

Hemodynamic and Neurohumoral Effects of Various Grades of Selective Adenosine Transport Inhibition in Humans

Implications for its Future Role in Cardioprotection

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Abstract

In 12 healthy male volunteers (27–53 yr), a placebo-controlled randomized double blind cross-over trial was performed to study the effect of the intravenous injection of 0.25, 0.5, 1, 2, 4, and 6 mg drafazine (a selective nucleoside transport inhibitor) on hemodynamic and neurohumoral parameters and ex vivo nucleoside transport inhibition. We hypothesized that an intravenous drafazine dosage without effect on hemodynamic and neurohumoral parameters would still be able to augment the forearm vasodilator response to intraarterially infused adenosine. Heart rate (electrocardiography), systolic blood pressure (Dinamap 1846 SX; Critikon, Portanije Electronica BV, Utrecht, The Netherlands) plasma norepinephrine and epinephrine increased dose-dependently and could almost totally be abolished by caffeine pretreatment indicating the involvement of adenosine receptors. Drafazine did not affect forearm blood flow (venous occlusion plethysmography). Intravenous injection of 0.5 mg drafazine did not affect any of the measured hemodynamic parameters but still induced a significant ex vivo nucleoside-transport inhibition of $31.5 \pm 4.1\%$ ($P < 0.05$ vs placebo). In a subgroup of 10 subjects the brachial artery was cannulated to infuse adenosine (0.15, 0.5, 1.5, 5, 15, and 50 $\mu\text{g}/100$ ml forearm per min) before and after intravenous injection of 0.5 mg drafazine. Forearm blood flow amounted 1.9 ± 0.3 ml/100 ml forearm per min for placebo and 1.8 ± 0.2 , 2.0 ± 0.3 , 3.8 ± 0.9 , 6.3 ± 1.2 , 11.3 ± 2.2 , and 19.3 ± 3.9 ml/100 ml forearm per min for the six incremental adenosine dosages, respectively. After the intravenous drafazine infusion, these values were 1.6 ± 0.2 ml/100 ml forearm per min for placebo and 2.1 ± 0.3 , 3.3 ± 0.6 , 5.8 ± 1.1 , 6.9 ± 1.4 , 14.4 ± 2.9 , and 23.5 ± 4.0 ml/100 ml forearm per min, respectively (Friedman ANOVA: $P < 0.05$ before vs after drafazine infusion). In conclusion, a 30–50% inhibition of adenosine transport significantly augments the fore-

arm vasodilator response to adenosine without significant systemic effects. These results suggest that drafazine is a feasible tool to potentiate adenosine-mediated cardioprotection in man. (*J. Clin. Invest.* 1995; 95:658–668.) Key words: nucleoside-transport inhibition • adenosine • autonomic nervous system • forearm • human pharmacology

Introduction

Adenosine has important cardioprotective properties that are mediated by stimulation of adenosine receptors, located on the outer cell membrane. In animals, infarct size is reduced when adenosine is infused either before ischemia or during the reperfusion period (1–3). Infusion of a selective adenosine receptor antagonist increases infarct size, indicating a role for endogenous adenosine as a cardioprotective autacoid (1). Adenosine is a mediator of ischemic preconditioning (1, 2, 4), defined as the increased tolerance of myocardium to a prolonged ischemic insult achieved by an initial brief exposure to ischemia and reperfusion (5). This phenomenon has originally been described in animals (6). Also, in humans, a preconditioning effect has been suggested (7, 8). At present, many potentially cardioprotective effects of adenosine are known, like inhibition of neutrophil activation with subsequent reduced free radical formation, inhibition of thrombocyte aggregation, vasodilation, presynaptic inhibition of norepinephrine release, opening of potassium channels, and repletion of purine stores (9). From a pharmacological point of view it seems of interest to develop agents with a comparable local effect on the myocardium (10). Within this concept, long-acting adenosine receptor agonists are not useful because these drugs elicit pharmacological effects, not only during ischemia but continuously, and not only in the myocardium but in nearly all organ systems, resulting in a large list of side effects (11, 12). Inhibition of the cellular uptake of extracellular adenosine might be an alternative approach to circumvent the disadvantages of intravenous adenosine infusions (13). In animals, this concept has been evaluated by the use of adenosine transport inhibitors like, for instance, dipyridamole. Dipyridamole appears to potentiate myocardial preconditioning (14). In humans, dipyridamole is not a suitable tool to potentiate the cardioprotective effect of endogenous adenosine (15, 16) and this might be due to its nonspecific actions like stimulation of prostacycline release and inhibition of phosphodiesterase (17, 18).

Recently, a new adenosine transport inhibitor, called drafazine, has become available for human investigation. This active (–)-enantiomer of the piperazine derivate R 75231 is a highly specific adenosine transport inhibitor with a tighter binding to the transporter and looser binding to plasma proteins

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Table I. Demographic Characteristics

<i>n</i> = 12	Mean (range)
Age (yr)	41 (27–53)
Weight (kg)	73.3 (62.3–88.5)
Length (cm)	179.5 (172.0–189.0)
Systolic blood pressure (mmHg)*	129 (112–140)
Diastolic blood pressure (mmHg)*	75 (62–88)
Heart rate (bpm) [‡]	63 (48–72)

* Auscultatory measurements with sphygmomanometer after 5 min of supine rest (systolic blood pressure: Korotkoff I; diastolic blood pressure: Korotkoff V). [‡] Measured by pulse frequency counting after 5 min of supine rest.

when compared with dipyridamole (19). In rabbits, R 75231 has shown to prevent death from catecholamine-induced cardiac toxicity (20) and to improve functional recovery after cardiac ischemia (21). In pigs, R 75231 reduces ischemia-induced arrhythmias (22). The present study explores the hemodynamic

53. Smits, P., T. Thien, and A. van't Laar. 1985. Circulatory effects of coffee port inhibition in man. Our results convincingly show that inhibition of adenosine transport by 70% or more increases blood pressure, heart rate, and plasma catecholamines. In contrast, low grade inhibition to 30–50% does not evoke any systemic hemodynamic changes but still potentiates the vasodilator effects of adenosine at a site of increased supply. These observations should be taken into account when draflazine is used as an “on demand” drug to potentiate the beneficial effects of adenosine as released during ischemia.

Methods

Subjects. After approval of the local ethics committee, 12 normotensive nonsmoking healthy Caucasian male volunteers signed written informed consent before participation in the study. Demographic data are shown in Table I. They had no history of hypertension, diabetes mellitus, or drug allergy, and did not use concomitant medication. In all volunteers a physical examination, routine laboratory investigation, and a 12-lead electrocardiography lead were performed to exclude cardiovascular, pulmonary, renal, liver, or neurologic disease.

Critical dose finding (part 1). In this study, the circulatory effects of six dosages of draflazine were investigated (0.25, 0.5, 1, 2, 4, and 6 mg).

First, in a double-blind randomized cross-over design, the hemodynamic and neurohumoral effects of placebo and 1, 2 or 4 mg draflazine were studied during four sessions per volunteer, that were separated by at least 1 wk. At least 1 wk thereafter, and after proven tolerance to the previous dosages, the effect of 6 mg draflazine was studied in a single-blind fashion. This 6-mg dose was not included in the randomized double-blind approach for safety reasons, on request of the ethics committee. After the randomization code had been broken, data analysis showed that all dosages, including the lowest, induced a statistically significant increase in heart rate. Therefore, the trial was prolonged with two visits per volunteer, separated by at least 1 wk, to study the hemodynamic effects of 0.25 and 0.5 mg draflazine in a double-blind randomized fashion.

Each experiment was performed in the morning after a 24-h abstinence from caffeinated products and an overnight fast of at least 10 h. Both arms were intravenously cannulated to infuse drug (left arm) and to collect blood (right arm). After an equilibration period of at least 45 min in supine position, placebo or drug was intravenously infused for

15 min using an automatic syringe infusion pump (type STC-521; Terumo Corp., Tokyo, Japan). From 5 min before until 60 min after the start of the infusion, heart rate was measured by standard electrocardiography and continuously recorded on tape (Oxford 9000 with ADgi preamplifier, band pass 0.05–40 Hz (–3 dB); Oxford Medical, Gorinchem, the Netherlands). Starting immediately before and continuing until 60 min after the start of the drug infusion, BP (Dinamap 1846 SX; Critikon, Portanje Electronica BV, Utrecht, The Netherlands) and forearm blood flow (FBF)¹ were measured at the left arm at 5- and 15-min intervals, respectively. FBF was registered by electrocardiography-triggered venous occlusion plethysmography using mercury-in-silastic strain gauges (EC4; D. E. Hokanson, Inc., Washington, DC). The upper arm collecting cuff was inflated using a rapid cuff inflator (E-20; D. E. Hokanson, Inc.). At least 1 min before the FBF measurements, the circulation of the left hand was occluded by inflation of a wrist cuff to 200 mmHg. FBF was recorded three times per min during 3 min.

Before the start of each intravenous injection, blood was collected for the measurement of the plasma caffeine concentration.

Immediately before and 15, 30, and 60 min after the start of the intravenous infusion of placebo, 1, 2, 4 or 6 mg draflazine, blood was collected to determine plasma epinephrine (EPI), NE, and adenosine concentration and to measure ex vivo adenosine transport inhibition (see Analytical Methods). Additionally, 15, 30, and 60 min after the start of the infusion, blood was collected to measure whole blood draflazine concentrations (see Analytical Methods). After administration of 0.25 and 0.5 mg draflazine, only adenosine transport inhibition and draflazine concentrations were determined.

