INO-8875, a Highly Selective A1 Adenosine Receptor Agonist: Evaluation of Chronotropic, Dromotropic, and Hemodynamic Effects in Rats

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Received October 2, 2012; accepted October 9, 2012

ABSTRACT

Selective pharmacological activation of the adenosine 1 receptor (A1R) is a promising new approach to achieve a potent block of atrioventricular (A–V)–nodal conduction without significant cardiovascular side effects. The purpose of the present study was to evaluate the cardiovasculor profile of INO-8875, a highly selective A1R agonist, and to compare its properties with N-[3(R)-tetracydrofuranyl]-6-aminopurine riboside (CVT-510), which has already been shown to induce negative dromotropic effects with minimal cardiovascular side effects in animals and in clinical studies. Dose-response experiments in the isolated hearts of rats were used to evaluate the functional selectivity of INO-8875 for the slowing of A-V–nodal conduction. Ventilated adult rats were used to study the effects of INO-8875, in vivo, on arterial blood pressure as well as on supraventricular electrophysiology. Ex vivo, INO-8875 (100 nM to 3 μM) progressively prolonged A-V–nodal conduction without reducing left ventricular function or coronary resistance. In vivo, INO-8875 up to a dose of 50 μg/kg did not reduce the carotid arterial blood pressure (n = 4). INO-8875 (1–50 μg/kg) and CVT-510 (20 and 50 μg/kg) both induced a dose-dependent decrease in heart rate and atrial refractoriness, as well as slowing of A-V–nodal conduction. However, compared with CVT-510, the activity of INO-8875 was more pronounced in A-V–nodal function. INO-8875 exhibited a greater duration of action, lasting up to 2.5 hours post dosing, whereas the effects of CVT-510 dissipated over 1 hour. INO-8875 demonstrates functional properties of a highly selective A1R agonist. INO-8875 exhibits an increased dromotropic effect and greater duration of action compared with CVT-510.

Introduction

Negative dromotropic effect is an important therapeutic goal in the majority of patients with supraventricular tachyarrhythmias. In patients with paroxysmal supraventricular tachycardias, pharmacological inhibition of atrioventricular (A-V)–nodal conduction is the main modality for acute termination of the re-entry cycle if the A-V node is part of the re-entry. Long-term inhibition of A-V–nodal conduction may also be used as a bridging modality or an alternative for ablation therapy (Blomstrom-Lundqvist et al., 2003). In patients with atrial fibrillation (AF) or atrial flutter/tachycardia, prolongation of A-V–nodal refractoriness is the main strategy to control ventricular response rate. Moreover, since current antiarrhythmic modalities are ineffective in a large proportion of AF patients, proper long-term control of ventricular response rate is critical for symptomatic relief, improvement of hemodynamic status, and prevention of tachycardia-induced cardiomyopathy (reviewed in Gopinathannair et al., 2009).

In many clinical instances, selective and potent negative dromotropic effect may be highly desirable during supraventricular tachyarrhythmias. This is specifically true for situations of acute reversible stress including: recent myocardial infarction, the postthoracic surgery setting, sepsis, and various other acute conditions in the emergency room/ intensive care environment. However, current pharmacological approaches to achieve negative dromotropic effect are
limited and nonselective. Digitalis acts rather slowly and is not effective in the face of the vagal withdrawal that often characterizes an acute stress situation. \(\beta\)-Adrenergic receptor blockers are often ineffective in acutely stressful situations and can have undesirable adverse effects on ventricular contractility and bronchial smooth muscle tone. Ca\(^{2+}\)-channel blockers can impair ventricular contractility and can cause hypotension, and their pharmacokinetics make sustained safe and effective doses difficult to achieve by intravenous administration (Fuster et al., 2006).

