

## PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.

<http://hdl.handle.net/2066/22775>

Please be advised that this information was generated on 2021-10-24 and may be subject to change.

# Presynaptic Inhibition of Norepinephrine Release From Sympathetic Nerve Endings by Endogenous Adenosine

Gerard A. Rongen, Jacques W.M. Lenders, Jorgo Lambrou, Jacques J. Willemsen, Herman Van Belle, Theo Thien, Paul Smits

**Abstract** ATP is coreleased with norepinephrine from sympathetic nerve endings and subsequently broken down to adenosine. In animal preparations, adenosine can inhibit norepinephrine release by stimulation of presynaptic receptors. We tested this feedback mechanism in humans by using a specific nucleoside transport inhibitor (draflazine) as a pharmacological tool to allow accumulation of endogenous adenosine in the synaptic cleft. In a dose-finding study on draflazine infusions into the brachial artery (n=10), we identified an optimal dose of 250 ng/min per deciliter of forearm tissue that induced considerable local nucleoside transport inhibition (approximately 40%) without systemic effects. In the main study, we investigated the effects of this draflazine dose on sympathetic-mediated norepinephrine spillover during lower body

negative pressure (-25 mm Hg) by use of the [<sup>3</sup>H]norepinephrine isotope dilution technique (n=25). Lower body negative pressure induced a significant increase in total body norepinephrine spillover, forearm norepinephrine appearance rate, forearm vascular resistance, and heart rate. During draflazine infusion into the brachial artery, the responses to lower body negative pressure were preserved for all parameters, with the exception of the median increase in forearm norepinephrine appearance rate, which was reduced from 54% to 2% ( $P<.05$ ). We conclude that accumulation of endogenous adenosine in the synaptic cleft during sympathetic stimulation can inhibit norepinephrine release from sympathetic nerve endings. (*Hypertension*. 1996;27:933-938.)

**Key Words** • norepinephrine • adenosine • receptors, presynaptic • nucleosides

In vitro observations have indicated that ATP is coreleased with norepinephrine from sympathetic nerve endings.<sup>1</sup> Extracellular ATP is rapidly degraded by ectophosphatases to AMP, which is subsequently dephosphorylated by 5'-nucleotidase to adenosine.<sup>2-4</sup> Then endogenous adenosine is rapidly cleared from the interstitium, at least in part by cellular uptake through specific nucleoside transporters.<sup>5</sup> In a variety of in vitro models, adenosine has been shown to inhibit norepinephrine release from sympathetic nerve endings.<sup>6-8</sup> Adenosine A<sub>1</sub> receptors are thought to be involved in this inhibitory effect.<sup>7</sup> In an elegant in vivo study in humans, Taddei et al<sup>9</sup> have provided indirect evidence for an adenosine-mediated reduction in norepinephrine release from sympathetic nerve endings in humans by demonstrating an augmented forearm vasoconstrictor response to sympathetic stimulation during administration of the adenosine receptor antagonist theophylline. However, they did not measure norepinephrine release in these experiments, and in theory, their observations could have been explained by alterations or interactions at the postsynaptic level.<sup>10,11</sup> Apart from the putative inhibitory action of adenosine at the

level of sympathetic nerve endings, it must be emphasized that adenosine can increase sympathetic nervous system activity in humans by stimulating afferent nerve endings in the vessel wall as well as in the carotid body.<sup>12-14</sup>

We performed this study to investigate the effect of endogenous adenosine on norepinephrine release from sympathetic nerve endings during stimulation of the sympathetic nervous system. To this end, a specific nucleoside transport inhibitor, draflazine, was used as a pharmacological tool to allow accumulation of endogenous adenosine in the synaptic cleft during sympathetic stimulation.<sup>5,14</sup> We performed appropriate pilot studies to determine the draflazine dose at which an optimal grade of nucleoside transport inhibition was induced. The current observations support the hypothesis that endogenous adenosine inhibits norepinephrine release at the sympathetic nerve ending.

## Methods

After the study had been approved by the local ethics committee, a total number of 35 normotensive, nonsmoking, healthy male volunteers signed written informed consent statements before participation in the study. Their demographic characteristics are summarized in Table 1. Ten of these subjects participated in the draflazine dose-finding study and 25 participated in the main study. Subjects had no history of hypertension, diabetes mellitus, or drug allergy and did not use concomitant medication. In all volunteers, a physical examination and 12-lead electrocardiography were performed to exclude cardiovascular, pulmonary, or neurological disease. The subjects were asked to abstain from caffeine-containing products for at least 24 hours because low concentrations of caffeine have been shown to attenuate the effects of adenosine in the forearm vascular bed.<sup>15</sup> All experiments were performed in the morning

Received May 29, 1995; first decision July 5, 1995; revision accepted December 7, 1995.

From the Departments of Medicine and Pharmacology (G.A.R., J.W.M.L., J.L., T.T., P.S.) and Department of Experimental and Chemical Endocrinology (J.J.W.), University Hospital Nijmegen (The Netherlands), and Janssen Research Foundation, Beerse, Belgium (H.V.B.).

Reprint requests to Paul Smits, Department of Pharmacology, University of Nijmegen, PO Box 9101, 6500 HB Nijmegen, Netherlands.

© 1996 American Heart Association, Inc.



## Selected Abbreviations and Acronyms

CI = 95% confidence interval
FBF = forearm blood flow
FVR = forearm vascular resistance
LBNP = lower body negative pressure

after a 10-hour fasting period, in a temperature-controlled room (24° to 25°C), and with subjects in the supine position. Since all drug and volume infusion rates were calculated per deciliter of forearm tissue, forearm volume was measured by water displacement for each individual.

After local anesthesia (2% xylocaine), the left brachial artery was cannulated with a 20-gauge catheter (Angiocath, Deseret Medical, Becton Dickinson) for both intra-arterial drug infusion (automatic syringe infusion pump, type STC-521, Terumo) and blood pressure recording (Hewlett-Packard GmbH). In the same arm, a deep antecubital vein was cannulated retrogradely for venous blood sampling. In the opposite arm, an antecubital vein was cannulated for blood sampling (dose-finding study) or infusion of tritiated norepinephrine (main study). FBF was recorded in both forearms by electrocardiography-triggered venous-occlusion plethysmography with the use of mercury-in-Silastic strain gauges (EC4, DE Hokanson). The upper-arm collecting cuffs were simultaneously inflated with a rapid cuff inflator (Hokanson E-20). At least 1 minute before FBF measurements were made, the hand circulation was occluded by inflation of the wrist cuffs to 200 mm Hg. FBF was recorded three times a minute. All experiments were started at least 30 minutes after intra-arterial cannulation. In all experiments, the total intra-arterial infusion rate was kept constant at 50  $\mu$ L/100 mL forearm per minute. Before the start of each experiment, venous blood was collected for measurement of plasma caffeine concentration.

