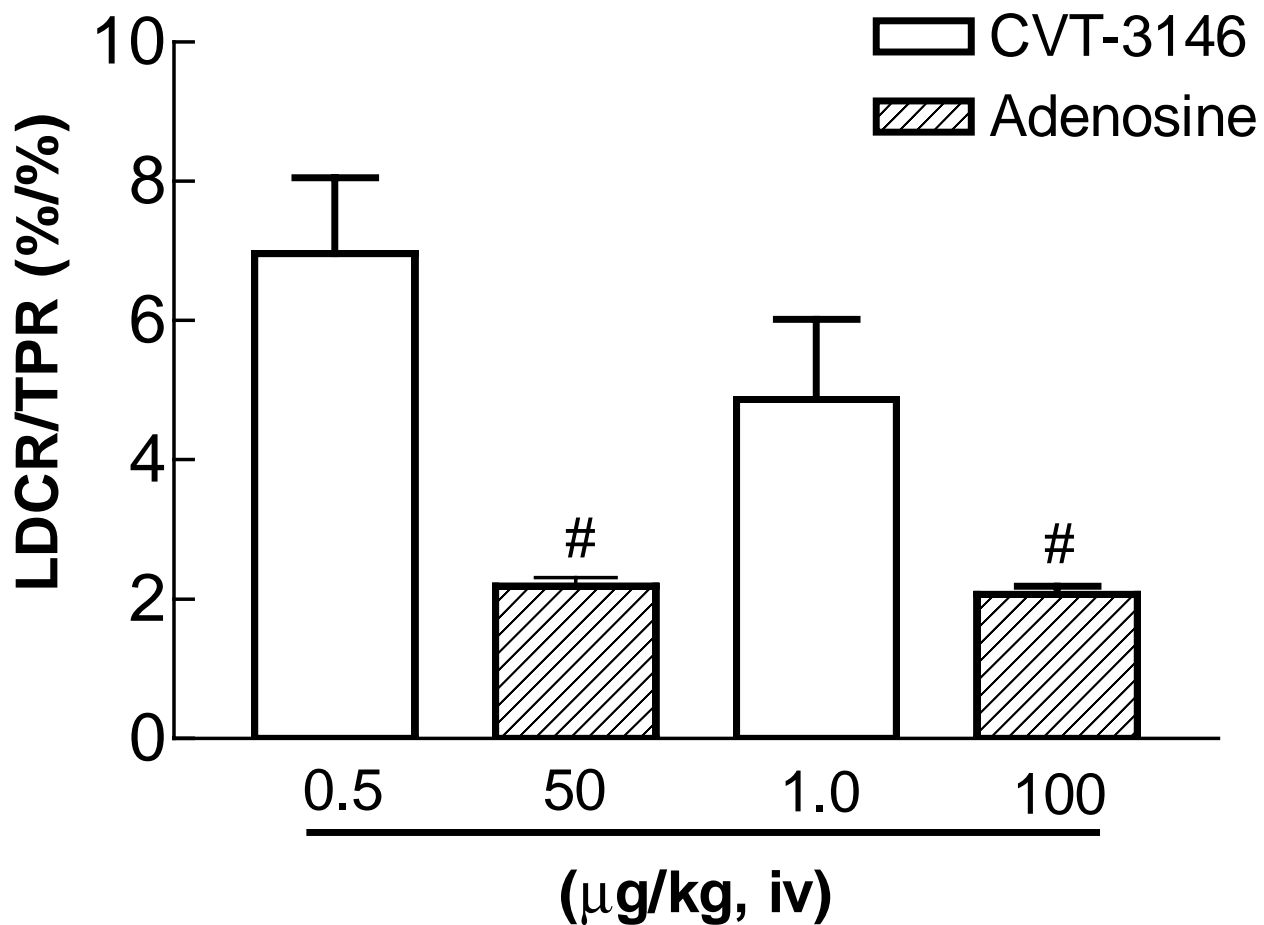
Ratio Between Decreases in LDCR and TPR

Figure 3