Effect of the adenosine receptor antagonist caffeine on hemodynamic and neurohumoral responses to draflazine (part 2). To investigate the contribution of adenosine-receptor stimulation in the hemodynamic and neurohumoral responses to draflazine, in a subgroup of 10 subjects, informed consent was obtained to repeat the intravenous injection of 4 mg draflazine during adenosine-receptor blockade with caffeine, an adenosine-receptor antagonist in humans (11). 40 min before the start of the draflazine injection, a 10-min intravenous caffeine infusion was started (4 mg/kg), as described previously (23). Blood pressure, heart rate, catecholamines, plasma caffeine concentration, and adenosine transport inhibition were measured immediately before the start of the caffeine infusion, immediately before the start of the draflazine infusion, and at regular time intervals thereafter as mentioned above. Draflazine-induced changes in hemodynamic and neurohumoral parameters were compared with those as obtained in part 1 of this study.

Effect of 0.5 mg draflazine on the forearm vasodilator response to adenosine (part 3). In 10 of the 12 subjects, informed consent was obtained for a third study part to investigate the effect of 0.5 mg draflazine (critical dose) on the forearm vasodilator response to intraarterially infused adenosine. This dose was chosen because it was the highest dose of draflazine which did not affect baseline hemodynamic or neurohumoral parameters. Before the start of the study, the subjects were asked to abstain from caffeine-containing products for at least 24 h. All tests were performed in the supine position after an overnight fast, starting at 8:00 a.m. After administration of a 2% local anesthesia (Xylocaine; Astea Pharmaceutical Products Inc., Worcester, MA), the left brachial artery was cannulated with a 20-gauge catheter (Angiocath; Deseret Medical, Inc., Becton Dickinson and Co., Sandy, UT) for both intraarterial adenosine infusion (automatic syringe infusion pump, [type STC-521; Terumo Corp.]) and blood pressure recording (Hewlett Packard GmbH, Böblingen, Germany). Forearm blood flow was registered simultaneously on both forearms by electrocardiography triggered venous occlusion plethysmography as stated above.

The experiment started with the measurement of baseline FBF during placebo infusion (NaCl, 0.9%). The effect of six increasing dosages of adenosine (0.15, 0.5, 1.5, 5, 15, and 50 µg/100 ml forearm per min) were compared with placebo (NaCl 0.9%). Prolonged occlusion of the

1. Abbreviations used in this paper: EPI, epinephrine; FBF, forearm blood flow.

hand circulation can cause discomfort with subsequent effects on blood pressure and heart rate. Therefore, a 5-min rest was allowed between the placebo infusion and the first adenosine infusion and between the third and fourth adenosine infusion. 45 min after the last adenosine infusion, the intraarterial placebo infusion was repeated and followed by a 15-min intravenous injection of 0.5 mg drafazine in the right arm. Subsequently, the vasodilator response to adenosine was studied again. Additionally, the ex vivo adenosine transport inhibition was measured at regular time intervals. During all procedures, total infusion was adjusted to forearm volume as measured by water displacement and kept at a constant rate of 100 μ l/100 ml forearm per min. Placebo and each adenosine dosage were infused for 4 min.

In a separate group of six healthy, nonsmoking male volunteers the same protocol was performed except for the intravenous infusion of drafazine (time control study).

Analytical methods. Samples for determination of plasma caffeine concentration were analyzed with a reversed-phase HPLC method (limit of detection: 0.2 μ g/ml) (24).

Plasma levels of EPI and NE were determined simultaneously by an HPLC method with fluorimetric detection, in which catecholamines are concentrated from plasma by liquid-liquid extraction and derivatized with the selective fluorescent agent 1,2-diphenylethylenediamine before chromatography as has been described in more detail elsewhere (25). The interassay coefficients of variation in our laboratory are 7.2% and 8.5% at a mean concentration of 0.15 and 1.02 nmol/liter for EPI and NE, respectively ($n = 52$).

To measure plasma adenosine concentration, 2 ml blood was collected and directly mixed during collection with 2 ml blocker solution using a specially designed device. The blocker solution contained the adenosine deaminase inhibitor erythro-9(2-hydroxy-3-nonyl)-adenosine (10 μ M), the adenosine transport inhibitor dipyridamole (20 μ M) and the thrombocyte aggregation inhibitor indomethacin (2 mg/liter). Immediately after blood collection the blood/blocker mixture was centrifuged (model 3200; Eppendorf North America, Inc., Madison, WI) at 3,000 rpm for 2 min and the plasma was deproteinized with perchloric acid as described before (26). The extract was kept frozen at -20°C until the adenosine concentration was determined in duplicate by reversed-phase, high performance liquid chromatography using a nonlinear gradient.

In five subjects 12 ml blood/blocker mixture was collected before administration of 0.25 or 0.5 mg drafazine and divided into six equal portions. Adenosine standards were added to five separate blocker/mixture portions. The six portions were handled as stated above. The expected increases in adenosine concentration, as compared with the control blood/blocker mixture were 0.029, 0.052, 0.110, 0.210, and 0.405 μ M. The averaged recoveries were 115.9 ± 31.3 , 144.7 ± 24.6 , 109.6 ± 11.6 , 114.5 ± 15.6 , and $126.7 \pm 12.2\%$ for the five increasing standards, respectively. The detected increase in plasma adenosine concentration tended to be overestimated, although this was not statistically significant ($P = 0.3$, $n = 5$; Friedman ANOVA). In each individual a maximal correlation of 1.0 was observed between expected and measured adenosine concentration. The individual regression coefficients ranged from 1.0 to 1.7 (mean \pm SE: 1.3 ± 0.1). The intraassay variability, calculated as the coefficient of variation in the duplicate-determinations at baseline (five duplicate determinations in 11 subjects) was $16.2 \pm 2.2\%$. The short-term intraindividual variability in measured adenosine concentration, calculated as the coefficient of variation of the four measurements during placebo infusion, was $20.7 \pm 7.2\%$.

Ex vivo adenosine transport inhibition was measured by standardized incubation of erythrocytes with adenosine. 4 ml blood was drawn into a vial containing 1 ml acid, citrate, and dextrose (85 mM trisodium citrate, 65 mM citric acid and 20 g/liter glucose) and further handled as described before (22). The percent inhibition of adenosine transport (ATI %) was calculated as:

$$\text{ATI}\% = (A_x - A_0) \times 100 / (1 - A_0)$$

in which A_0 represents the adenosine concentration as proportion of the sum of the concentration of adenosine, inosine, and hypoxanthine as

determined in the sample collected just before the drug infusion and A_x represents this proportion as determined in the sample collected after the start of the drug infusion.

Whole-blood drafazine concentration was detected by HPLC (limit of detection: 5.0 ng/ml).

Drugs and solutions. Sterile solutions of drafazine or placebo in a formulation with 5% hydroxypropyl- β -cyclodextrine (Janssen Pharmaceutica Inc., Beerse, Belgium), were prepared with NaCl 0.9% on the morning of the study day by a specially trained research nurse, who was not otherwise involved in the practical performance of the trial. The randomization code was broken at the end of the trial, after all calculations on forearm blood flow were performed. Sterile solutions of caffeine (OPG Pharma, Utrecht, The Netherlands) and adenosine (Sigma Chemical Co., St. Louis, MO) were freshly prepared by the investigator with NaCl 0.9% as solvent.

Statistics. Heart rate, as derived from continuously recorded R-R intervals, was averaged for each consecutive 5-min interval. The 5-min interval just before drafazine or placebo infusion was taken as baseline. For the other hemodynamic and neurohumoral parameters, the values obtained just before the intravenous infusion were taken as baseline. For each experiment, the effect of drug administration was calculated at each time point as change from baseline. Since hemodynamic parameters appeared to be normally distributed ($P > 0.1$; Shapiro-Wilk test for normality), differences in changes from baseline between placebo and drafazine administration were assessed by an ANOVA for repeated measurements with the drug dosage, and time as within-subject factors. The neurohumoral parameters were not normally distributed ($P < 0.1$; Shapiro-Wilk test for normality). If an overall analysis by Friedman two-way nonparametric ANOVA showed significant differences in responses ($P < 0.05$, by means of Chi-square approximation), the paired Wilcoxon signed rank test was used to detect which drafazine dosages were different from placebo. During the intraarterial study (part 3), mean arterial pressure was measured continuously during each recording of FBF and averaged per FBF registration. Forearm vascular resistance was calculated from simultaneously measured mean arterial pressure and FBF and expressed as arbitrary units. Additionally, the ratio of each simultaneously measured FBF (FBF infused/FBF control arm) was calculated. FBFs, the calculated flow ratios and forearm vascular resistances obtained during each 4 min of placebo infusion or during the last 2 min of each drug infusion, were averaged to one value. Adenosine-induced effects were expressed both as absolute and percent change from preceding placebo infusion. The overall effect of drafazine on the adenosine dose response curve was analyzed by Friedman two-way nonparametric ANOVA. All results are expressed as mean \pm SE unless indicated otherwise; $P < 0.05$ (two sided) was considered to indicate statistical significance.

Results

Plasma caffeine levels were determined to check the compliance with respect to the caffeine abstinence. For part 1 of this study, in four subjects, the plasma caffeine concentration was below the limit of detection for all visits. In six subjects the plasma caffeine concentration remained below 1 mg/liter. In one subject caffeine was only detectable before the infusion of 1 mg drafazine (1.4 mg/liter) and in another subject, caffeine was detectable during five of the seven visits ranging from 0.5 to 1.6 mg/liter. For part 2 of this study, plasma caffeine was detectable in two subjects being 0.2 mg/liter for both. In part 3 of this study, plasma caffeine was detectable in three subjects being 0.8, 0.3, and 0.25 mg/liter. These plasma caffeine concentrations indicate a good compliance with regard to the caffeine abstinence. In the second part of the study, caffeine was administered in a dose of 4 mg/kg. 30 min after the caffeine infusion and immediately before the start of the drafazine infusion, plasma caffeine concentration was on average 5.7 ± 0.2 mg/liter.