### Draflazine Dose-Finding Study

The aim of the dose-finding study was to determine the intra-arterial draflazine dose that resulted in a sufficient level of nucleoside transport inhibition in the forearm vascular bed without causing systemic effects and without inducing regional vasodilation. We reasoned that prevention of draflazine-induced vasodilation was important because baseline vascular tone is an important determinant of the magnitude of the response to vasomotor stimuli.<sup>16</sup>

In 10 subjects, the effect of five increasing doses of draflazine, infused into the brachial artery, on forearm vascular tone and local and systemic ex vivo nucleoside transport inhibition was studied. The experiment started with measurement of baseline FBF, mean arterial pressure, and FVR during the last 4 minutes of a 5-minute saline infusion. The effect of five

increasing doses of draflazine (100, 250, 500, 1000, and 2000 ng/100 mL forearm per minute) on FBF and FVR were compared with the effect of saline. Each draflazine dose was infused for 20 minutes. At the end of saline infusion and each draflazine infusion, venous blood was sampled from both arms for ex vivo measurements of nucleoside transport inhibition (see "Analytic Methods"). Prolonged occlusion of the hand circulation can cause discomfort; therefore, wrist cuffs were inflated only during the last 10 minutes of draflazine infusion, and FBF was measured during the last 8 minutes of each draflazine dose.

### Main Study

In 25 subjects, the lower body was sealed in an air-tight Plexiglas box. The applied LBNP was recorded by a manometer connected to the inside of the box. In each subject, a 15-minute LBNP at -25 mm Hg was applied twice. The second LBNP was performed 50 minutes after the first one. In a pilot study, we have shown that the mean hemodynamic response to LBNP was identical when repeated within 1 day, with a fall in FBF of  $1.3 \pm 1.0$  and  $1.1 \pm 0.8$  mL/100 mL forearm per minute for the first and second LBNP, respectively (n=15, unpublished data, 1995).

Intra-arterial infusion of placebo (0.9% NaCl) started 10 minutes before each LBNP. The first LBNP was performed during ongoing intra-arterial placebo infusion. In contrast, 5 minutes before the second LBNP, intra-arterial placebo was switched to draflazine (250 ng/100 mL forearm per minute), which was infused until the end of LBNP. This draflazine dose was based on the results of the dose-finding study. The wrist cuffs were inflated during the 10 minutes before each LBNP and during the last 10 minutes of LBNP. FBF was measured during the placebo infusions, during the draflazine infusion, and during the last 8 minutes of each LBNP. Venous and arterial blood samples were obtained from the infused arm immediately before and at the end of each LBNP for determination of norepinephrine kinetics. Additionally, arterial and venous blood was sampled at the end of the second placebo infusion for detection of a possible effect of draflazine on baseline norepinephrine kinetics.

Levo-[ring-2,5,6-<sup>3</sup>H]norepinephrine (specific activity, 30 to 60 Ci/mmol) was infused intravenously for assessment of plasma norepinephrine kinetics. The radiotracer was administered intravenously at a constant rate of 1.0  $\mu$ Ci/min, and this infusion was started 20 minutes before the onset of each LBNP. Samples of the infusate were taken at the end of the infusion for determination of the exact infusion rate and the original activity of [<sup>3</sup>H]norepinephrine in a 1.0-mL plasma sample.

### Analytic Methods

Samples for determination of plasma caffeine concentration were analyzed with a reversed-phase high-performance liquid chromatographic method (detection limit, 0.2  $\mu$ g/mL), as described previously.<sup>17</sup>

Blood samples for determination of plasma norepinephrine were collected in prechilled tubes containing glutathione (0.2 mol/L) and EGTA (0.25 mol/L). The tubes were centrifuged at 4°C, and the plasma was separated and stored at -20°C. Measurements for concentrations of norepinephrine and [<sup>3</sup>H]-norepinephrine of all plasma samples and infusates occurred within 2 months after sampling, using high-performance liquid chromatography with fluorimetric detection after precolumn derivatization with the fluorescent agent 1,2-diphenylethyl-enediamine. A fraction collector (model 201-202, Gilson Medical Electronics), that was connected to an automatic sample injector (Wisp 710B), was used for collection of [<sup>3</sup>H]norepinephrine into scintillation vials according to the retention time of the norepinephrine standard solution.

Ex vivo nucleoside transport inhibition was measured by standardized incubation of erythrocytes with adenosine. Blood (4 mL) was drawn into a vial containing 1.0 mL acid/citrate/

TABLE 1. Baseline Characteristics of the Study Groups

Parameter	Dose-Finding Study	Main Study
n	10	25
Age, y	29.4 $\pm$ 11.9	34.6 $\pm$ 16.8
Weight, kg	73.3 $\pm$ 11.5	76.0 $\pm$ 10.4
Height, cm	180.5 $\pm$ 9.4	181.4 $\pm$ 6.6
Body mass index, kg/m <sup>2</sup>	22.4 $\pm$ 2.0	23.0 $\pm$ 2.5
Systolic BP, mm Hg	128 $\pm$ 8.0	126 $\pm$ 8.9
Diastolic BP, mm Hg	74.0 $\pm$ 9.6	72.5 $\pm$ 8.9
Heart rate, bpm	70.0 $\pm$ 12.1	66.4 $\pm$ 9.7
FVR, AU	55 $\pm$ 11	42 $\pm$ 15.6
Forearm volume, L	1.01 $\pm$ 0.16	1.11 $\pm$ 0.13

BP indicates blood pressure; AU, arbitrary units. BP was measured with subjects in the supine position by the Riva-Rocci technique using Korotkoff phases I and V for systolic and diastolic BPs, respectively. Heart rate was measured by pulse frequency counting (radial artery). Values are mean $\pm$ SD.



dextrose (65 mmol/L citric acid, 85 mmol/L trisodium citrate, and 20 g/L glucose) and further handled as described previously.<sup>14</sup> After incubation, the concentrations of adenosine, inosine, and hypoxanthine were determined in the supernatant by a chemiluminescence technique. The percent inhibition of nucleoside transport was calculated as  $(A_x - A_0) \times 100 / (1 - A_0)$ , where  $A_0$  represents the adenosine concentration as a 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.