Adenosine, an endogenous nucleoside that activates several cell-surface receptor subtypes (Jacobson and Gao, 2006), is a potent inhibitor of A-V–nodal conduction. This beneficial effect of adenosine is mediated by activation of the adenosine 1 receptor (A1R), which also causes slowing of the sinus rate, depression of atrial (but not ventricular) contractility, and attenuation of the stimulatory effects of catecholamines on the myocardium (Belardinelli and Lerman, 1990; Shryock and Belardinelli, 1997). The A-V–nodal effects of adenosine are related to the opening of \(\text{Gi}_\text{b}-\text{coupled K}^+\) channels as well as to a depression of \(I_{\text{Ca,L}}\) (Wang et al., 1996). As a result, adenosine causes rate-dependent impairment of A-V–nodal function that results in substantial and effective block at rapid rates (Nayebpour et al., 1993; Lai et al., 1994). Nevertheless, the therapeutic utility of adenosine is limited to the acute treatment of A-V–nodal-dependent Paroxysmal supraventricular tachycardia owing to its extremely short physiological half-life and its hemodynamic side effects, which are mainly related to the activation of A2A receptors (Wilbur and Marchlinski, 1997). Selective activation of A1R is considered a promising new approach to achieve potent slowing of A-V–nodal conduction without decreasing cardiac contractility. \(N\)-(3[\(\text{R}\)]-tetrahydrofuranyl)-6-aminopurine riboside (CVT-510), a pharmacological agent designed for this purpose, was found to have a selective negative dromotropic effect in the guinea pig heart when compared with diltiazem (Snowdy et al., 1999). Moreover, phase I and II clinical studies have also shown promising results with a relatively low side-effect profile in the setting of PSVT (reviewed in Cheung and Lerman, 2003) and a possible benefit for acute rate control in patients with AF.

INO-8875 is a potent \((K_i = 0.97 \text{ nM})\) and selective \(\text{A}_1\)R agonist (Etzion et al., 2008b; Kim et al., 2009), the structure of which is shown in Supplemental Fig. 1 according to its publication in patent PCT/EP2011/064829 (Kalar et al., 2012). INO-8875 administered in a topical ocular formulation is currently under clinical evaluation for the treatment of elevated intraocular pressure associated with glaucoma (Kim et al., 2009). However, the possible role of this compound as a modulator of A-V–nodal conduction has not yet been studied. In the present work, we evaluated in detail the cardiovascular effects of INO-8875 in rats. By use of a recently described modality that we developed for evaluation of rodent supraventricular electrophysiology (EP) in vivo (Etzion et al., 2008a), we directly compared the effects of INO-8875 and CVT-510 on the supraventricular EP of rats.

**Materials and Methods**

A total of 44 rats were used in the present study. All experiments were approved by the Institutional Ethics Committee, Faculty of Health Sciences, Ben-Gurion University of the Negev, Israel. At the end of all in vivo experiments, animals were killed using i.v. injection of KCl under deep isoflurane anesthesia.

**Chemicals**

CVT-510 and INO-8875 were provided in lyophilized form (Inotek Pharmaceuticals Corporation, Lexington, MA). CVT-510 was prepared as aliquots of 0.2 mg/ml in 0.9% saline and stored at \(-20^\circ\text{C}\). For each experiment, a new aliquot was dissolved in 0.9% saline according to the animal weight immediately before use. The final concentration was calculated so that at a flow rate of 1 ml/h, the desired dose of the drug was applied to the animal over a period of 20 minutes. INO-8875 viles were stored at \(4^\circ\text{C}\) in lyophilized form. For each experiment, a new vile was freshly dissolved in 0.9% saline to form a stock solution of 0.2 mg/ml that was thereafter further diluted in 0.9% saline and applied to the animal as described previously for CVT-510. For ex vivo experiments, INO-8875 was freshly diluted in the isolated heart Tyrode’s solution to form escalating concentrations of the drug that were serially applied to the heart preparation following baseline recordings. The concentrations of INO-8875 are noted in molar terms for the ex vivo experiments and in micrograms per kilogram for the in vivo experiments. For comparison, it should be noted that 1 \(\mu\)g of INO-8875 equals 2.63 nM.