Table II. Baseline Characteristics Before Each Drug Infusion (Mean \pm SE)

<i>n</i> = 12	Drafazoline dosage						
	placebo	0.25 mg	0.5 mg	1 mg	2 mg	4 mg	6 mg
Systolic BP (mmHg)	108.2 \pm 3.0	108.5 \pm 2.5	108.5 \pm 2.9	107.5 \pm 3.0	105.4 \pm 2.8	107.9 \pm 2.7	104.8 \pm 3.7
Diastolic BP (mmHg)	68.6 \pm 1.6	69.3 \pm 1.6	69.5 \pm 1.5	66.4 \pm 1.4	66.1 \pm 1.9	69.0 \pm 1.9	66.1 \pm 2.2
MAP (mmHg)	83.4 \pm 2.0	82.9 \pm 1.8	83.7 \pm 1.8	81.3 \pm 1.5	80.4 \pm 2.3	83.2 \pm 2.2	80.3 \pm 2.8
Heart rate (bpm)	60.6 \pm 2.0	61.7 \pm 1.5	60.8 \pm 1.9	60.6 \pm 2.3	61.5 \pm 1.9	59.8 \pm 1.8	61.4 \pm 2.5
FBF (ml/100 ml per min)	1.8 \pm 0.3	2.1 \pm 0.5	1.6 \pm 0.3	1.4 \pm 0.2	1.5 \pm 0.2	1.3 \pm 0.2	1.8 \pm 0.2
NE (nmol/liter)	1.2 \pm 0.1	—	—	1.1 \pm 0.1	1.2 \pm 0.1	1.3 \pm 0.2	1.3 \pm 0.2
EPI (nmol/liter)	0.10 \pm 0.02	—	—	0.11 \pm 0.02	0.09 \pm 0.01	0.09 \pm 0.01	0.08 \pm 0.02
Adenosine (μ mol/liter)	0.17 \pm 0.07	—	—	0.22 \pm 0.08	0.39 \pm 0.13	0.39 \pm 0.18	0.35 \pm 0.17

Subjective side effects to drafazoline. Up to 2 mg drafazoline, no subjective side effects occurred. In one subject, the 4-mg dose induced a slight headache and a feeling of dyspnea. Previous intravenous injection of caffeine completely prevented these complaints. In 8 of the 12 subjects, the 6-mg dose induced temporary subjective side effects ranging from a feeling of excitement ($n = 4$) and/or headache ($n = 4$) to nausea ($n = 3$) that was accompanied by vomiting and chest pain (without electrocardiographic changes) in one subject. These subjective side effects might have affected the recordings, resulting in less accurate measurements during and after the administration of 6 mg drafazoline as compared with the lower dosages.

Critical dose finding. Table II shows mean baseline values for all measured parameters. Overall, no statistically significant differences in baseline values were observed. Fig. 1 shows the time course of changes in blood pressure and heart rate during the varying drafazoline infusions as compared with placebo. Up to 0.5 mg, drafazoline did not induce a significant increase in heart rate. Heart rate increased by maximally 2.0 ± 1.1 for placebo and 1.8 ± 0.5 , 3.3 ± 0.8 , 6.1 ± 0.7 , 15.2 ± 2.3 , 29.2 ± 2.6 , and 32.5 ± 2.8 bpm for 0.25, 0.5, 1, 2, 4, and 6 mg drafazoline, respectively ($P < 0.01$ vs placebo for 1, 2, 4 and 6 mg drafazoline, $n = 12$). Up to 1 mg drafazoline, systolic blood pressure was not significantly affected. At higher dosages, the maximal mean systolic blood pressure response increased dose-dependently by 9.1 ± 1.4 , 12.5 ± 3.2 , and 18.0 ± 3.1 mmHg for 2, 4, and 6 mg drafazoline, respectively ($P < 0.05$ vs placebo, $n = 12$). The most important increase in systolic blood pressure occurred during the first 15 min after starting the drafazoline infusion. Neither diastolic blood pressure, mean arterial pressure nor forearm blood flow were significantly affected by drafazoline. When forearm vascular tone was expressed as vascular resistance (ratio of mean arterial pressure and forearm blood flow), still no effect of drafazoline on forearm vasculature could be observed.

The effect of drafazoline on plasma NE and EPI concentration are shown in Fig. 2. Starting with 2 mg, drafazoline induced a dose-dependent increase in plasma NE concentration that reached a maximum at 30 min after starting the infusion. At this time point, the increase in NE concentration was 0.7 ± 0.1 , 1.0 ± 0.2 , and 1.7 ± 0.3 nmol/liter for 2, 4, and 6 mg drafazoline, respectively ($P < 0.05$ vs placebo, $n = 12$).

After infusion of 1 mg drafazoline, the EPI concentration was not significantly affected. Infusion of 2 mg drafazoline induced a small increase in EPI concentration of 0.02 ± 0.01 nmol/liter at 15 min after starting the drafazoline infusion ($P < 0.05$ vs placebo, $n = 12$). Up to 60 min after the start of the infusion

of 4 or 6 mg drafazoline, the EPI concentration continuously increased maximally by 0.15 ± 0.03 and 0.31 ± 0.06 nmol/liter, respectively ($P < 0.05$ vs placebo, $n = 12$).

The course of ex vivo adenosine transport inhibition is shown in Fig. 3. 15 min after the start of each drafazoline infusion, maximal adenosine transport inhibition was achieved being 0.2 ± 0.7 for placebo and 10.2 ± 2.3 , 31.5 ± 4.1 , 70.4 ± 1.5 , 80.6 ± 0.9 , 89.6 ± 1.1 , and $92.6 \pm 0.6\%$ for 0.25, 0.5, 1, 2, 4, and 6 mg drafazoline, respectively ($P < 0.05$ for each dosage vs placebo). 60 min after starting the infusion, there still remained

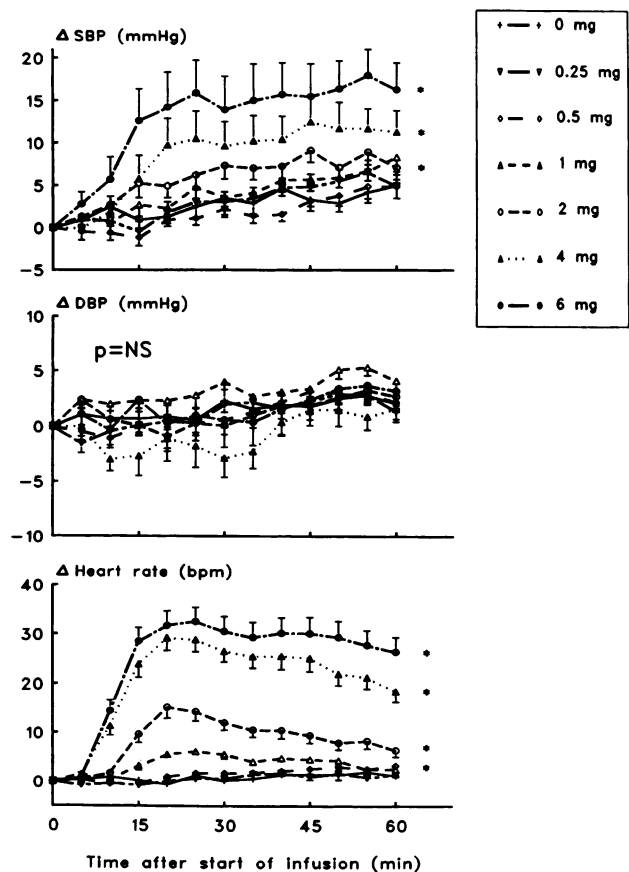


Figure 1. Effect of drafazoline on systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate. *, Significant difference with placebo ($n = 12$).

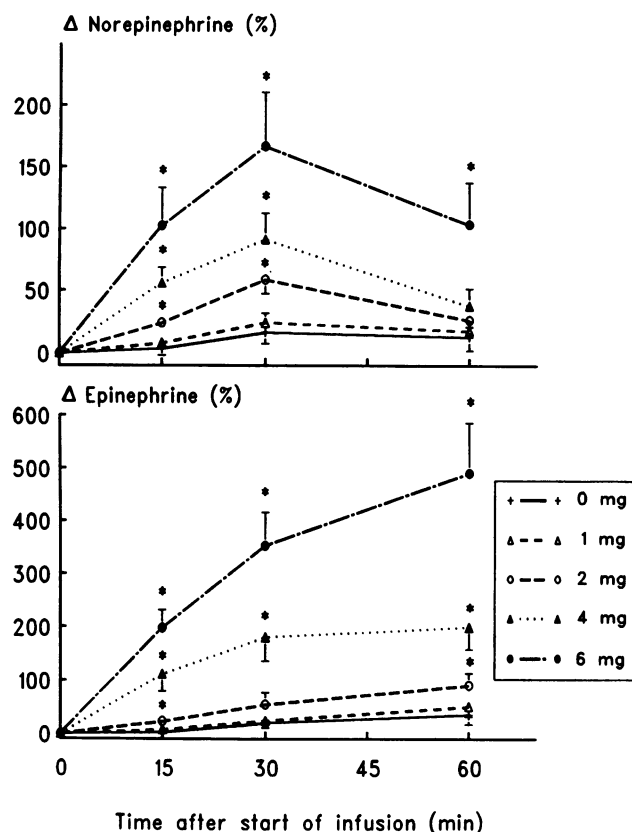


Figure 2. Effect of draflazine on plasma catecholamines. *, Significant difference with placebo ($n = 12$).

a significant adenosine transport inhibition being -0.9 ± 0.8 for placebo and 6.0 ± 0.8 , 14.2 ± 1.1 , 22.3 ± 3.0 , 42.5 ± 1.7 , 59.8 ± 1.9 , and $68.3 \pm 2.0\%$ for the six draflazine doses, respectively ($P < 0.05$ for each dosage vs placebo). For each subject, the relation between drug concentration and ex vivo adenosine transport inhibition was assessed by computer assisted curve fitting using the Hill equation (Fig. 4). For this analysis, minimal and maximal ex vivo adenosine transport inhibition was set constant at 0 and 100% respectively. The best fitting drug concentration with 50% ex vivo adenosine transport inhibition and Hill coefficient were 74.1 ± 2.4 ng/ml (range: 55.5–88.1 ng/ml; $n = 12$) and 3.2 ± 0.1 (range: 2.5–4.0; $n = 12$), respectively. According to this model, $95 \pm 1\%$ of the variation in ex vivo nucleoside transport inhibition could on average be explained by the blood draflazine concentration.