## Drugs and Solutions

Tritiated norepinephrine was obtained from DuPont–New England Nuclear. The radionuclide was sterilized with a 0.22- $\mu$ m filter and diluted in 0.9% NaCl containing acetic (0.2 mol/L) and ascorbic (1 mg/mL) acids. Aliquots of approximately 70  $\mu$ Ci/mL of [<sup>3</sup>H]norepinephrine were stored at  $-80^\circ\text{C}$  until use. Sterilization, dilution, and aliquoting were carried out under nitrogen. Just before use, an aliquot was diluted in 0.9% NaCl.

Sterile draflazine solutions were freshly prepared from 10-mL vials containing 5 mg draflazine diluted in 0.9% NaCl (Janssen Pharmaceutica Inc). The specificity of this drug as a nucleoside transport inhibitor and its ability to increase endogenous adenosine levels in humans have been extensively described before.<sup>5,14</sup>

## Data Analysis

Mean arterial blood pressure was measured continuously during each recording of FBF and averaged per FBF measurement. FVR was calculated as the quotient of the simultaneously registered mean arterial blood pressure and FBF and is expressed as arbitrary units (AU). For the draflazine dose-finding study, the hemodynamic registrations obtained during placebo infusion and the last 6 minutes of each draflazine infusion were each averaged to one value. Draflazine-induced effects were expressed as both absolute and percent changes from the preceding placebo infusion. For the main study, the hemodynamic parameters were averaged to one value for the following consecutive periods: placebo-1, placebo plus LBNP, placebo-2, draflazine, and draflazine plus LBNP. The data obtained during the last 6 minutes of each LBNP were used.

Arterial and venous concentrations of [<sup>3</sup>H]norepinephrine and norepinephrine were used for calculations of the various parameters of norepinephrine kinetics, as previously described.<sup>18-20</sup> Total body norepinephrine spillover, the estimated rate of appearance of endogenous norepinephrine in arterial plasma, was calculated from arterial plasma norepinephrine concentration ( $NE_a$ ), the arterial steady-state plasma concentration of [<sup>3</sup>H]norepinephrine ( $[^3\text{H}]NE_a$ ), and the infusion rate of [<sup>3</sup>H]norepinephrine according to the equation

$$\text{Total Body Norepinephrine Spillover (ng/min)}$$

$$= NE_a(\text{ng/L})$$

$$\times \{ \text{Infusion Rate (dpm/min)} / [^3\text{H}]NE_a(\text{dpm/L}) \}$$

where dpm is disintegrations per minute. The local forearm norepinephrine appearance rate was estimated from

$$\text{Forearm Norepinephrine Appearance Rate}$$

$$(\text{pg}/100 \text{ mL forearm per minute})$$

$$= \{ (\text{FPF} \times NE_a \times f_{NE}) + [\text{FPF} \times (NE_v - NE_a)] \} / (1 - f_{NE})$$

where  $f_{NE}$  is the fractional extraction of the tracer across the forearm, calculated as  $([^3\text{H}]NE_a - [^3\text{H}]NE_v) / [^3\text{H}]NE_a$ ;  $[^3\text{H}]NE_v$  and  $[^3\text{H}]NE_a$  are the venous and arterial plasma concentrations

of [<sup>3</sup>H]norepinephrine, respectively; and FPF is the forearm plasma flow (milliliters per 100 mL forearm per minute), calculated from FBF and hematocrit as  $\text{FBF} \times (1 - \text{Ht})$ .

Changes in the hemodynamic and kinetic parameters induced by LBNP during placebo were compared with those induced by LBNP during draflazine.

All hemodynamic results are presented as mean  $\pm$  SE unless indicated otherwise. The data on forearm norepinephrine appearance rate and norepinephrine total body spillover are presented as median with CI. To avoid multiple comparison, within-subject effects were first assessed by ANOVA for repeated measurements and further analyzed with paired Student's *t* test if appropriate (dose-finding study). The results of the main study were analyzed by paired Student's *t* tests because these data sets showed gaussian distributions according to the Shapiro-Wilks test. To minimize multiple comparisons, LBNP-induced changes from baseline were not tested statistically. For the same reason, only the key parameters were included in the statistical analysis (FVR, forearm norepinephrine appearance rate, and total body spillover). Differences were considered to be statistically significant at a value of  $P < .05$  (two-sided).

## Results

We determined plasma caffeine levels to check compliance with respect to caffeine abstinence. In all volunteers, plasma caffeine concentrations were below the limit of detection, indicating excellent compliance to the caffeine-free diet. No subjective side effects occurred during the intra-arterial infusion of draflazine.

## Dose-Finding Study

Baseline FBF was  $2.1 \pm 0.3$  mL/100 mL forearm per minute in the cannulated arm and increased to  $2.2 \pm 0.4$ ,  $2.4 \pm 0.3$ ,  $3.0 \pm 0.4$ ,  $3.7 \pm 0.5$ , and  $4.2 \pm 0.5$  mL/100 mL forearm per minute at the end of the five increasing draflazine doses, respectively. Expressed as percent change from baseline, FBF was increased by  $4.9 \pm 7.4\%$ ,  $18.0 \pm 6.1\%$ ,  $51.1 \pm 13.6\%$ ,  $94.1 \pm 20.8\%$ , and  $123.2 \pm 24.5\%$  at the end of the five increasing draflazine doses, respectively ( $n=10$ ,  $P < .05$  for the four highest doses). FBF in the contralateral arm was not significantly affected during any of the infusions. Baseline FVR was  $54.6 \pm 10.7$  AU in the cannulated arm. Draflazine decreased FVR by  $5.3 \pm 10.1\%$ ,  $5.7 \pm 5.7\%$ ,  $20.4 \pm 6.7\%$ ,  $34.8 \pm 7.1\%$ , and  $41.7 \pm 6.7\%$  for 100, 250, 500, 1000, and 2000 ng draflazine/100 mL forearm per minute, respectively ( $n=10$ ,  $P < .05$  for the three highest doses). FVR in the contralateral arm was not significantly affected (Fig 1).