**Isolated Perfused Heart Preparation**

Experiments were conducted on hearts of adult male rats (250–350 g). After injection of heparin (500 units/kg i.p.), each rat was anesthetized with an injection of ketamine/xylazine (75/5 mg/kg i.p.), and the heart was isolated from the chest. The aorta was cannulated and perfused was initiated with oxygenated Tyrode’s buffer solution consisting of the following: 126 mM NaCl, 5.4 mM KCl, 1 mM MgCl\(_2\), 2 mM CaCl\(_2\), 0.39 mM Na\(_2\)PO\(_4\), 10 mM glucose, and 10 mM HEPES (titrated to pH 7.4 with NaOH). Hearts were hung on a Langendorff apparatus and perfused with oxygenated, preheated \((37^\circ\text{C})\) Tyrode’s solution at a constant rate of 10 ml/min. The left atrium was excised and a fluid-filled latex balloon was inserted into the left ventricle (LV) through the mitral valve. Once inside the LV, the balloon was inflated to an end-diastolic pressure of 5 mm Hg. After placement of the LV balloon, a miniature-bipolar hook electrode (Etzion et al., 2008a) was attached to the right atrial epicardium, and the atria were continuously paced at a cycle length of 150 ms (400 beats per minute) throughout the experiment (2-ms square pulses; Pulsar 6bp-as; FHC, Bowdoinham, ME). An additional miniature-bipolar hook electrode was attached to the ventricular epicardium for recording of the delay between the stimulus artifact and the initiation of the ventricular signal (SV interval; see Fig. 1A) as a measure of A-V conduction (Snowdy et al., 1999). LV pressure (0–2 kHz) was recorded by a pressure amplifier (ETH-256C amplifier and PB-100 probe; iWorx, Dover, NH). Electrical signals were filtered \((1–2\text{ kHz})\) and recorded by a voltage amplifier (Model 1700 AC amplifier; A-M Systems, Carlsborg, WA). Both signals were interfaced with a personal computer using an analog to digital converter (PCI-6024E; National Instruments, Austin, TX) and a home-made program developed by one author (Y.E., using LabView 7.1, National Instruments) to control signal acquisition, data saving, and off-line analysis of the SV interval, pressure derivatives \((-\text{dP/dt}, -\text{dP/dt})\), and developed pressure. Coronary perfusion pressure was measured using an analog manometer attached to the perfusion apparatus and was used to calculate the coronary conductance throughout the experiment.

**Anesthetized Rat Preparation**

**Anesthesia and Ventilation.** Male Sprague-Dawley rats (250–350 g) were anesthetized, intubated, and ventilated with a rodent respirator (Inspira; Harvard Apparatus, Holliston, MA) as previously described (Etzion et al., 2008a). A positive-end expiratory pressure of 3 cm H\(_2\)O was applied continuously and increased to 5 cm of H\(_2\)O upon thoracotomy (see the following section). Maintenance of
anesthesia was applied with 2.5% isoflurane. Body temperature was continuously monitored with a rectal thermometer and was maintained at 37°C by a heating pad. An intravenous line was inserted in the tail vein, and saline injection at a rate of 1 ml/h was constantly applied using a syringe pump. The animals were connected to an electrophysiological system (Nihon Kohden, Tokyo, Japan), and surface ECG was monitored using cutaneous clips fixed on each limb.

Electrophysiological Recordings. EP recordings were done as previously described (Etzion et al., 2008a). In brief, through a right-lateral thoracotomy, two pairs of mini-bipolar hook electrodes were inserted on the upper and lower lateral aspects of the right atrium. The upper electrode was used for stimulation and the lower for recording of the atrial activity. A third bipolar electrode was inserted on the right ventricle for assessment of the ventricular response. Electrical stimulation consisted of square current pulses of 2-ms duration applied through an isolation unit (Iso-Flex; AMPI, Jerusalem, Israel). EP protocols included basal recordings of the RR, PR, and QT intervals (if easily detected in the ECG). Thereafter, programmed S1-S2 stimulation protocol (in 1-ms steps) was used to determine the A-V-node refractory period and the atrial effective refractory period.

Fig. 1. Concentration-dependence effects of INO-8875 in the isolated rat heart. Isolated rat hearts were exposed to escalating doses of INO-8875 under a constant coronary flow rate (10 ml/min) and constant atrial pacing (150-ms cycle length). (A) example of LV pressure (LVP) recordings (upper traces) and ventricular electrical recordings (lower traces) in a preparation under baseline conditions (control) and following exposure to 1000 nM INO-8875. Note the increased SV interval, which indicates a negative dromotropic effect of the drug. (B) summary of the concentration-dependence effects of INO-8875 in five similar experiments. Note that INO-8875 progressively increased the SV interval (●) without a significant effect on LV developed pressure (●) or coronary conductance (▲). Washout of 15 minutes almost totally reversed the activity of INO-8875 on the SV interval (arrow). Statistical significance was determined using one-way analysis of variance of repeated measures. *P < 0.05.
(AERP). A decremental atrial pacing protocol starting from a 150-ms cycle length (CL) down to a 20-ms CL (in 5-ms steps) was thereafter used. Each step consisted of a 5-second-long train of 2-ms pulses. This protocol was used to determine the A-V–nodal Wenckebach conduction block (A-V–Wenck).