For each subject, the relation between draflazine-induced heart rate response (difference with response after placebo infusion) and ex vivo adenosine transport inhibition was assessed at 15, 30, and 60 min after starting the draflazine infusion (Fig. 5). Up to $\sim 50\%$ inhibition of adenosine transport, heart rate was unaffected. An equation in the form of $Y = AX^3$ appeared to describe the relation between heart rate response and adenosine transport inhibition most accurately. Nonlinear regression analysis revealed a mean value for A of $(3.0 \pm 0.3) \cdot 10^{-5}$, $(6.1 \pm 0.4) \cdot 10^{-5}$, and $(7.7 \pm 0.8) \cdot 10^{-5}$ at 15, 30 and 60 min, respectively. With this model, 77 ± 2 , 90 ± 2 , and $81 \pm 3\%$ of the intraindividual variation in heart rate response could be explained by ex vivo nucleoside transport inhibition at 15, 30 and 60 min after starting the draflazine infusion, respectively.

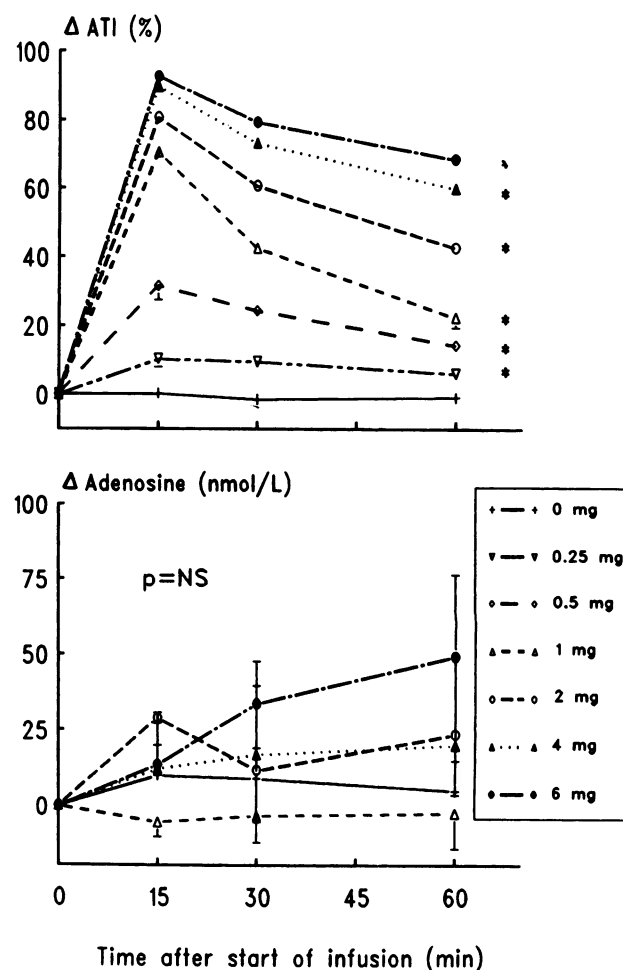


Figure 3. Effect of draflazine on ex vivo nucleoside transport inhibition (top) and plasma-adenosine concentration (bottom), respectively. *, Curves that were significantly different from placebo at all time points ($n = 12$).

Apart from ex vivo adenosine transport inhibition, Fig. 3 shows the effect of draflazine on plasma adenosine concentration. Draflazine did not induce significant changes in plasma adenosine concentration ($P > 0.5$, $n = 11$).

Effect of the adenosine receptor antagonist caffeine on hemodynamic and neurohumoral responses to draflazine. Baseline values after caffeine infusion were significantly increased for systolic, diastolic, and mean arterial pressure and plasma epinephrine concentration and reduced for heart rate when compared with baseline values without caffeine pretreatment (Table III). Fig. 6 shows the effect of intravenous caffeine infusion (4 mg/kg) on the course of systolic blood pressure, heart rate, epinephrine, and norepinephrine after draflazine infusion. Caffeine pretreatment reduced the heart rate response to the 4-mg dose of draflazine from 29.3 ± 3.8 bpm ($32.6 \pm 3.0\%$) to 8.8 ± 1.0 ($13.9 \pm 1.4\%$) ($P < 0.01$ vs placebo and vs draflazine without caffeine pretreatment). The systolic blood pressure response to draflazine was reduced from 11.7 ± 3.7 mmHg ($10.7 \pm 3.3\%$) without caffeine pretreatment to 4.9 ± 1.7 mmHg ($4.3 \pm 1.5\%$) after caffeine pretreatment ($P = \text{NS}$ versus placebo, $P < 0.05$ vs draflazine without caffeine pretreatment).

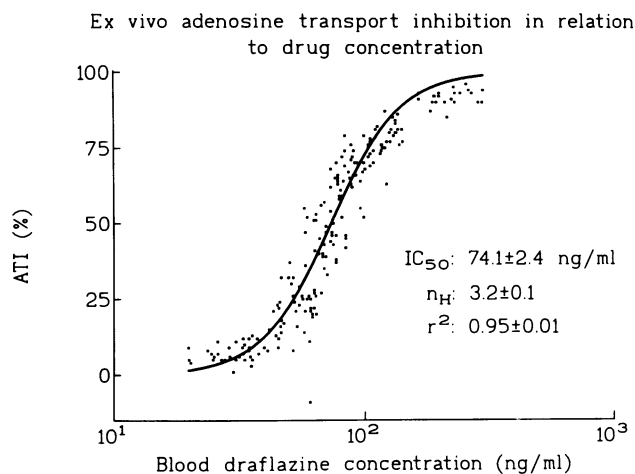


Figure 4. Relation between percent inhibition in adenosine transport and whole-blood draflazine concentration. This relation was evaluated for each subject using the Hill equation. For each individual, the best fitting Hill coefficient and IC_{50} (draflazine concentration that exhibits 50% of the maximal effect) were calculated by computer-assisted curve fitting ($n = 12$).

Caffeine pretreatment reduced the increase in NE concentration after the 4-mg dose of draflazine from 1.1 ± 0.23 nmol/liter ($105.6 \pm 22.6\%$) to 0.2 ± 0.1 nmol/liter ($23.7 \pm 4.1\%$) ($P = NS$ vs placebo and $P < 0.05$ vs draflazine infusion without caffeine pretreatment). The increase in plasma epinephrine concentration after 4 mg draflazine was reduced from 0.14 ± 0.04 nmol/liter ($177.0 \pm 42.2\%$) without caffeine pretreatment to 0.09 ± 0.02 nmol/liter ($73.1 \pm 12.9\%$) after caffeine pretreatment ($P = NS$ vs placebo and $P < 0.05$ vs draflazine without caffeine pretreatment).

Draflazine induced a significant inhibition of nucleoside transport of 89.3 ± 1.1 , 71.5 ± 1.3 , and $59.4 \pm 1.8\%$ at 15, 30, and 60 min after the start of the draflazine infusion, respectively, and these figures did not significantly differ from adenosine transport inhibition as observed after infusion of 4 mg draflazine without caffeine pretreatment.

Effect of 0.5 mg draflazine on forearm vasodilator response to adenosine. Intraarterial infusion of adenosine increased forearm blood flow dose dependently. The baseline forearm blood flow was 1.9 ± 0.3 and 1.6 ± 0.2 ml/100 ml forearm per min in the infused and control arm, respectively. During the subsequent six incremental adenosine infusions, the forearm blood flow in the infused arm amounted to 1.8 ± 0.2 , 2.0 ± 0.3 , 3.8 ± 0.9 , 6.3 ± 1.2 , 11.3 ± 2.2 , and 19.3 ± 3.9 ml/100 ml per min, respectively. In the control arm, forearm blood flow remained unchanged. Draflazine did not affect forearm blood flow: in the infused arm, forearm blood flow was 1.8 ± 0.2 and 1.6 ± 0.2 ml/100 ml per min immediately before and after the draflazine infusion, respectively (control arm: 1.3 ± 0.1 and 1.2 ± 0.2 ml/100 ml per min). However, draflazine significantly augmented the adenosine-induced changes in forearm blood flow. After the draflazine infusion, the forearm blood flow in the infused arm amounted 2.1 ± 0.3 , 3.3 ± 0.6 , 5.8 ± 1.1 , 6.9 ± 1.4 , 14.4 ± 2.9 , and 23.5 ± 4.0 ml/100 ml forearm per min during the six incremental adenosine infusions, respectively ($P < 0.05$ vs adenosine infusions before draflazine treatment). In the control arm, forearm blood flow remained unchanged. Fig. 7 shows the effect of