In the infused arm, ex vivo nucleoside transport inhibition was  $7.0 \pm 2.4\%$ ,  $42.4 \pm 8.8\%$ ,  $69.7 \pm 7.7\%$ ,  $80.8 \pm 4.8\%$ , and  $87.1 \pm 2.5\%$  during the five increasing doses, respectively ( $n=10$ ,  $P < .05$  for each dose versus baseline; see Fig 1). Likewise, in the nonexperimental arm, ex vivo nucleoside transport inhibition increased dose dependently by  $3.9 \pm 1.6\%$ ,  $6.8 \pm 3.4\%$ ,  $11.9 \pm 3.7\%$ ,  $17.8 \pm 3.5\%$ , and  $51.2 \pm 3.0\%$ , respectively ( $n=10$ ,  $P < .05$  for each dose versus baseline; see Fig 1).

On the basis of these results, the draflazine dose of 250 ng/100 mL forearm per minute was chosen for the main study because this dose induced a sufficient nucleoside transport inhibition in the forearm vascular bed (approximately 40%), without a relevant increase in this parameter at the contralateral site (approximately 7%) and without causing a significant vasodilator response at the site of infusion.



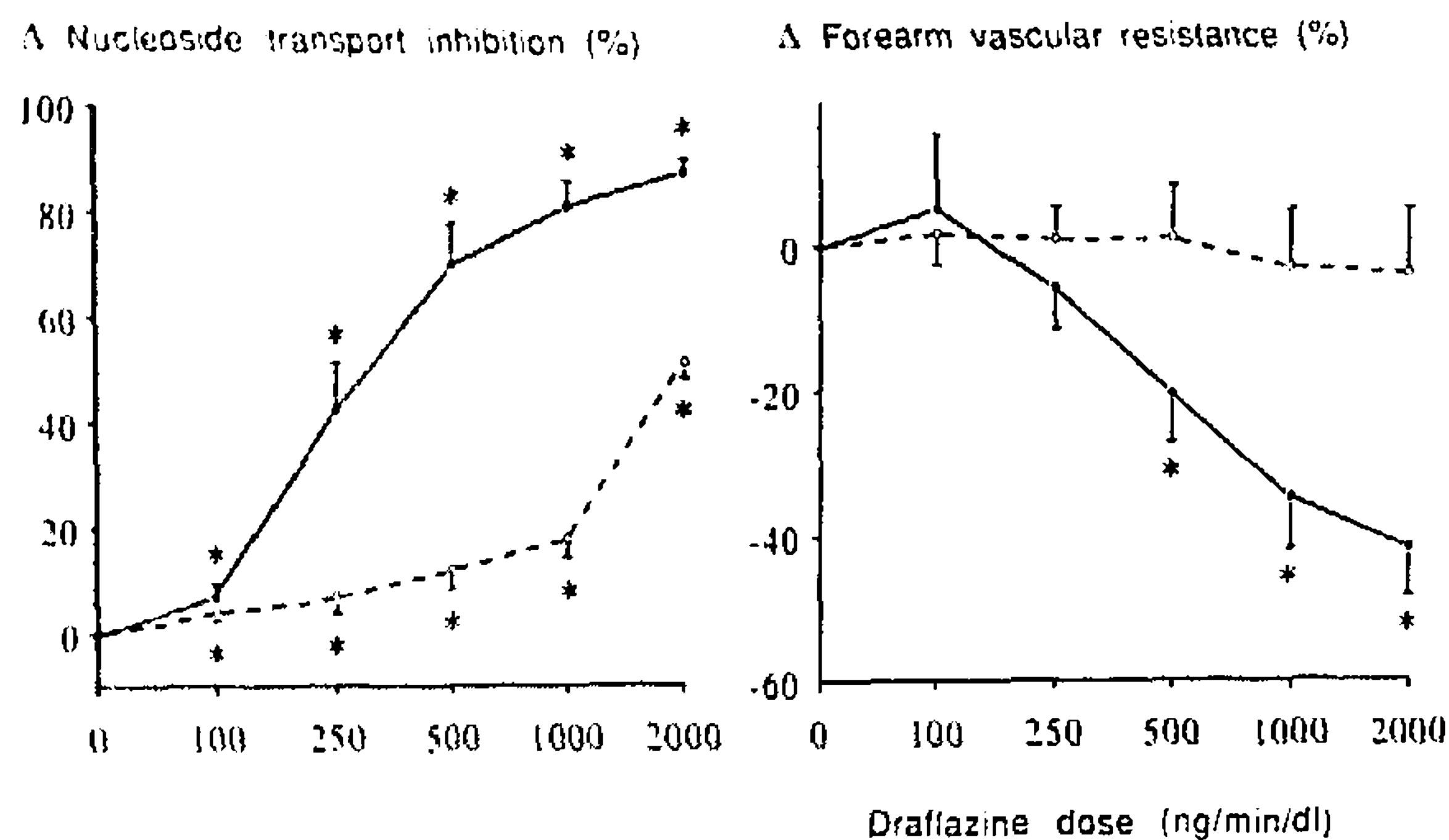


FIG 1. Percent changes (mean $\pm$ SE) in nucleoside transport inhibition (left) and FVR (right) induced by incremental doses of draflazine infused into the brachial artery. The solid line represents data at the side of draflazine infusion; the dashed line, data from the contralateral side. \* $P$ <.05 vs baseline.

## Main Study

### Effect of Sympathetic Stimulation by LBNP

Table 2 shows the hemodynamic and neurohumoral effects of LBNP with and without concomitant intra-arterial draflazine infusion. During placebo, LBNP induced a fall in FBF from  $2.6\pm 0.3$  to  $1.5\pm 0.2$  mL/100 mL forearm per minute at the experimental side and from  $2.5\pm 0.3$  to  $1.5\pm 0.2$  mL/100 mL forearm per minute at the contralateral side. Mean arterial blood pressure did not change, but heart rate increased by  $5.2\pm 1.6\%$  as a result of the first LBNP. Total body norepinephrine spillover increased by 26.2% (CI, 11.3% to 41.5%), whereas the forearm norepinephrine appearance rate increased by 53.5% (CI, -0.6% to 97.9%). Fifty minutes after the first LBNP was stopped, all parameters had returned toward baseline level (placebo-1).