Vasodilation in   Different Vascular Beds by CVT-3146 and Adenosine in Conscious Dogs
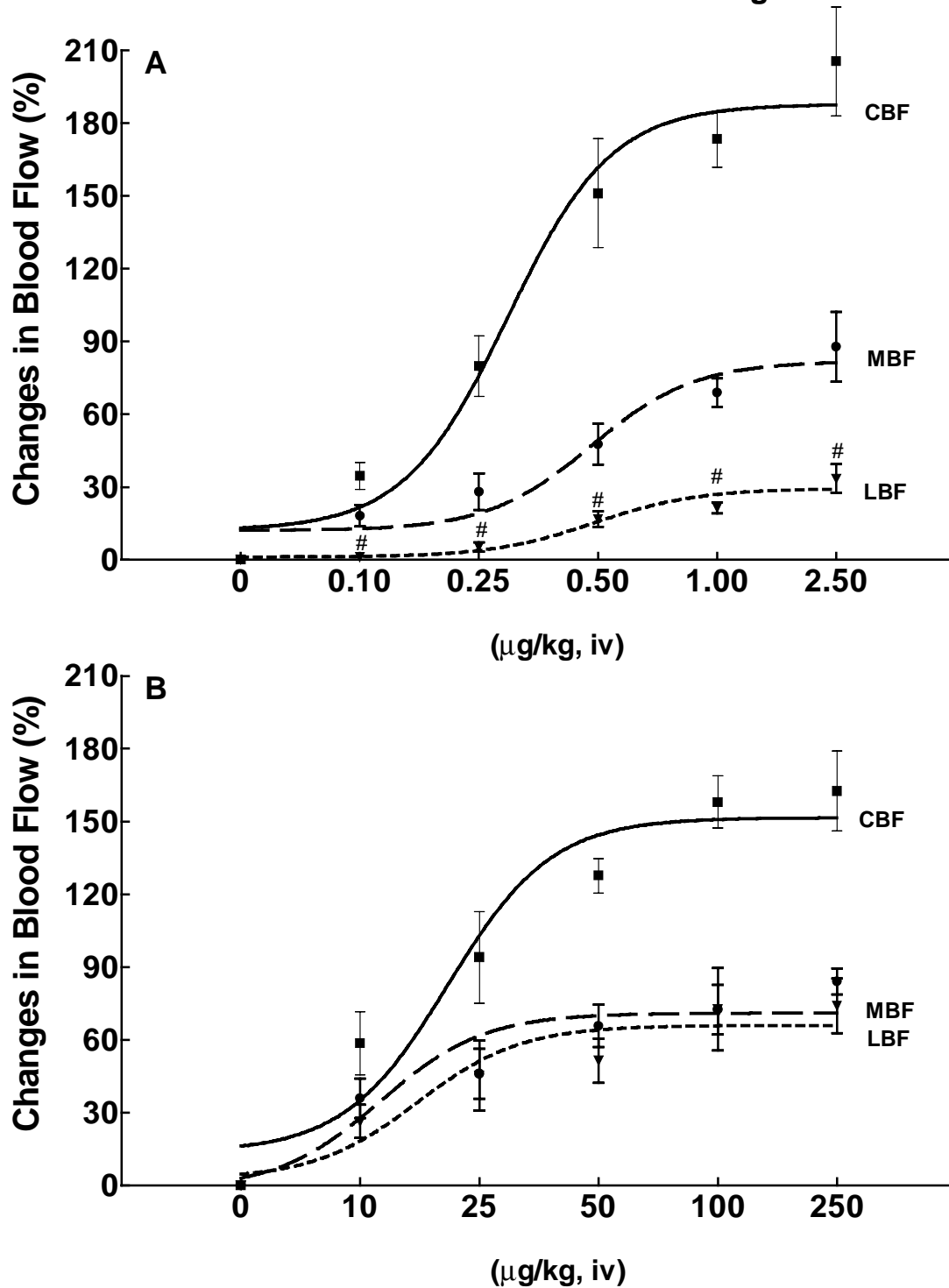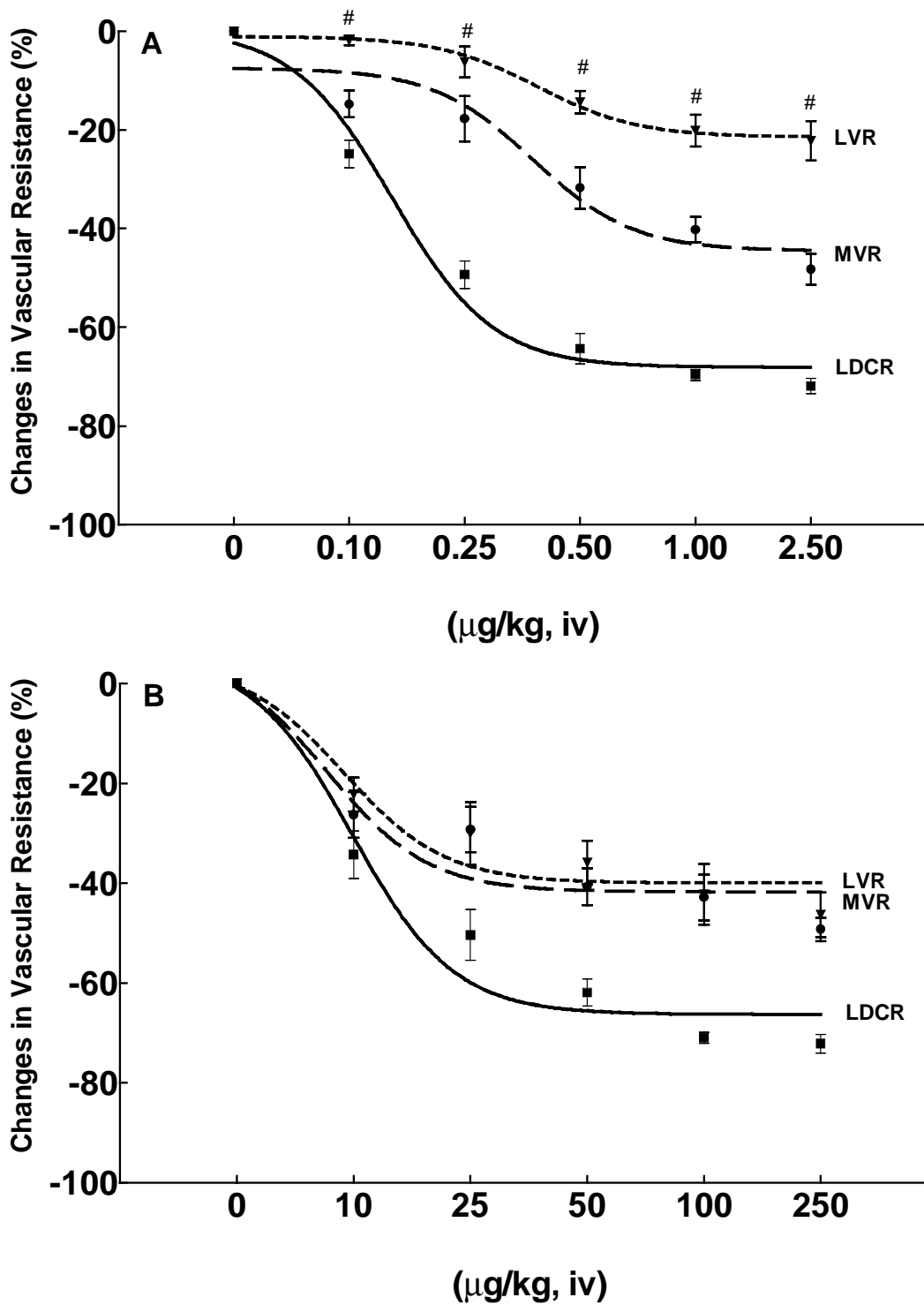
JPET #53306