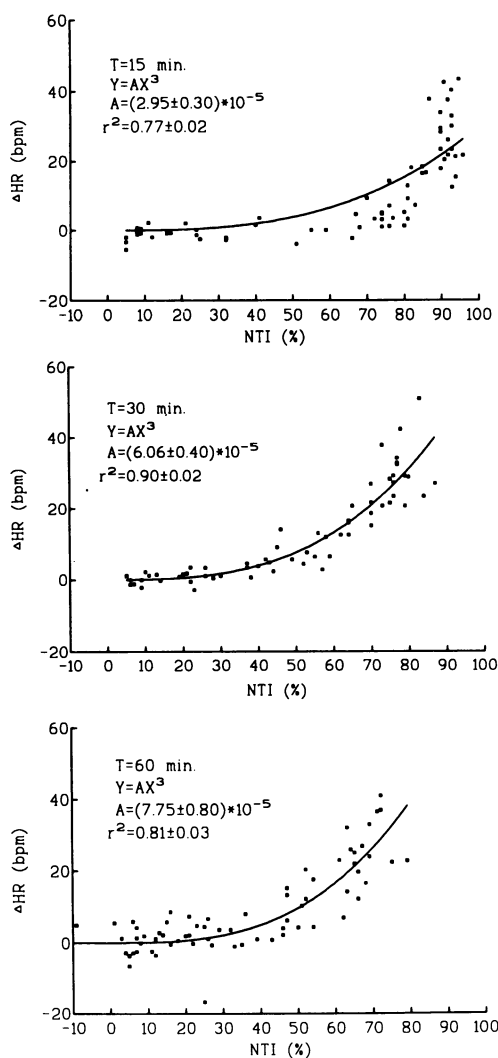


Figure 5. Relation between heart rate response and adenosine transport inhibition. At each time point and for each individual, this relation was evaluated by computer assisted curve fitting. An equation in the form of $Y = AX^3$ appeared to result in the best fitting curves. The individual values for A and r^2 (squared correlation coefficient) were averaged and are depicted in the figure as mean \pm SE ($n = 12$).

draflazine on the forearm vasodilator response to adenosine, expressed as percent change in forearm vascular resistance. The same effect of draflazine was observed when results were expressed as forearm vascular resistance or flow ratio both expressed as absolute as well as percent changes from baseline. The time control study did not reveal a significant change in adenosine-induced forearm vasodilation, excluding significant carry-over effects (see lower panel of Fig. 7). Heart rate was 57.5 ± 1.7 and 58.4 ± 1.4 beats per minute (bpm) immediately before and after draflazine infusion, respectively ($P > 0.2$), confirming that the used draflazine dosage did not induce systemic effects.

Ex vivo nucleoside transport inhibition was 47.9 ± 3.2 , 21.6 ± 1.6 , and $15.0 \pm 1.5\%$ at the end of the draflazine infusion, and at the end of the third and sixth intraarterial adenosine dose, respectively.

Table III. Effect of Caffeine on Baseline Parameters

	Before placebo		Before 4 mg draflazine	
	Without caffeine pretreatment	With caffeine pretreatment	Without caffeine pretreatment	With caffeine pretreatment
<i>n</i> = 10				
Systolic BP (mmHg)	108.8±3.4	108.5±3.1	113.0±2.1**	
Diastolic BP (mmHg)	68.9±1.9	68.9±2.3	73.1±1.6**	
MAP (mmHg)	83.8±2.3	83.5±2.5	87.3±1.7†	
Heart rate (bpm)	59.0±2.2	58.4±1.4	53.9±1.4**	
NE (nmol/liter)	1.2±0.1	1.3±0.2	1.0±0.1**	
EPI (nmol/liter)	0.11±0.02	0.09±0.02	0.14±0.02§	

* and † Significantly different from baseline values before placebo and 4 mg (without caffeine treatment), respectively, § Friedman two-way ANOVA: *P* = 0.08; Wilcoxon matched-pairs, signed-ranks test: *P* = 0.03 vs 4 mg draflazine (without caffeine pretreatment).

Discussion

This study was aimed at finding a draflazine dosage without unwanted hemodynamic and neurohumoral effects that still potentiates the beneficial effects of adenosine at sites of increased formation. Therefore, a dose-response trial was performed that revealed a dose-dependent increase in systolic blood pressure, heart rate, and plasma catecholamines in subjects that refrained from caffeine-containing products for at least 24 h. Preceding caffeine infusion abolished all draflazine-mediated effects, confirming that they were mediated by adenosine receptor stimulation. Heart rate appeared to be the most sensitive parameter

to detect unwanted effects. An intravenous dosage of 0.5 mg draflazine did not affect heart rate, but still induced an ex vivo transport inhibition by ~ 30%. To test the hypothesis that this critical dose is able to potentiate the vasodilatory effects of adenosine in humans at sites of increased formation, the perfused forearm technique was used as a model of local adenosine formation. Intravenous infusion of 0.5 mg draflazine appeared to augment the forearm vasodilator response to adenosine three-fold, suggesting that a low grade adenosine transport inhibition is a feasible approach to exploit the beneficial effects of endogenous adenosine in humans.

Hemodynamic and neurohumoral effects of draflazine. With regard to the cardiovascular system, adenosine can induce direct and indirect effects. The direct effects of adenosine include negative inotropic, chronotropic, and dromotropic effects (27), relaxation of vascular smooth muscle (except in the pulmonary and renal vascular bed where vasoconstriction is observed) (28, 29), pre- and postsynaptic inhibition of adrenergic neurotransmission (30, 31), reduction of renal renin release (32–34), stimulation of vascular angiotensin II production (35) and inhibitory effects on cardiovascular centers in the brainstem, except for the nucleus tractus solitarius that is excited by adenosine, resulting in an increased sensitivity of the baroreflex (36). These effects are thought to be involved in the hypotensive response to intravenously administered adenosine as observed in animals and anesthetized humans. The indirect effects are mediated by adenosine-induced stimulation of afferent nerves, including renal (37, 38) and myocardial afferent nerves (39a), carotid and aortic chemoreceptors (12, 39), and forearm (muscle) afferent nerves (40). Stimulation of these afferents results in activation of the sympathetic nervous system and respiratory system (11, 41) and a subsequent increase in systolic blood

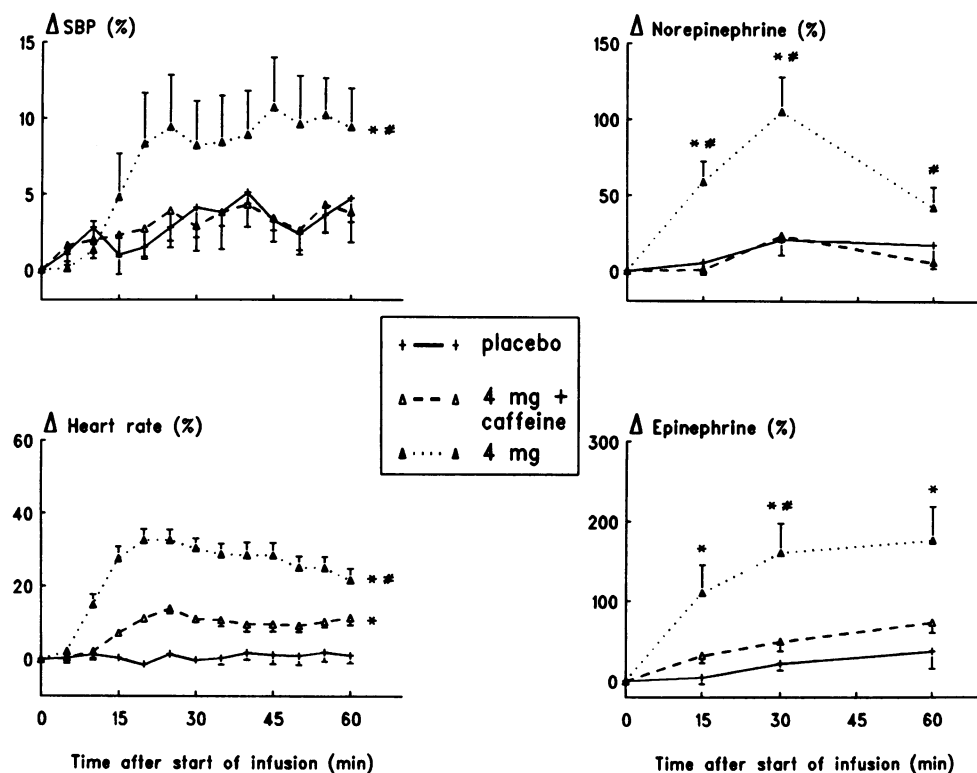


Figure 6. Effect of caffeine pretreatment (4 mg/kg) on the draflazine (4 mg)-induced increases in systolic blood pressure (SBP), heart rate, NE, and EPI. *And †, significant differences with placebo or draflazine after caffeine pretreatment, respectively (*n* = 10).