### Effects of Draflazine Infusion

In accordance with the results of the dose-finding study, intra-arterial infusion of draflazine at a rate of 250 ng/100 mL forearm per minute hardly affected vascular tone (Table 2). Although total body norepinephrine spillover was not altered, draflazine caused an increase in forearm norepinephrine appearance rate of 19.4% (CI, 6.3% to 47.0%) ( $P$ <.01 versus baseline).

### Effects of Draflazine on the Response to Sympathetic Stimulation by LBNP

Application of LBNP during draflazine exerted a hemodynamic response that did not differ from the

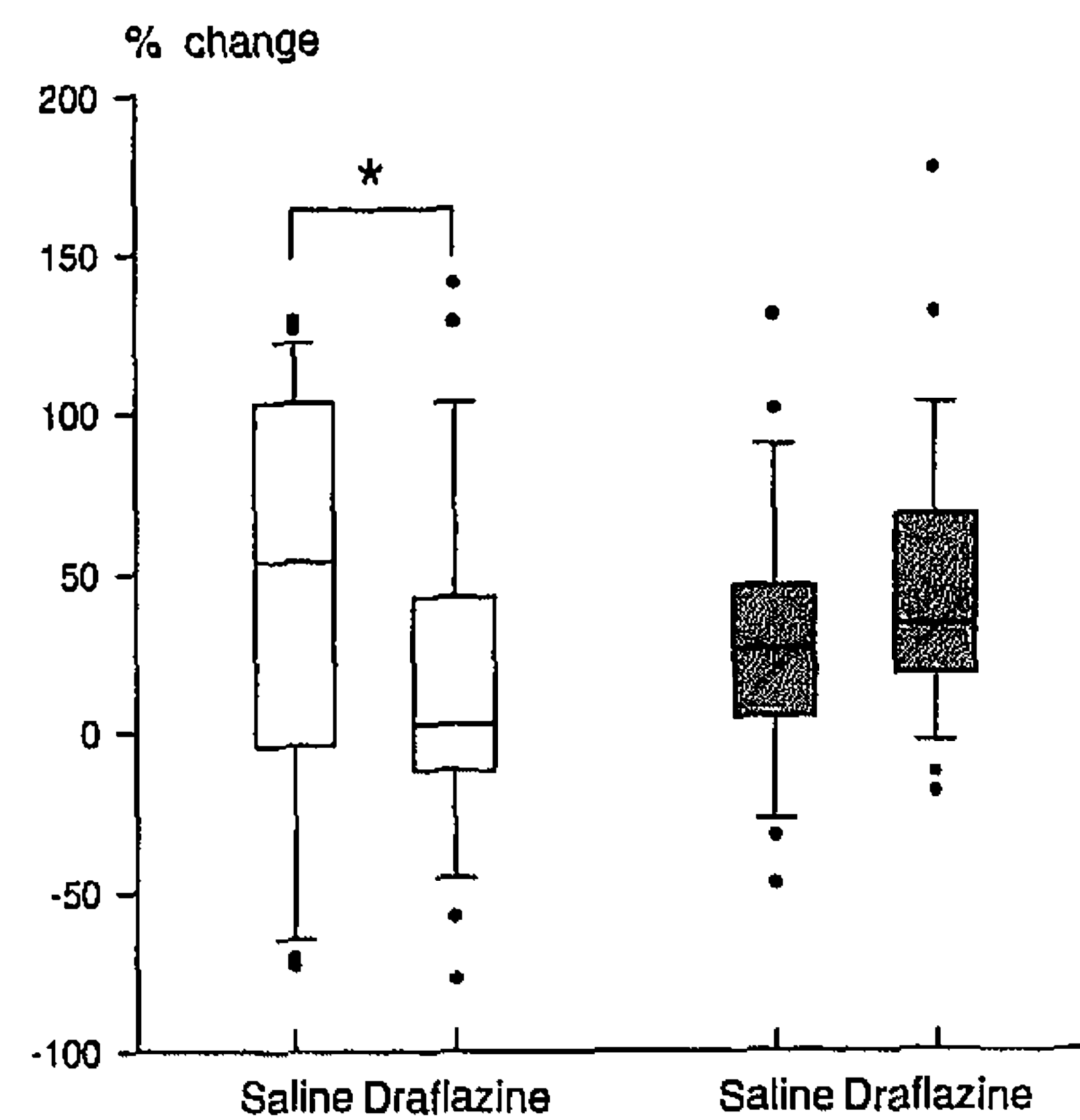


FIG 2. LBNP-induced percent changes in forearm norepinephrine appearance rate (open boxes) and total body norepinephrine spillover (shaded boxes). Boxes represent the interquartile range; capped bars, 10th and 90th percentiles of individual data. Dots mark all data outside the 5th and 95th percentiles. \*Statistically significant difference between saline and draflazine ( $P$ <.05, two-tailed paired Student's  $t$  test).

response to the first LBNP, with no change in mean arterial blood pressure and a significant increase in heart rate of  $7.3\pm 2.0\%$ . During draflazine infusion, LBNP induced a fall in FBF from  $3.2\pm 0.4$  to  $2.1\pm 0.3$  mL/100 mL forearm per minute at the experimental side and from  $2.8\pm 0.3$  to  $1.9\pm 0.2$  mL/100 mL forearm per minute at the contralateral side. FVR showed a bilateral fall without a difference between the experimental and contralateral sides, although the response was lower on both sides compared with the first LBNP.

Fig 2 shows the LBNP-induced percent changes from baseline for the data on norepinephrine. Again, total body norepinephrine spillover increased by a median percent change of 32.7% (CI, 19.8% to 62.8%) as opposed to 26.2% (CI, 11.3% to 41.5%) during the first LBNP (first versus second LBNP,  $P$ =.2). In contrast, the response of forearm norepinephrine appearance rate to LBNP was almost abolished during draflazine, this response being significantly lower compared with that to the first LBNP (2.2% [CI, -11.4% to 40.4%] versus 53.5% [CI, -0.6% to 97.9%];  $P$ <.05).