**Blood Pressure Measurements.** Invasive blood pressure measurements were done using cannulation of the right carotid artery with a polyethylene catheter connected to a pressure transducer and amplifier (Nihon Koden). The signal was digitized and stored on a PC for off-line analysis.

**Drug Application.** Following baseline EP recordings or blood pressure measurements, saline injection was switched with the drug solution. The solution containing the drug of interest was placed in a different line of the syringe pump so that a rapid switch to drug application was possible using a three-port stopcock directly connected to the i.v. catheter. For EP recordings, the desired dose of A1R agonist (CVT-510 or INO-8875) was applied at a rate of 2.5 µg/kg/min up to a cumulative dose of 100 µg/kg (over a 40-minute period). Blood pressure measurements were performed every 10 minutes during drug application (see Results for details).

**Statistical Analysis**

Values are expressed as the mean ± S.E. Student’s t test or one-way analysis of variance was used as required (SigmaStat 3.1; Systat Software, Inc, Point Richmond, CA). Statistical significance was set at P < 0.05.

**Results**

**Effects of INO-8875 in the Isolated Rat Heart**

As a first step in characterizing the cardiovascular profile of INO-8875, the concentration-dependence effect of the drug was evaluated in isolated perfused rat hearts (n = 5). In these experiments, continuous atrial pacing (150-ms cycle length) was employed and the coronary flow was maintained at 10 ml/min. Under these conditions, the hearts were exposed to increased concentrations of INO-8875 (0.5 nM up to 3000 nM). Fig. 1, which summarizes these experiments, demonstrates that INO-8875 caused a concentration-dependent increase in the recorded SV interval, a finding consistent with a negative dromotropic effect of the drug (Snowdy et al., 1999). However, under this wide range of concentrations, no effect of INO-8875 was noted on cardiac contractility (as measured by LV-developed pressure) or on coronary conductance (as calculated from the coronary perfusion pressure measurements).

**Effect of INO-8875 on Arterial Blood Pressure in Anesthetized Rats**

To further evaluate the hemodynamic profile of INO-8875, arterial blood pressure measurements were obtained in anesthetized rats and INO-8875 was applied up to a cumulative dose of 100 µg/kg. A1R activation in rodents is known to potently affect sinoatrial (S-A) node function, leading to reduced heart rates (Froldi and Belardinelli, 1990). Therefore, constant atrial pacing (150-ms CL) was applied in these experiments to evaluate the direct cardiovascular effects of INO-8875 and avoid a bradycardia-related decrease in cardiac output. The obtained results indicated that INO-8875 up to a dose of 50 µg/kg over 20 minutes (the maximal dose applied for electrophysiological evaluation; see next section) did not significantly reduce systolic or diastolic blood pressure (Fig. 2). Further application of the drug up to a dose of 100 µg/kg (over 40 minutes) did induce a mild decrease in systolic and diastolic blood pressure (Fig. 2A), leading to a 17.1 ± 4.1% decrease in the mean blood pressure at a cumulative dose of 75 µg/kg and a 21.9 ± 4.4% decrease in the mean blood pressure at a cumulative dose of 100 µg/kg (Fig. 2B). Application of the nonselective adenosine receptor antagonist aminophylline could instantly reverse the blood pressure reduction noted following a high dose of INO-8875 (Fig. 2).