Figure 4

**Changes in Resistance in Different Vascular Beds
by CVT-3146 and Adenosine in Conscious Dogs**

JPET #53306   Figure 5

Change in Renal Blood Flow and Vascular Resistance by CVT-3146 and Adenosine in Conscious Dogs
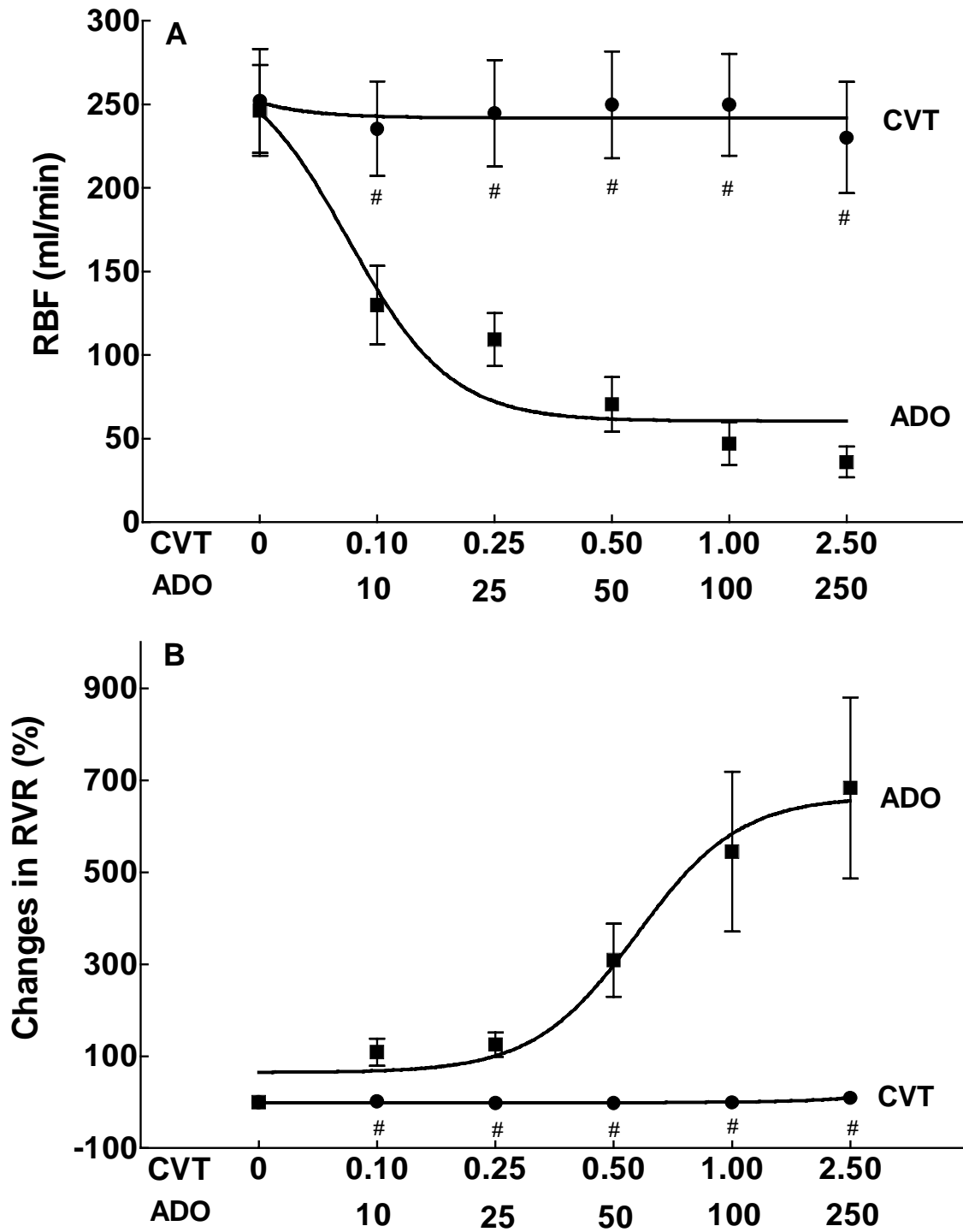
JPET #53306   Figure 6