## Discussion

The main observation of this study is that infusion of the specific nucleoside transport inhibitor draflazine into the forearm skeletal muscle vascular bed resulted in a

TABLE 2. Hemodynamic and Neurohumoral Effects of LBNP With and Without Concomitant Intra-arterial Draflazine Infusion

Parameter	First Placebo	First LBNP	Second Placebo	Draflazine	Second LBNP
FVR infused arm, AU	41.5 $\pm$ 3.1	86.7 $\pm$ 12.6 (100.7 $\pm$ 16.4%)	39.7 $\pm$ 3.2	38.1 $\pm$ 3.0 (-3.4 $\pm$ 1.9%)	64.5 $\pm$ 7.5 (65.9 $\pm$ 10.5%)†
FVR control arm, AU	44.0 $\pm$ 3.8	92.3 $\pm$ 14.5 (102.6 $\pm$ 19.4%)	49.2 $\pm$ 6.5	45.9 $\pm$ 5.2 (-3.6 $\pm$ 2.8%)	78.3 $\pm$ 11.4 (68.5 $\pm$ 12.2%)†
Total body norepinephrine spillover, ng/min	344.5	439.1 (26.2%; CI, 11.3-41.5%)	352.2	356.5 (-0.6%; CI, -8-3.2%)	507.1 (32.7%; CI, 19.8-62.8%)
Forearm norepinephrine appearance rate, (pg/100 mL)/min	549.0	732.1 (53.5%; CI, -0.6-97.9%)	692.0	766.5 (19.4%; CI, 6.3-47%)*	922.3 (2.2%; CI, -11.4-40.4%)†

AU indicates arbitrary units. Values are mean $\pm$ SE for FVR data and median for norepinephrine data. Percent changes from baseline are shown in parentheses (mean $\pm$ SE for FVR data and median plus CI for norepinephrine data).

\*Statistically significant changes from baseline (tested only for draflazine); †statistically significant differences in percent response between first and second LBNP.



blunted response of the local norepinephrine appearance rate during sympathetic stimulation. This attenuation occurred only at the side of draflazine infusion, as simultaneous measurements of total body norepinephrine spillover convincingly showed that the overall response of the sympathetic nervous system to LBNP during draflazine (median, 32.7%) was certainly not lower than that to LBNP during placebo (median, 26.2%). The absence of an effect on total body norepinephrine spillover agrees with the almost negligible level of systemic nucleoside transport inhibition, which was far below the threshold for interference with the sympathetic nervous system.<sup>14</sup> The specificity of draflazine has been tested in various *in vitro* systems.<sup>21</sup> Intravenous infusion of draflazine in conscious humans elicits hemodynamic, neurohumoral, and ventilatory responses that closely resemble the effects of intravenous infusion of adenosine.<sup>12,14,22-25</sup> This occurred at higher systemic draflazine blood concentrations than currently used locally, as assessed by *ex vivo* nucleoside transport inhibition. By use of caffeine as an adenosine receptor antagonist, we were able to show in a previous study that the circulatory effects of nucleoside transport inhibition by draflazine resulted entirely from adenosine receptor stimulation.<sup>14</sup> Consequently, our current data provide evidence for an adenosine receptor-mediated reduction in norepinephrine release from efferent nerve endings during sympathetic stimulation. This reduction in the observed LBNP-induced norepinephrine release from the forearm vascular bed was expected to be accompanied by a parallel reduction in the local vasoconstrictor response to sympathetic stimulation. However, we did not find any evidence for an impaired forearm vasoconstrictor response at the side of draflazine infusion (Table 2). This does not necessarily argue against our interpretation of the results, because the forearm vasoconstrictor response also depends on a variety of other factors, including the postsynaptic interaction between adenosine and norepinephrine,<sup>10,11</sup> eventual alterations in receptor populations, and changes in the release of co-transmitters such as ATP and neuropeptide Y.<sup>1,26,27</sup> Of course, possible actions of draflazine unrelated to nucleoside transport inhibition may contribute to the effects seen in this study.

Our interpretations are mainly based on assessment of the spillover of norepinephrine according to the isotope dilution technique as described by Esler et al.<sup>19</sup> The tracer [<sup>3</sup>H]norepinephrine is used for determination of the amount of norepinephrine extracted during passage across a vascular bed, in this case, that of the forearm skeletal muscles. However, forearm norepinephrine spillover as calculated from these parameters strongly depends on FBF.<sup>18</sup> Recently, Chang et al<sup>20</sup> proposed a correction for this calculated forearm norepinephrine spillover by taking into account the amount of released norepinephrine that is also extracted from the forearm vascular bed. This so-called forearm norepinephrine appearance rate should be a more accurate measurement of the regional release of norepinephrine, especially for our study because of the induced changes in FBF as a result of sympathetic stimulation.

In our dose-finding study, intra-arterial infusion of draflazine resulted in a significant dose-dependent inhibition of *ex vivo* nucleoside transport that was considerably higher in the infused arm compared with the