**Fig. 2.** Effect of INO-8875 on arterial blood pressure in anesthetized rats. Carotid artery pressure measurements were applied in anesthetized rats (n = 4) subjected to atrial pacing at a constant rate of 400 beats per minute (150-ms CL). INO-8875 was applied at a rate of 2.5 µg/kg/min up to a cumulative dose of 100 µg/kg. At the end of the experiments, the nonselective adenosine receptor antagonist aminophylline (3 mg/kg) was applied. (A) systolic and diastolic blood pressure (BP) values at different cumulative doses of INO-8875. (B) mean blood pressure changes presented as the percentage of baseline recordings. Statistical significance was determined using one-way analysis of variance of repeated measures. **P < 0.01.
Electrophysiological Effects of INO-8875 and CVT-510 in Anesthetized Rats

In vivo evaluation of supraventricular EP in small animals is a challenging task, and therefore most data on the EP effects of adenosine receptor agonists currently come from ex vivo and in vitro experiments in these species (Froldi and Belardinelli, 1990; Snowdy et al., 1999; Song et al., 2002). We recently demonstrated that, utilizing an array of bipolar mini-hook electrodes implanted through small lateral thoracotomy, evaluation of the supraventricular EP of rodents can be performed consistently (Etzion et al., 2008a). In the present study, we used this new setup to explore, in detail, the EP effects of INO-8875 (Fig. 3). Application of INO-8875 (1–50 µg/kg) induced a dose-dependent negative chronotropic effect as well as a negative dromotropic effect (Fig. 4). In addition, a small but significant shortening of the AERP was also observed. The QT interval was unaffected by increasing doses of INO-8875.

Since the EP effects of the A1R agonist CVT-510 were previously studied in detail in the guinea pig isolated heart as well as in human subjects (reviewed in Cheung and Lerman, 2003), direct comparison between INO-8875 and CVT-510 is of interest. The effects of CVT-510 were evaluated in the present study at two different doses (20 and 50 µg/kg) equivalent to those studied with INO-8875. The results indicated that both drugs demonstrate rather similar effects on the various evaluated EP parameters (Fig. 5A). However, CVT-510 seemed to have a stronger negative chronotropic effect and a weaker negative dromotropic effect as compared with INO-8875. Indeed, at the highest tested dose (50 µg/kg), INO-8875 increased the RR interval to 165 ± 12% of the control and prolonged the A-V–Wenck to 131 ± 78%, whereas CVT-510 increased the RR interval to 180 ± 4% of the control, but prolonged the A-V–Wenck to 119 ± 5% only (Fig. 5A). To assess more quantitatively the possibility of different drug potencies in regard to chronotropic versus dromotropic effects, these two effects were plotted against each other for all tested animals with both drugs. For CVT-510, which was tested only at high doses, this analysis also included recordings obtained following 20 minutes of drug washout. The obtained results (Fig. 5B) demonstrated a clear linear relationship between the chronotropic and the dromotropic effect induced by both INO-8875 and CVT-510.
drugs. However, INO-8875 activity was clearly more pronounced in A-V–nodal conduction compared with CVT-510, as measured by the regression slope of the A-V–nodal effect/S-A–nodal effect (0.42 and 0.27 for INO-8875 and CVT-510, respectively; Fig. 5B). In addition, when the percent increase in A-V–Wenck/percentage increase in RR were calculated for all animals exposed to equivalent doses of both drugs (Fig. 5C), it was significantly higher for INO-8875 compared with CVT-510 (0.42 ± 0.04 vs. 0.22 ± 0.03, respectively; \( P < 0.05 \))

It is noteworthy that a similar calculation of the percent increase in A-V–Wenck/percent decrease in AERP (Fig. 5C) also indicated a tendency for higher values in animals exposed to INO-8875 compared with CVT-510 (1.96 ± 0.40 vs. 0.79 ± 0.13, respectively; \( P = 0.08 \)). Therefore, it appears that INO-8875 has a higher functional selectivity to the slowing of A-V–nodal conduction in rats compared with CVT-510 (see Discussion).

Another marked difference between CVT-510 and INO-8875 was the time course of drug action. The chronotropic and the dromotropic effects of CVT-510 were significantly reduced 20 minutes following drug application. In contrast, the effects of INO-8875 were still fully noted 20 minutes following drug application (Fig. 6A). To further and more precisely assess the time course of action of INO-8875 compared with CVT-510 (1.96 ± 0.40 vs. 0.79 ± 0.13, respectively; \( P = 0.08 \)), a different set of animals was subjected to continuous atrial pacing at a 150-ms CL (to avoid bradycardia-related hemodynamic changes that can affect drug metabolism). Under these conditions, animals were exposed to a rapid bolus of the drug followed by serial testing of the RR interval and SV interval over a period of 2 hours. Figure 6B summarizes these experiments and demonstrates the prolonged time of action of INO-8875. Indeed, following 120 minutes, both parameters were still above the baseline for applied doses of 10–60 \( \mu g/kg \) of INO-8875 (RR interval at 120 minutes: 180.0 ± 21.5% for 60 \( \mu g/kg \), 143.5 ± 11.8% for 30 \( \mu g/kg \), and 117.7 ± 0.75% for 10 \( \mu g/kg \); \( P < 0.05 \) for all).

