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# High-Grade Nucleoside Transport Inhibition Stimulates Ventilation in Humans

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*In 6 healthy male volunteers a placebo-controlled, double-blind, randomized, crossover trial was done to assess the effect of 1, 2, 4, and 6 mg of draflazine, a specific nucleoside transport inhibitor, on ventilation. Draflazine increased thoracic excursions dose-dependently by maximally (median with 95% confidence interval) 114.0% (38.3–184.8%) without affecting breathing rate. Ex vivo adenosine transport was inhibited by 0% (0–1%) after placebo, and 70% (59–74%), 81% (76–85%), 90% (86–93%), and 93% (90–96%) after the 4 increasing draflazine dosages, respectively ( $P < .05$  for each draflazine dosage versus placebo). These results indicate that endogenously released adenosine may play a role in the regulation of ventilation.*

Intravenous injection of adenosine has been reported to stimulate ventilation in humans.<sup>1–4</sup> The adenosine receptor antagonist theophylline does not reduce ventilation in healthy volunteers,<sup>5</sup> suggesting that endogenous adenosine does not play an important role in the regulation of ventilation. However, theophylline crosses the blood-brain barrier, and the contrasting central and peripheral effects of adenosine may offer an explanation for the lack of effect of theophylline on ventilation.<sup>6</sup> Alternatively, the physiologic importance of adenosine can be evaluated by inhibiting its cellular uptake. Although dipyridamole augmented the ventilatory effects of intravenously injected adenosine,<sup>7,8</sup> it did not affect ventilation during isocapnic hypoxia when given orally.<sup>5</sup> In contrast, it has recently been reported that intravenously injected dipyridamole stimulates ventilation in humans.<sup>9</sup> Besides the variable oral avail-

ability of dipyridamole,<sup>10</sup> nonspecific actions such as phosphodiesterase inhibition and stimulation of prostacyclin release<sup>11,12</sup> may have interfered, possibly accounting for these contrasting results. Based on these previous observations, we hypothesized that endogenously released adenosine is able to stimulate ventilation in humans. To test this hypothesis, we decided to study the effect of intravenously injected draflazine, a specific nucleoside transport inhibitor, on ventilation. Draflazine, the active (–)-enantiomer of R 75 231, has a tight binding to the nucleoside transporter.<sup>13</sup> Phase I studies in humans reveal a long duration of action: 50% ex vivo adenosine transport inhibition at 4 hours after a total dose of 10 mg of R 75 231 (resembling 5 mg draflazine) intravenously.<sup>14</sup> The advantage of using draflazine instead of dipyridamole is that draflazine does not possess nonspecific confounding effects as does dipyridamole. Drug effectivity was monitored by measuring ex vivo nucleoside transport inhibition.

## SUBJECTS AND METHODS

### Subjects

After approval of the local ethics committee, 6 normotensive, nonsmoking, healthy, Caucasian, non-obese, male volunteers [age: 44 (29–53) years; height:

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180 (172–189) cm] signed written informed consent before participation. They had no history of pulmonary disease and did not use concomitant medication.

### Study Design

The ventilatory effects of placebo and 1, 2, 4, or 6 mg of draflazine were studied during 5 sessions per volunteer, that were separated by at least 1 week. The administration of placebo or draflazine was randomized and double-blind, except for the highest dose (6 mg). The 6-mg dose was always given in the final session in a single-blind way for safety reasons, because at the time of these experiments this dosage had not been given before. Each experiment was done in the morning after a 24-hour abstinence from caffeine-containing products and an overnight fast of at least 10 hours. Both arms were intravenously cannulated to infuse the drug (left arm) and to collect blood (right arm). Immediately before and after the 15-minute drug infusion, blood was collected for measurement of plasma adenosine<sup>15</sup> and *ex vivo* nucleoside transport.<sup>16</sup> Ventilation was measured by registration of each thoracic excursion that occurred during three minutes before the start of draflazine infusion (baseline period) and immediately after draflazine administration.<sup>4</sup>

### Measurement of Ventilation

Thoracic excursions were measured using mercury-in-Silastic strain gauge plethysmography (Hokanson EC4, D.E. Hokanson, Inc.; Washington, DC). A 26-cm mercury-in-Silastic strain gauge was wrapped around the thorax at the midsternal level. To bridge the remaining gap, both ends of the Silastic tube were attached to an unextensible band at the back of the volunteer. As the thorax expands, the length of the gauge is changed by a corresponding amount. The resulting variations in the voltage drop across the gauge will reflect changes in thoracic circumference. In a separate group of 6 healthy nonobese male volunteers [age: 29 (22–36) years; height: 180 (172–190) cm], this method was validated against simultaneous spirometric recordings (Figure 1). Although confidence intervals were considerably higher for the plethysmographic technique, an increase in tidal volume as recorded by spirometry almost always accompanied an increase in thoracic excursion for each individual.