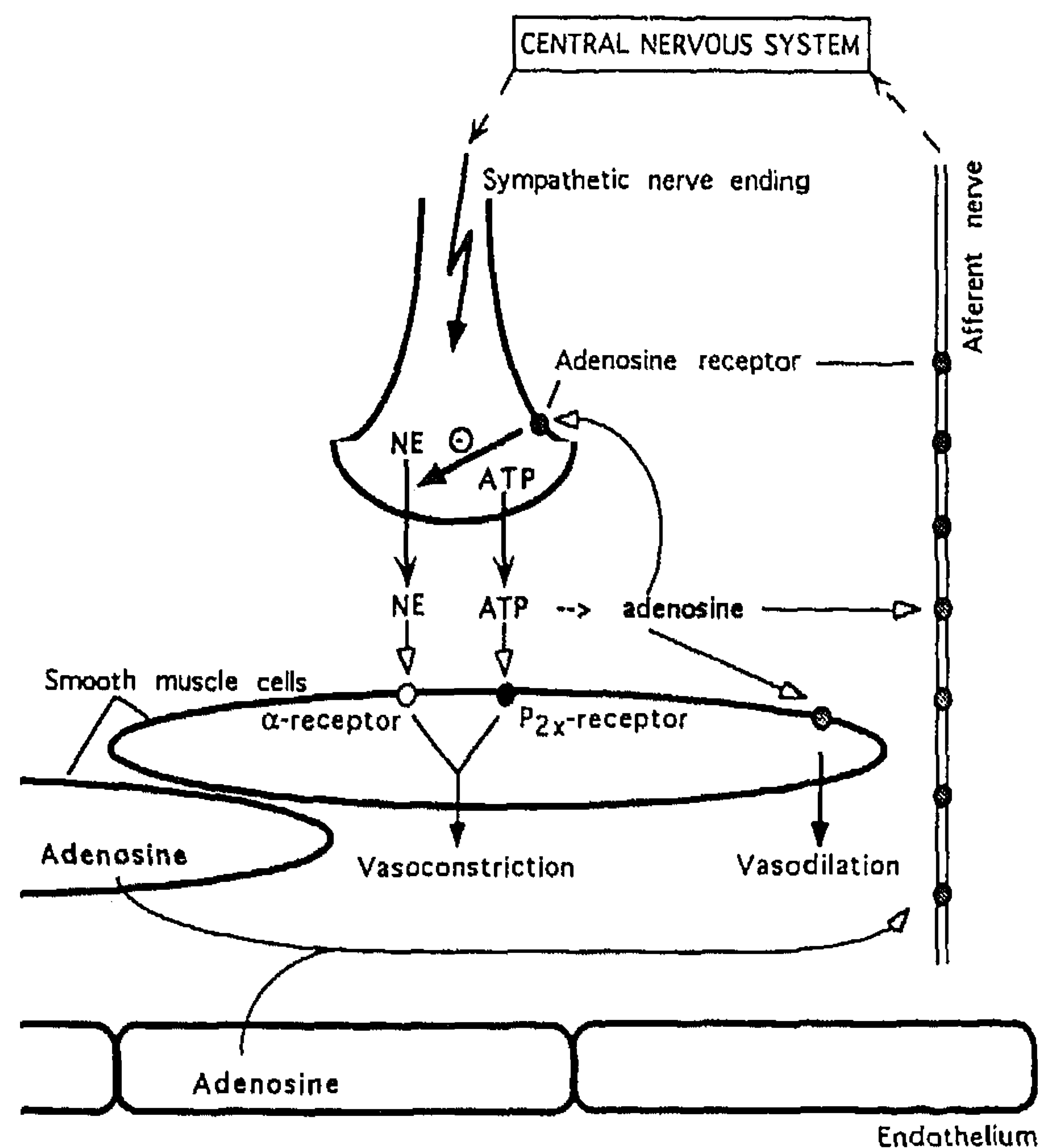


FIG 3. Outline of the potential interactions between endogenous adenosine and the sympathetic nervous system. NE indicates norepinephrine.

noninfused contralateral arm. Draflazine doses of 500 ng/100 mL forearm per minute or more elicited vasodilation in the infused arm that was not observed in the control arm, indicating a local vasodilator action of draflazine in humans. As demonstrated previously, the effects of draflazine are mediated by adenosine accumulation in the extracellular space and subsequent adenosine receptor stimulation.<sup>14</sup> Therefore, the vasodilator action of draflazine indicates extracellular adenosine accumulation in the nonischemic human forearm vascular bed. Possible sources of this endogenous adenosine include endothelium, vascular smooth muscle cells, and sympathetic nerve endings where adenosine is formed extracellularly by enzymatic degradation of the neurotransmitter ATP.<sup>28-31</sup>

Unexpectedly, the main study showed that baseline forearm norepinephrine appearance rate increased significantly during draflazine infusion without changes in total body norepinephrine spillover. Actually, the dose of 250 ng/100 mL forearm per minute was chosen because it was expected to inhibit nucleoside transport inhibition to a sufficient extent without affecting baseline hemodynamic or humoral parameters. Of course, we realized that increasing endogenous adenosine levels may stimulate sympathetic nervous system activity by stimulation of afferent nerves.<sup>12-14</sup> Recently, this has been shown in particular for the forearm skeletal muscle vascular bed.<sup>32</sup> However, dose-response data on draflazine have suggested that this afferent stimulation should not occur at the level of nucleoside transport inhibition reached in our main study.<sup>14</sup> Nonetheless, the present finding indicates that the baseline norepinephrine release from sympathetic nerve endings in the forearm can be slightly stimulated by low doses of intra-arterially infused draflazine. We have no clear explanation for this observation. If stimulation of adenosine-sensitive afferent nerve endings in the forearm vascular bed plays a



role in this observation, we must assume that the response of the efferent sympathetic nervous system was a differential side-selective response because total body norepinephrine spillover and systemic hemodynamics were unaffected. Along these lines, the contrasting effects of drafazine on baseline versus stimulated forearm norepinephrine appearance rate may be mediated by interaction of endogenous adenosine with the sympathetic nervous system at two distinct levels. Fig 3 summarizes this hypothesis.

First, accumulation of continuously formed interstitial adenosine may increase sympathetic nervous system activity as a result of stimulation of afferent nerve endings in the forearm vascular bed. Second, during sympathetic stimulation, drafazine allows accumulation of endogenous adenosine, as a breakdown product of ATP coreleased in the synaptic cleft that is able to reduce norepinephrine release from sympathetic nerve endings locally by presynaptic inhibition of neurotransmitter release. Apparently, the first effect of adenosine dominates during baseline conditions, when baseline sympathetic nervous system activity is low. However, the importance of the second interaction increases during sympathetic stimulation by LBNP as adenosine builds up in the synaptic cleft. It has to be emphasized that this dual action of adenosine has already been extensively described for exogenous adenosine.<sup>33</sup> The current results extrapolate this concept to the effects of endogenous adenosine.