Discussion

The results of this study indicate that INO-8875, a novel A1R agonist, has the functional selectivity in rats that enables this agent to slow S-A–nodal activity and to prolong A-V–nodal conduction time and refractoriness at concentrations much lower than those causing coronary vasodilatation or depressed LV contractility. Direct in vivo comparison with CVT-510, an A1R agonist that was previously shown to have a selective dromotropic effect in humans, was a key element in the present study. This comparison indicated that INO-8875 possesses a rather similar potency, but a higher selectivity, to the slowing of A-V–nodal conduction in rats compared with CVT-510 (see Discussion).

Isolated Heart Data. Based on adenosine receptor-binding assays, INO-8875 is reported to possess properties of a potent and highly selective A1R agonist with a binding affinity of \( K_I = 0.97 \) nM for A1 receptors (Kim et al., 2009) and \( A_1/A_2A \) receptor-binding affinity >10,000 (Etzion et al., 2008b). As a first step in the present study, we aimed to determine whether the cardiac effects of INO-8875 are indeed consistent with those expected from a potent and highly selective A1R agonist. The isolated heart is an ideal tool to evaluate such functional selectivity (Snowdy et al., 1999), because it allows simultaneous evaluation of the A-V–nodal...
slowing effect (A1R-dependent) and coronary conductance increase (A2A-dependent). In addition, a possible effect on left ventricular contractility, which is not expected by selective A1R activation, can be evaluated (Belardinelli et al., 1989; Cheung and Lerman, 2003; Tikh et al., 2006). For CVT-510, Snowdy et al. (1999) reported a 5-fold difference between the A-V–nodal slowing effect and the coronary conductance increase as measured in the isolated guinea pig heart (Snowdy et al., 1999). In the present study, the concentration-response results recorded for INO-8875 in the isolated rat heart indicate significant A-V–nodal slowing at a concentration of 100 nM, whereas coronary conductance and LV function were not

Fig. 5. Comparison of the EP effects of INO-8875 and CVT-510 in anesthetized rats. (A) summary of the results obtained from rats exposed to INO-8875 and CVT-510 at doses of 20 µg/kg (n = 5 and n = 5 for INO-8875 and CVT-510, respectively) and 50 µg/kg (n = 5 and n = 3 for INO-8875 and CVT-510, respectively). Data are presented as the percentage of baseline value obtained prior to drug application. Statistical difference was determined for each parameter from the baseline values of the same animals using paired Student’s t test. (B) evaluation of chronotropic versus dromotropic effect induced by INO-8875 and CVT-510. For all animals exposed to the different drug doses, the percent increase in A-V–Wenck (dromotropic effect) was plotted as a function of the percent increase in RR interval (chronotropic effect). For CVT-510, which was tested only at higher doses, this analysis also included recordings obtained following 20 minutes of drug washout. Note the clear linear relationship between both effects for both drugs. Linear regression analysis (dashed line) indicated a 1.58-fold higher slope for INO-8875 compared with CVT-510 (0.427 vs. 0.27, respectively). (C) for all animals receiving INO-8875 or CVT-510, the ratio between the dromotropic effect (defined as the percent change in A-V–Wenck compared with baseline) and the chronotropic effect (the percent change in RR interval) was calculated and compared (left bar graph). Similar analysis comparing the dromotropic effect with changes in atrial refractoriness (AERP) was also performed (right bar graph). *P < 0.05 and **P < 0.01.
affected up to a 30-fold higher concentration (3000 nM, the maximum tested in the present study). Although some concern may be noted regarding the difference in species that were used to evaluate this point (rats in the present study for INO-8875 vs. guinea pigs in the study by Snowdy et al. (1999) for CVT-510), it is worth noting that in a previous report (Ueeda et al., 1991), the sensitivities of the A-V node and the coronary vasculature were directly compared in rats versus guinea pigs using a wide variety of selective and nonselective adenosine receptor agonists. The results of that study indicated an approximately 7-fold-higher sensitivity of the guinea pig A-V node compared with the rat, whereas coronary vasodilation was only 2-fold more sensitive in the guinea pig. Therefore, it seems unlikely that a difference in species can account for the total absence of change in coronary conductance that was noted in the present study in a concentration up to 30-fold higher than the concentration that affected the A-V node. In terms of magnitude, the A-V-nodal slowing effect of INO-8875 (~20% at 100 nM and a 25% increase at higher doses) can be interpreted as potent considering the relative insensitivity of the rat A-V node to adenosine (Froldi and Belardinelli, 1990; Ueeda et al., 1991). Indeed, the effect of 10 to 20 μM adenosine on the rat A-V node could not reach 15% in the study by Froldi and Belardinelli (1990) (see Fig. 2A in Froldi and Belardinelli, 1990). Moreover, since the noted results of Froldi and Belardinelli (1990) are based on S-H interval recordings whereas our data are based on S-V measurements, some underestimation of the actual effect on the A-V node is intrinsically inherent in our results. In conclusion, our isolated heart data are consistent with a functional activity of INO-8875 as a potent and highly selective A1Ra agonist.