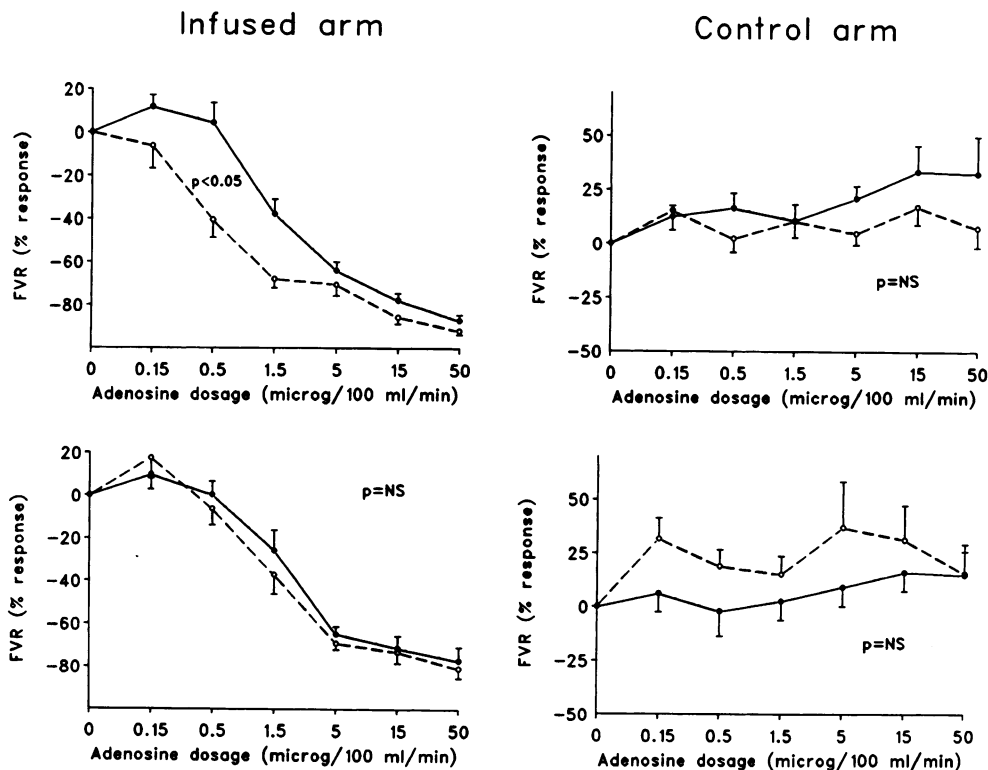


Figure 7. Adenosine-induced forearm vasodilation expressed as FVR (quotient of MAP and FBF) in infused and control arm. (Top) effect of intravenous draflazine treatment ($n = 10$); — before draflazine infusion; --- after draflazine infusion. (Bottom) Time control study ($n = 6$); — first dose response curve; --- second dose response curve. P values indicate level of significance for the difference between both curves ($n = 10$).

pressure, and plasma renin activity (11, 12, 41–45). The increase in heart rate is probably mediated by concomitant deactivation of the parasympathetic nervous system since it can be antagonized by atropine, but not by propranolol (46). These indirect effects of intravenous adenosine infusion are dependent on an intact autonomic reflex arc (12), which probably explains why these effects are blunted in anesthetized humans and animals (47–49). The increase in systolic blood pressure is not always observed during intravenous infusion of adenosine in healthy volunteers (46, 50–52). This apparent discrepancy in the literature can be explained by differences in caffeine abstinence which is always relatively short or totally absent in the studies in which no increase in systolic blood pressure is observed. After ingestion of two cups of regular coffee, plasma caffeine concentrations are in the range of 4–5 mg/liter (53), which is sufficiently high to antagonize the hemodynamic effects of intravenous adenosine infusion (11). The plasma half-life time of caffeine after ingestion of two cups of coffee ranges from 2 to 8.5 h (53). Therefore, a 24-h period of caffeine abstinence is important to prevent underestimation of adenosine-induced hemodynamic and neurohumoral effects. An increase in systolic blood pressure, heart rate, and plasma catecholamines has also been observed in resting humans after administration of the adenosine transport inhibitor dipyridamole (23, 54) and could be inhibited by previous administration of caffeine or theophylline (23, 55), suggesting that adenosine is formed under baseline conditions. In addition to the variable oral bioavailability of dipyridamole and its lack of specificity (17, 18, 56, 57), these excitatory effects of dipyridamole may be an explanation for its disappointing effect on cardiovascular mortality in large trials (15, 16).

It was expected from the previous experiments with adenosine and dipyridamole in healthy, conscious human volunteers,

that draflazine, too, could induce hemodynamic and neurohumoral effects that would be unfavorable in patients with ischemic heart disease. The present study indeed confirms most of these observations and substantiates them by showing their dependency on the degree of ex vivo adenosine transport inhibition. Moreover, we observed an increase in both EPI and NE during high degrees of selective adenosine transport inhibition, supporting the involvement of the sympathetic nervous system in this excitatory response. The increase in systolic blood pressure without a change in mean arterial pressure excludes baroreflex activation as a possible mechanism for the increased plasma catecholamine concentrations. Activation of sympathetic tone by draflazine-induced myocardial ischemia is very unlikely because the study was performed in healthy subjects without a history of cardiovascular disease. Additionally, continuous electrocardiography did not demonstrate myocardial ischemia. However, a yet unknown direct effect of draflazine on the kinetics of catecholamines cannot be excluded.

Effect of caffeine pretreatment on draflazine-induced hemodynamic and neurohumoral responses. Caffeine pretreatment almost completely abolished the effect of draflazine on systolic blood pressure, heart rate, and plasma catecholamines. This antagonism occurred at a plasma caffeine concentration of 5.7 mg/liter, a concentration that can occur after drinking one cup of coffee (53). The property of caffeine as a competitive adenosine receptor antagonist has been well documented in human in vivo studies (11, 29, 58, 59). Therefore, the observed antagonism of draflazine-induced hemodynamic and neurohumoral responses by caffeine indicates the involvement of adenosine receptors in draflazine-induced effects.

30 min after caffeine pretreatment, baseline values of systolic, diastolic, mean arterial pressure, and plasma epinephrine concentration were increased and baseline heart rate was re-

duced. These findings are in agreement with previous reports about the effect of caffeine (24). The changes in baseline blood pressure, heart rate, and epinephrine that were associated with caffeine pretreatment could have interfered with the subsequent effects of draflazine infusion. However, the magnitude of the increased baseline levels after caffeine are almost negligible when compared with the effects of draflazine.

Draflazine-induced adenosine transport inhibition was not significantly affected by caffeine pretreatment. Therefore, the effect of caffeine on draflazine-induced hemodynamic and neurohumoral responses cannot be explained by differences in the degree of adenosine transport inhibition.

In theory, inhibition of 5'-nucleotidase by caffeine could have diminished the formation of extracellular adenosine (60). However, inhibition of the ecto-5'-nucleotidase, as obtained from rat brain, occurs at a caffeine concentration of 1.0 mM which is considerably higher than the plasma caffeine concentration of 5.7 mg/liter (equivalent to 0.03 mM) as observed in this study. Therefore, caffeine-induced inhibition of extracellular adenosine formation is not likely in the present study.

Draflazine and plasma adenosine concentration. Draflazine failed to increase plasma adenosine concentrations. Two possible explanations will be discussed: (a) the lack of an observed plasma adenosine increase is due to a measurement error and (b) adenosine concentrations are increased in the interstitium only.

(a) The precision of the adenosine determinations in the present study does not essentially differ from that reported by others (61). As stated in Methods, the recovery of adenosine added to plasma was high and appeared to be linear within the physiological range. Therefore, a systematic measurement error is unlikely. Both German et al. and Sollevi et al. observed a doubling in plasma adenosine concentration after administration of the adenosine transport inhibitor dipyridamole (55, 62). However, in both studies, adenosine formation, uptake, and breakdown after blood sampling were not optimally antagonized. German et al. did not use a blocker solution (62, 63), while Sollevi et al. used a blocker solution that was mixed with blood immediately after, instead of during, blood sampling (55). Therefore, in these studies, baseline plasma adenosine concentrations may have been underestimated. The administered dipyridamole may have affected adenosine uptake by erythrocytes after blood sampling resulting in an artificial increase in measured plasma adenosine concentration. The present study does not rule out a small draflazine-induced increase in plasma adenosine levels since intrasubject variability of the adenosine detection method is relatively high, especially when compared with detection methods of other endogenous compounds like, for instance, (nor)epinephrine. Plasma adenosine concentrations were measured up to 60 min after the start of the draflazine infusion. Therefore, our data do not exclude a detectable increase in plasma adenosine levels after this time point or during more sustained nucleoside transport inhibition.

(b) The most likely explanation for adenosine receptor-mediated hemodynamic and neurohumoral effects without any change in plasma adenosine concentrations is that selective adenosine transport inhibition results in accumulation of endogenous adenosine within the interstitium. As discussed in detail elsewhere, the vascular endothelium may act as a strong barrier against passage of metabolites (64–66). In normal tissue, the nucleoside transporter bypasses this barrier but nucleoside transport inhibition may transform the endothelium to a functional

barrier preventing adenosine to leave the interstitial space (66). Interstitial adenosine may originate from ATP that is released from sympathetic nerve endings (67) and subsequently dephosphorylated to adenosine by ecto-phosphatases and ecto-5'-nucleotidase located on the membranes of vascular smooth muscle cells and endothelium. Since ATP is released from sympathetic nerve endings by exocytosis, this source of adenosine is not blocked by adenosine transport inhibition.

Ex vivo nucleoside transport inhibition in relation to draflazine concentration and heart rate response. The relation between blood drug concentration and ex vivo adenosine transport inhibition could well be described by the Hill equation. Since draflazine concentration was measured in whole blood, this is not necessarily a reflection of draflazine concentration at the level of the nucleoside transporter in the erythrocyte membrane. Therefore, the accuracy and pharmacological importance of the Hill coefficient and the 50% inhibiting drug concentration, as determined in this study, should not be overvalued. Nevertheless, the high correlation between ex vivo nucleoside transport inhibition and whole-blood draflazine concentration provides an experimental base to use this functional parameter as a tool to monitor draflazine treatment. This is further supported by the high correlation between ex vivo adenosine transport inhibition and heart rate response. The relation between heart rate response and ex vivo nucleoside transport inhibition became steeper over time, reflecting a time delay between draflazine-induced ex vivo nucleoside transport inhibition and its effect on heart rate. Two processes may account for this delay. First, intravenous infusion of draflazine rapidly inhibits nucleoside transport at the erythrocyte membrane while diffusion of draflazine into the interstitial space and inhibition of adenosine transport in the vascular wall may take more time. Second, it probably takes some time for endogenous adenosine to accumulate and to reach concentrations that are sufficiently high to evoke its full effect on heart rate.