## References

- Burnstock G, Sneddon P. Evidence for ATP and noradrenaline as cotransmitters in sympathetic nerves. *Clin Sci*. 1985;68(suppl 10): 89s-92s.
- Pearson J, Carleton J, Gordon J. Metabolism of adenine nucleotides by ectoenzymes of vascular endothelial and smooth-muscle cells in culture. *Biochem J*. 1980;190:421-429.
- Juul B, Lüscher ME, Aalkjaer C, Plesner L. Nucleotide hydrolytic activity of isolated intact mesenteric small arteries. *Biochim Biophys Acta*. 1991;1067:201-207.
- Welford LA, Cusack NJ, Hourani SMO. The structure-activity relationships of ectonucleotidases and of excitatory P2-purinoceptors: evidence that dephosphorylation of ATP analogues reduces pharmacological potency. *Eur J Pharmacol*. 1987;141:123-130.
- Van Belle H. Nucleoside transport inhibition: a therapeutic approach to cardioprotection via adenosine? *Cardiovasc Res*. 1993; 27:68-76.
- Kubo T, Su C. Effects of adenosine on [3H]norepinephrine release from perfused mesenteric arteries of SHR and renal hypertensive rats. *Eur J Pharmacol*. 1983;87:349-352.
- von Kugelgen I, Spath L, Starke K. Stable adenine nucleotides inhibit [H-3]-noradrenaline release in rabbit brain cortex slices by direct action at presynaptic adenosine A1-receptors. *Naunyn Schmiedebergs Arch Pharmacol*. 1992;346:187-196.
- Wennmalm M, Fredholm BB, Hedqvist P. Adenosine as a modulator of sympathetic nerve-stimulation-induced release of noradrenaline from the isolated rabbit heart. *Acta Physiol Scand*. 1988; 132:487-494.
- Taddei S, Pedrinelli R, Salvetti A. Sympathetic nervous system-dependent vasoconstriction in humans: evidence for mechanistic role of endogenous purine compounds. *Circulation*. 1990;82: 2061-2067.
- Burnstock G, Kennedy C. A dual function for adenosine 5'-triphosphate in the regulation of vascular tone: excitatory cotransmitter with noradrenaline from perivascular nerves and locally released inhibitory intravascular agent. *Circ Res*. 1985;58:319-330.
- Smits P, Lenders JW, Willemsen JJ, Thien T. Adenosine attenuates the response to sympathetic stimuli in humans. *Hypertension*. 1991; 18:216-223.
- Biaggioni I, Olafsson B, Robertson RM, Hollister AS, Robertson D. Cardiovascular and respiratory effects of adenosine in conscious man: evidence for chemoreceptor activation. *Circ Res*. 1987;61: 779-786.
- Engelstein ED, Lerman BB, Somers VK, Rea RF. Role of arterial chemoreceptors in mediating the effects of endogenous adenosine on sympathetic nerve activity. *Circulation*. 1994;90:2919-2926.
- Rongen GA, Smits P, Ver Donck K, Willemsen JJ, de Abreu RA, Van Belle H, Thien T. Hemodynamic and neurohumoral effects of various grades of selective adenosine transport inhibition in humans: implications for its future role in cardioprotection. *J Clin Invest*. 1995;95:658-668.
- Smits P, Lenders JW, Thien T. Caffeine and theophylline attenuate adenosine-induced vasodilation in humans. *Clin Pharmacol Ther*. 1990;48:410-418.
- Myers HA, Honig CR. Influence of initial resistance on magnitude of response to vasomotor stimuli. *Am J Physiol*. 1969;216:1429-1436.
- Smits P, Hoffmann H, Thien T, Houben H, van't Laar A. Hemodynamic and humoral effects of coffee after beta-1-selective and nonselective beta-blockade. *Clin Pharmacol Ther*. 1983;34:153-158.
- Grossman E, Chang PC, Hoffman A, Tamrat M, Kopin IJ, Goldstein DS. Tracer norepinephrine kinetics: dependence on regional blood flow and the site of infusion. *Am J Physiol*. 1991;260: R946-R952.
- Esler M, Jennings G, Korner P, Willett I, Dudley F, Hasking G, Anderson W, Lambert G. Assessment of human sympathetic nervous system activity from measurements of norepinephrine turnover. *Hypertension*. 1988;11:3-20.
- Chang PC, Kriek E, van Brummelen P. Sympathetic activity and presynaptic adrenoceptor function in patients with longstanding essential hypertension. *J Hypertens*. 1994;12:179-190.
- Van Belle H, Verheyen W, Donck KV, Janssen PAJ, Robertson JIS. Prevention of catecholamine-induced cardiac damage and death with a nucleoside transport inhibitor. *J Cardiovasc Pharmacol*. 1992;20: 173-178.
- Smits P, Schouten J, Thien T. Respiratory stimulant effects of adenosine in man after caffeine and enprofylline. *Br J Clin Pharmacol*. 1987;24:816-819.
- Smits P, Boekema P, Thien T, de Abreu R, van't Laar A. Evidence for an antagonism between caffeine and adenosine in the cardiovascular system in man. *J Cardiovasc Pharmacol*. 1987;10:136-143.
- Biaggioni I, Killian TJ, Mosqueda Garcia R, Robertson RM, Robertson D. Adenosine increases sympathetic nerve traffic in humans. *Circulation*. 1991;83:1668-1675.
- Rongen GA, Smits P, Bootsma G, Ver Donck K, de Vries A, Thien T. High-grade nucleoside transport inhibition stimulates ventilation in humans. *J Clin Pharmacol*. 1995;35:357-361.
- Clarke J, Benjamin N, Larkin S, Webb D, Maseri A, Davies G. Interaction of neuropeptide Y and the sympathetic nervous system in vascular control in man. *Circulation*. 1991;83:774-777.
- Taddei S, Salvetti A, Pedrinelli R. Persistence of sympathetic-mediated forearm vasoconstriction after alpha-blockade in hypertensive patients. *Circulation*. 1989;80:485-490.
- Deussen A, Moser G, Schrader J. Contribution of coronary endothelial cells to cardiac adenosine production. *Pflugers Arch*. 1986; 406:608-614.
- Schutz W, Schrader J, Gerlach E. Different sites of adenosine formation in the heart. *Am J Physiol*. 1981;240:H963-H970.
- Borst MM, Schrader J. Adenine nucleotide release from isolated perfused guinea pig hearts and extracellular formation of adenosine. *Circ Res*. 1991;68:797-806.
- Burnstock G, Griffith SG, Sneddon P. Autonomic nerves in the precapillary vessel wall. *J Cardiovasc Pharmacol*. 1984;6(suppl 2):S344-S353.
- Costa F, Biaggioni I. Role of adenosine in the sympathetic activation produced by isometric exercise in humans. *J Clin Invest*. 1994;93:1654-1660.
- Biaggioni I. Contrasting excitatory and inhibitory effects of adenosine in blood pressure regulation. *Hypertension*. 1992;20:457-465.