In Vivo Findings. Invasive blood pressure measurements, which were obtained in the presence of continuous atrial pacing, are consistent with the isolated heart data and indicate a favorable hemodynamic profile of INO-8875. Nevertheless, at the higher cumulative doses of the drug (75 and 100 μg/kg; Fig. 2), a mild reduction in blood pressure was noted. Based on the isolated heart data, which did not reveal any effect on cardiac contractility at concentrations up to 30-fold higher than those causing a dromotropic effect, it is reasonable to speculate that this in vivo effect of INO-8875 may not be related to a direct effect on cardiac function.
The cardiac effects of adenosine are highly species-dependent (Froldi and Belardinelli, 1990). In rats, the typical supraventricular effects of A1R activation include a potent negative chronotropic effect as well as milder effects on A-V–nodal conduction and atrial refractoriness. Our results (Figs. 3 and 4) indicate that INO-8875 indeed caused all of these effects as expected. Direct in vivo comparison with CVT-510 indicates that both drugs (at equivalent concentrations) cause rather similar effects (Fig. 5A). However, more detailed analysis shows a higher functional selectivity of INO-8875 in causing a negative dromotropic effect compared with CVT-510 (Fig. 5, B and C). Similar differences in the functional activity of different adenosine analogs were already described in the past when the A1R-dependent effects of the drugs were compared in the atria versus the A-V node (i.e., shortening of the AERP vs. prolongation of the S-H interval). In this study, the differences were suggested to result from different receptor reserve in the different sites of action (Snowdy et al., 1999; Table 2). However, other possibilities, such as tissue selectivity of A1R binding for different agents, or differential activation of downstream effectors in the different tissues, cannot be excluded at the present. Interestingly, a higher functional selectivity for A-V–nodal effect compared with shortening of the AERP was correlated with higher A1R selectivity and was found to have the highest level for CVT-510 compared with adenosine, CCPA (2-chloro-N6-cyclopentyl-adenosine), and R-PIA [N6(2-phenylisopropyl)-adenosine R(−)-isomer] (Snowdy et al., 1999). Therefore, based on this correlative analysis of Snowdy et al. (1999), our EP results are consistent with a higher A1R selectivity of INO-8875 compared with CVT-510. Another difference between INO-8875 and CVT-510 was the prolonged effect of INO-8875. The source of this difference is not clear at present and may result from various factors such as differences in drug distribution and metabolism. However, since blood measurements of drug levels were not available in the present study, this issue will necessitate further and more focused analysis in future studies. In addition, it will be important to evaluate whether the prolonged action of INO-8875 noted here can be observed in other species as well.

Authorship Contributions

Participated in research design: Mor, Shalev, Dror, Etzion, Moran, Katz.
Conducted experiments: Mor, Shalev, Dror, Pikovsky.
Performed data analysis: Mor, Shalev, Dror, Pikovsky, Beharier, Etzion.
Wrote or contributed to the writing of the manuscript: Mor, Shalev, Beharier, Moran, Katz, Etzion.

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