Effect of 0.5 mg draflazine on forearm vasodilator response to adenosine. The forearm vasodilator response to adenosine was significantly augmented by a draflazine dosage that did not induce unwanted hemodynamic or neurohumoral side effects. These results indicate that the cellular uptake of luminal adenosine is inhibited, and that the increased adenosine concentrations are available for stimulation of adenosine receptors that mediate vascular relaxation. The reduced augmentation of adenosine-mediated vasodilation at the three highest adenosine concentrations, could be the result of the decreased nucleoside transport inhibition at the time that the highest adenosine concentration was infused. This is supported by the ex vivo nucleoside transport inhibition which amounted to only 15% at the end of the sixth adenosine infusion, compared with 22% at the end of the third adenosine infusion.

Limitations of the study and conclusions. Three limitations of the study should be mentioned. First, the studies were performed in healthy volunteers. We are not informed about the influence of age and varying disease states on the systemic effects of draflazine. Therefore, it cannot be excluded that in the elderly or in patients with conditions like hypertension and cardiovascular disease, a different dose-response pattern to draflazine exists. Second, the hemodynamic and neurohumoral responses to the adenosine transport inhibitor draflazine were studied after a 24-h abstinence from caffeine-containing products. This abstinence period was included to prevent adenosine antagonism by caffeine. In clinical practice, such a situation will

almost never occur. Adenosine receptor upregulation during caffeine use may have occurred and could have exaggerated the effects of adenosine uptake inhibition after short-term abstinence from caffeine (68–70). Third, we studied the acute effects of short-term nucleoside transport inhibition. Our data do not exclude the development of tolerance to increased levels of endogenous adenosine that may arise during long-term nucleoside transport inhibition.

Before it can definitely be concluded that drafazine is a feasible tool to potentiate the cardioprotective effects of adenosine in humans, our findings should be confirmed in the coronary vasculature. Furthermore, the present observation of in vivo adenosine transport inhibition holds for intraarterially applied adenosine and should be verified for interstitially formed adenosine as during ischemia.

In conclusion, in healthy male volunteers, drafazine is able to inhibit adenosine transport significantly both *ex vivo* as well as *in vivo*. Heart rate appeared to be the most sensitive parameter to detect systemic hemodynamic or neurohumoral effects. When a drafazine dosage is injected that does not affect heart rate, the forearm vasodilator response to adenosine is still potentiated. Clinical trials are needed to investigate the possible beneficial effect of adenosine uptake inhibition in the prevention of ischemic injury. In these trials, caffeine intake should be monitored, the optimal drafazine dosage can be determined using heart rate as a marker of unwanted systemic effects, and *ex vivo* adenosine transport inhibition can be used as a tool to monitor efficacy and compliance.

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References

- Toombs, C. F., D. S. McGee, W. E. Johnston, and J. Vinten-Johansen. 1992. Myocardial protective effects of adenosine: infarct size reduction with pretreatment and continued receptor stimulation during ischemia. *Circulation*. 86:986–994.
- Thornton, J. D., G. S. Liu, R. A. Olsson, and J. M. Downey. 1992. Intravenous pretreatment with A1-selective adenosine analogues protects the heart against infarction. *Circulation*. 85:659–665.
- Norton, E. D., E. K. Jackson, M. B. Turner, R. Virmani, and M. B. Forman. 1992. The effects of intravenous infusions of selective adenosine A1-receptor and A2-receptor agonists on myocardial reperfusion injury. *Am. Heart J.* 123:332–338.
- Zhao, Z. Q., D. S. McGee, K. Nakanishi, C. F. Toombs, W. E. Johnston, M. S. Ashar, and J. Vinten-Johansen. 1993. Receptor-mediated cardioprotective effects of endogenous adenosine are exerted primarily during reperfusion after coronary occlusion in the rabbit. *Circulation*. 88:709–719.
- Mullane, K. 1992. Myocardial preconditioning: part of the adenosine revival. *Circulation*. 85:845–847.
- Murry, C. E., R. B. Jennings, and K. A. Reimer. 1986. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation*. 74:1124–1136.
- Deutsch, E., M. Berger, W. G. Kussmaul, J. W. Hirshfeld, H. C. Herrmann, and W. K. Laskey. 1990. Adaptation to ischemia during percutaneous transluminal coronary angioplasty. *Circulation*. 82:2044–2051.
- Yellon, D. M., A. M. Alkhalaf, and W. B. Pugsley. 1993. Preconditioning the human myocardium. *Lancet*. 342:276–277.
- Forman, M. B., C. E. Velasco, and E. K. Jackson. 1993. Adenosine attenu-

ates reperfusion injury following regional myocardial ischemia. *Cardiovasc. Res.* 27:9–17.

- Cohen, M. V., and J. M. Downey. 1993. Ischaemic preconditioning: can the protection be bottled? *Lancet*. 342:6.
- Smits, P., P. Boekema, T. Thien, R. de Abreu, and A. van't Laar. 1987. Evidence for an antagonism between caffeine and adenosine in the cardiovascular system in man. *J. Cardiovasc. Pharmacol.* 10:136–143.
- Biaggioni, I., B. Olafsson, R. M. Robertson, A. S. Hollister, and D. Robertson. 1987. Cardiovascular and respiratory effects of adenosine in conscious man. Evidence for chemoreceptor activation. *Circ. Res.* 61:779–786.
- Van Belle, H. 1993. Specific metabolically active antiischemic agents: adenosine and nucleoside transport inhibitors. In *Cardiovascular Pharmacology and Therapeutics*. B. Singh, V. Dzan, P. Vanhoutte, and R. Woosley, editors. Churchill-Livingstone Inc., New York. 217–235.
- Miura, T., T. Ogawa, T. Iwamoto, K. Shimamoto, and O. Iimura. 1992. Dipyrindamole potentiates the myocardial infarct size-limiting effect of ischemic preconditioning. *Circulation*. 86:979–985.
- PARIS II Research Group. 1986. Persantine-aspirin reinfarction study. Part II. Secondary coronary prevention with persantine and aspirin. *J. Am. Coll. Cardiol.* 7:251–269.
- The Persantine-Aspirin Reinfarction Study Research Group. 1980. Persantine and aspirin in coronary heart disease. *Circulation*. 62:449–461.
- Blass, K. E., H. U. Block, W. Forster, and K. Ponick. 1980. Dipyrindamole: a potent stimulator of prostacyclin (PGI₂) biosynthesis. *Br. J. Pharmacol.* 68:71–73.
- McElroy, F. A., and R. B. Philp. 1975. Relative potencies of dipyrindamole and related agents as inhibitors of cyclic nucleotide phosphodiesterases: possible explanation of mechanism of inhibition of platelet function. *Life Sci.* 17:1479–1493.
- Van Belle, H., and P. A. J. Janssen. 1991. Comparative pharmacology of nucleoside transport inhibitors. *Nucleosides & Nucleotides*. 10:975–982.
- Van Belle, H., W. Verheyen, K. V. Donck, P. A. J. Janssen, and J. I. S. Robertson. 1992. Prevention of catecholamine-induced cardiac damage and death with a nucleoside transport inhibitor. *J. Cardiovasc. Pharmacol.* 20:173–178.
- Masuda, M., A. Demeulemeester, C. C. Chen, M. Hendrikx, H. Van Belle, and W. Flameng. 1991. Cardioprotective effects of nucleoside transport inhibition in rabbit hearts. *Ann. Thorac. Surg.* 52:1300–1305.
- Wainwright, C. L., J. R. Parratt, and H. Van Belle. 1993. The antiarrhythmic effects of the nucleoside transporter inhibitor, R75231, in anesthetized pigs. *Br. J. Pharmacol.* 109:592–599.
- Smits, P., C. Straatman, E. Pijpers, and T. Thien. 1991. Dose-dependent inhibition of the hemodynamic response to dipyrindamole by caffeine. *Clin. Pharmacol. & Ther.* 50:529–537.
- Smits, P., H. Hoffmann, T. Thien, H. Houben, and A. van't Laar. 1983. Hemodynamic and humoral effects of coffee after beta-1-selective and nonselective beta-blockade. *Clin. Pharmacol. & Ther.* 34:153–158.
- van der Hoorn, F. A. J., F. Boomsma, A. J. Man in't Veld, and M. A. D. H. Schalekamp. 1989. Determination of catecholamines in human plasma by high-performance liquid chromatography: comparison between a new method with fluorescence detection and an established method with electrochemical detection. *J. Chromatogr.* 487:17–28.
- de Abreu, R. A., and J. M. van Baal. 1982. High-performance liquid chromatographic determination of purine and pyrimidine bases, ribonucleosides, deoxyribonucleosides and cyclic ribonucleotides in biological fluids. *J. Chromatogr.* 229:67–75.
- Belardinelli, L., and R. M. Berne. 1989. The cardiac effects of adenosine. *Prog. Cardiovasc. Dis.* 32:73–97.
- Olsson, R. A., and J. D. Pearson. 1990. Cardiovascular purinoceptors. *Physiol. Rev.* 70:761–845.
- Smits, P., J. W. Lenders, and T. Thien. 1990. Caffeine and theophylline attenuate adenosine-induced vasodilation in humans. *Clin. Pharmacol. & Ther.* 48:410–418.
- Kubo, T., and C. Su. 1983. Effects of adenosine on [³H]norepinephrine release from perfused mesenteric arteries of SHR and renal hypertensive rats. *Eur. J. Pharmacol.* 87:349–352.
- Smits, P., J. W. Lenders, J. J. Willemsen, and T. Thien. 1991. Adenosine attenuates the response to sympathetic stimuli in humans. *Hypertension (Dallas)*. 18:216–223.
- Arend, L. J., A. Haramati, C. I. Thompson, and W. S. Spielman. 1984. Adenosine-induced decrease in renin release: dissociation from hemodynamic effects. *Am. J. Physiol.* 247:F447–F452.
- Kurtz, A., R. Della Bruna, J. Pfeilschifter, and C. Bauer. 1988. Role of cGMP as second messenger of adenosine in the inhibition of renin release. *Kidney Int.* 33:798–803.
- Taddei, S., F. Arzilli, P. Arrighi, and A. Salvetti. 1992. Dipyrindamole decreases circulating renin-angiotensin system activity in hypertensive patients. *Am. J. Hypertens.* 5:29–31.
- Taddei, S., A. Virdis, S. Favilla, and A. Salvetti. 1992. Adenosine activates a vascular renin-angiotensin system in hypertensive subjects. *Hypertension (Dallas)*. 19:672–675.