Time Course of Changes in HR by CVT-3146 and Adenosine in Conscious Dogs

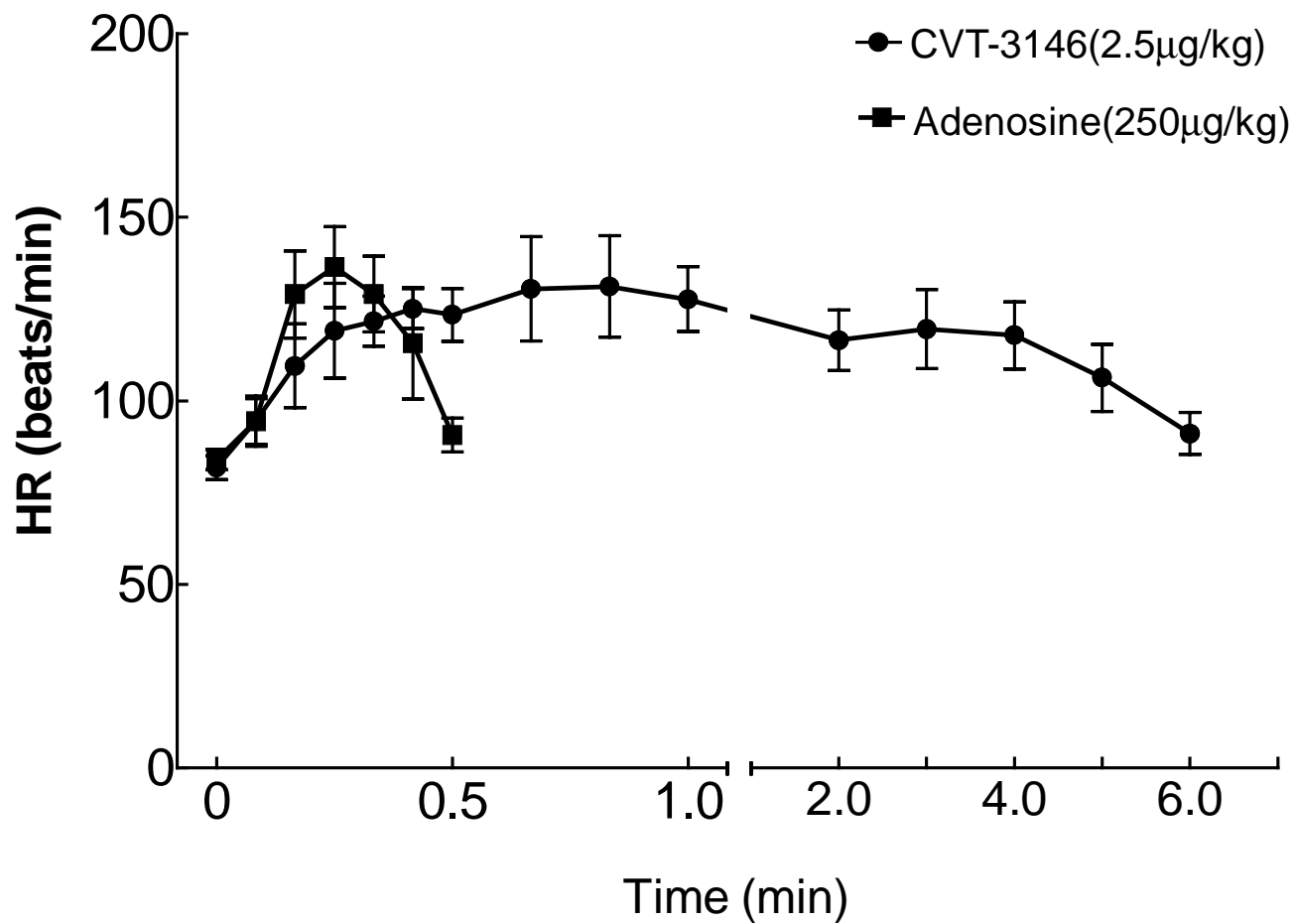Figure 7

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250	105±3	75±5*	89±3	152±10*

MAP: mean arterial blood pressure, HR: heart rate. Mean±SEM, n=13, *P<0.05, compared with the baseline, #P<0.05, compared with the response to adenosine.