36. Biaggioni, I. 1992. Contrasting excitatory and inhibitory effects of adenosine in blood pressure regulation. *Hypertension (Dallas)*. 20:457-465.
37. Katholi, R. E., G. R. Hageman, P. L. Whitlow, and W. T. Woods. 1983. Hemodynamic and afferent renal nerve responses to intrarenal adenosine in the dog. *Hypertension (Dallas)*. 5:1149-1154.
38. Katholi, R. E., P. L. Whitlow, G. R. Hageman, and W. T. Woods. 1984. Intrarenal adenosine produces hypertension by activating the sympathetic nervous system via the renal nerves in the dog. *J. Hypertens.* 2:349-359.
39. Watt, A. H., P. G. Reid, M. R. Stephens, and P. A. Routledge. 1987. Adenosine-induced respiratory stimulation in man depends on site of infusion. Evidence for an action on the carotid body? *Br. J. Clin. Pharmacol.* 23:486-490.
- 39a. Dibner-Dunlap, M. E., T. Kinugawa, and M. D. Thames. 1993. Activation of cardiac sympathetic afferents—effects of exogenous adenosine and adenosine analogues. *Am. J. Physiol.* 265:H395-H400.
40. Costa, F., and I. Biaggioni. 1994. Role of adenosine in the sympathetic activation produced by isometric exercise in humans. *J. Clin. Invest.* 93:1654-1660.
41. Biaggioni, I., T. J. Killian, R. Mosqueda Garcia, R. M. Robertson, and D. Robertson. 1991. Adenosine increases sympathetic nerve traffic in humans. *Circulation*. 83:1668-1675.
42. Biaggioni, I., J. Onrot, A. S. Hollister, and D. Robertson. 1986. Cardiovascular effects of adenosine infusion in man and their modulation by dipyridamole. *Life Sci.* 39:2229-2236.
43. Fuller, R. W., D. L. Maxwell, T. B. G. Conradson, C. M. S. Dixon, and P. J. Barnes. 1987. Circulatory and respiratory effects of infused adenosine in conscious man. *Br. J. Clin. Pharmacol.* 24:309-317.
44. Edlund, A., A. Sollevi, and B. Linde. 1990. Haemodynamic and metabolic effects of infused adenosine in man. *Clin. Sci. (Lond.)*. 79:131-138.
45. Reid, P. G., A. H. Watt, P. A. Routledge, and A. P. Smith. 1987. Intravenous infusion of adenosine but not inosine stimulates respiration in man. *Br. J. Clin. Pharmacol.* 23:331-338.
46. Conradson, T. B., B. Clarke, C. M. Dixon, R. N. Dalton, and P. J. Barnes. 1987. Effects of adenosine on autonomic control of heart rate in man. *Acta Physiol. Scand.* 131:525-531.
47. Fukunaga, A. F., W. E. Flacke, and B. C. Bloor. 1982. Hypotensive effects of adenosine and adenosine triphosphate compared with sodium nitroprusside. *Anesth. Analg.* 61:273-278.
48. Lappe, R. W., J. H. Sheldon, and B. F. Cox. 1992. Selective adenosine-2 agonist produces both direct and reflex tachycardia in normotensive rats. *J. Cardiovasc. Pharmacol.* 19:460-463.
49. Sollevi, A., M. Lagerkranser, L. Irestedt, E. Gordon, and C. Lindquist. 1984. Controlled hypotension with adenosine in cerebral aneurysm surgery. *Anesthesiology*. 61:400-405.
50. Conradson, T. B. G., C. M. S. Dixon, B. Clarke, and P. J. Barnes. 1987. Cardiovascular effects of infused adenosine in man: potentiation by dipyridamole. *Acta Physiol. Scand.* 129:387-391.
51. Maxwell, D. L., R. W. Fuller, T. B. Conradson, C. M. S. Dixon, V. Aber, J. M. B. Hughes, and P. J. Barnes. 1987. Contrasting effects of two xanthines, theophylline and enprofylline, on the cardio-respiratory stimulation of infused adenosine in man. *Acta Physiol. Scand.* 131:459-465.
52. Bush, A., C. M. Busst, B. Clarke, and P. J. Barnes. 1989. Effect of infused adenosine on cardiac output and systemic resistance in normal subjects. *Br. J. Clin. Pharmacol.* 27:165-171.
53. Smits, P., T. Thien, and A. van't Laar. 1985. Circulatory effects of coffee in relation to the pharmacokinetics of caffeine. *Am. J. Cardiol.* 56:958-963.
54. Lucarini, A. R., E. Picano, C. Marini, S. Favilla, A. Salvetti, and A. Distante. 1992. Activation of sympathetic tone during dipyridamole test. *Chest*. 102:444-447.
55. Sollevi, A., J. Ostergren, B. Fagrell, and P. Hjerdahl. 1984. Theophylline antagonizes cardiovascular responses to dipyridamole in man without affecting increases in plasma adenosine. *Acta Physiol. Scand.* 121:165-171.
56. Stringer, K. A., J. M. Branconi, R. Abadier, J. H. Wilton, and S. H. Royal. 1992. Disposition of oral dipyridamole in patients undergoing Thallium 201 myocardial imaging. *Pharmacotherapy*. 12:83-87.
57. Rardon, D. P., R. J. Kovacs, and J. C. Bailey. 1984. Adenosine and prostacyclin independent electrophysiological effects of dipyridamole in guinea-pig papillary muscles and canine cardiac Purkinje fibers. *J. Pharmacol. Exp. Ther.* 231:206-213.
58. Biaggioni, I., S. Paul, A. Puckett, and C. Arzubaga. 1991. Caffeine and theophylline as adenosine receptor antagonists in humans. *J. Pharmacol. Exp. Ther.* 258:588-593.
59. Smits, P., J. Schouten, and T. Thien. 1987. Respiratory stimulant effects of adenosine in man after caffeine and enprofylline. *Br. J. Clin. Pharmacol.* 24:816-819.
60. Fredholm, B. B., and E. Lindgren. 1983. Inhibition of soluble 5'-nucleotidase from rat brain by different xanthine derivatives. *Biochem. Pharmacol.* 32:2832-2834.
61. Feldman, M. D., C. R. Ayers, M. R. Lehman, H. E. Taylor, V. L. Gordon, P. J. Sabia, D. Ras, T. C. Skalak, and J. Linden. 1992. Improved detection of ischemia-induced increases in coronary sinus adenosine in patients with coronary artery disease. *Clin. Chem.* 38:256-262.
62. German, D. C., N. M. Kredich, and T. D. Bjornsson. 1989. Oral dipyridamole increases plasma adenosine levels in human beings. *Clin. Pharmacol. & Ther.* 45:80-84.
63. German, D. C., and N. M. Kredich. 1984. A radioenzymatic assay for plasma adenosine. *Anal. Biochem.* 142:536-541.
64. Mohrman, D. E., and L. J. Heller. 1990. Transcapillary adenosine transport in isolated guinea pig and rat hearts. *Am. J. Physiol.* 259:H772-H783.
65. Kroll, K., M. K. Kelm, K. F. Burring, and J. Schrader. 1989. Transendothelial transport and metabolism of adenosine and inosine in the intact rat aorta. *Circ. Res.* 64:1147-1157.
66. Van Belle, H. 1993. Nucleoside transport inhibition: a therapeutic approach to cardioprotection via adenosine? *Cardiovasc. Res.* 27:68-76.
67. Ralevic, V., and G. Burnstock. 1991. Roles of P2-purinoceptors in the cardiovascular system. *Circulation*. 84:1-14.
68. Mosqueda Garcia, R., C. J. Tseng, I. Biaggioni, R. M. Robertson, and D. Robertson. 1990. Effects of caffeine on baroreflex activity in humans. *Clin. Pharmacol. & Ther.* 48:568-574.
69. von Borstel, R. W., R. J. Wurtman, and L. A. Conlay. 1983. Chronic caffeine consumption potentiates the hypotensive action of circulating adenosine. *Life Sci.* 32:1151-1158.
70. Robertson, D., D. Wade, R. Workman, and R. L. Woosley. 1981. Tolerance to the humoral and hemodynamic effects of caffeine in man. *J. Clin. Invest.* 67:1111-1117.