### Analytical Methods

To measure plasma adenosine concentration, 2 mL of blood was collected and directly mixed during col-

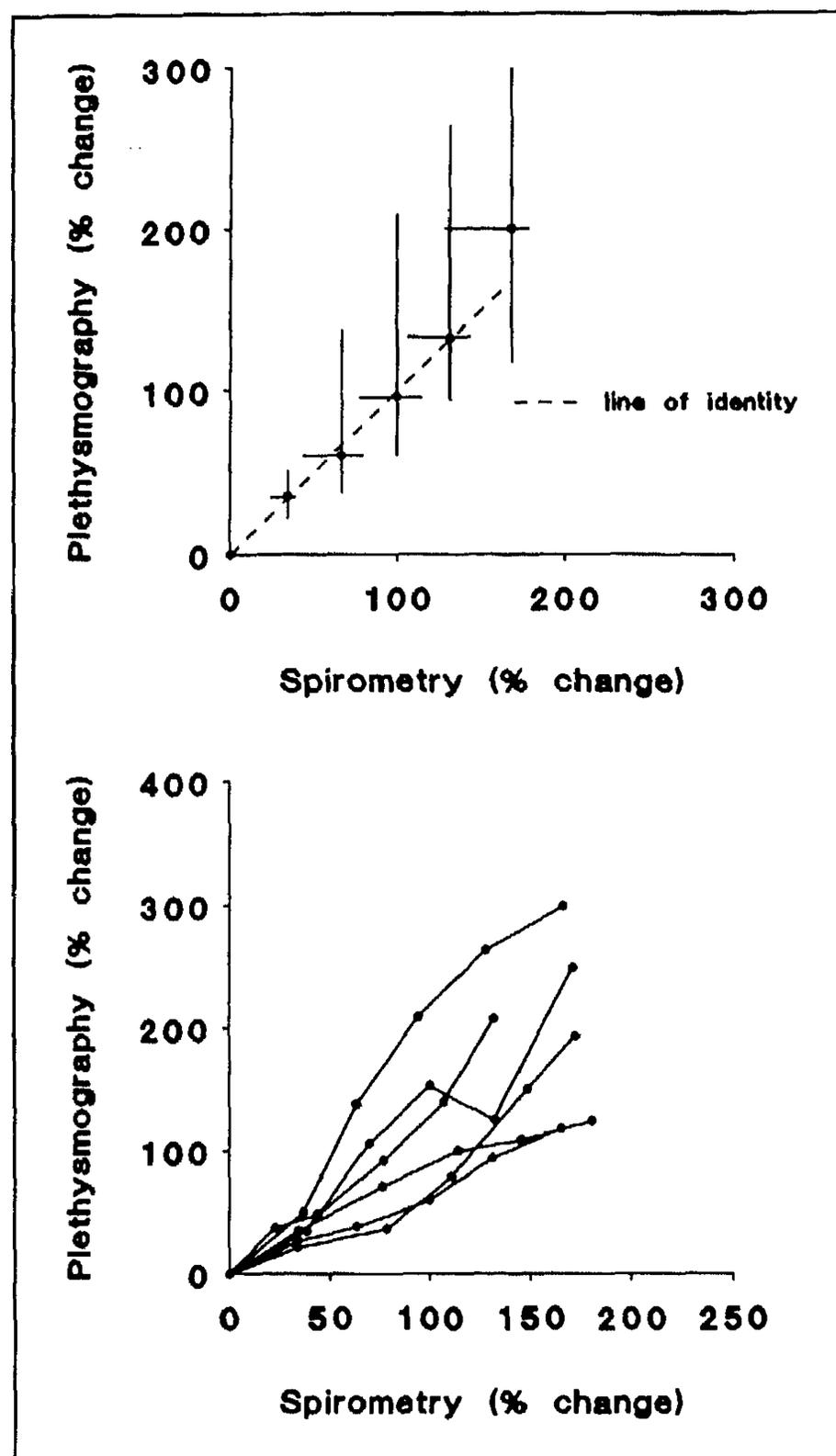


Figure 1. Six subjects were asked to breathe with a tidal volume of approximately 600 (baseline), 800, 1000, 1200, 1400, and 1600 mL. They adjust their ventilation by watching a continuous registration of their tidal volume by spirometry. Changes in real tidal volume (spirometry) and thoracic excursions (strain gauge plethysmography) were expressed as percentage change from baseline.

The upper panel shows the group average changes in thoracic excursion (plethysmography) in comparison with changes in tidal volume (spirometry) expressed as median with 95% confidence interval. The lower panel shows individual changes in these two parameters.

lection with 2 mL of "blocker solution" using a specially designed device. The blocker solution contained the adenosine deaminase inhibitor erythro-9(2-hydroxy-3-nonyl)-adenosine (EHNA, 10  $\mu$ mol/L), the adenosine transport inhibitor dipyridamole

(20  $\mu\text{mol/L}$ ), and the thrombocyte aggregation inhibitor indomethacin (2 mg/L). Immediately after blood collection, the blood/blocker mixture was centrifuged (Eppendorf centrifuge, Model 3200; Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany,) at 3000 rpm for 2 minutes, and the plasma was deproteinated with perchloric acid as previously described.<sup>15</sup> The extract was kept frozen at  $-20^{\circ}\text{C}$  until the adenosine concentration was determined in duplo by reversed-phase high-performance liquid chromatography using a nonlinear gradient. This adenosine determination has an intra-assay coefficient of variation of  $16.2 \pm 2.2\%$  (11 subjects, in each subject 5 duplo determinations) and an intra-individual coefficient of variation of  $20.7 \pm 7.2\%$  (11 subjects, 4 samples in each subject).

*Ex vivo* adenosine transport inhibition was measured by standardized incubation of erythrocytes with adenosine. Four milliliters of blood was drawn into a vial containing 1 mL of citrate/acid/dextrose (85  $\mu\text{mol/L}$  of trisodium citrate, 65  $\mu\text{mol/L}$  of citric acid, and 20 g/L of glucose) and further handled as previously described.<sup>16</sup> The percentage of adenosine transport inhibition (ATI%) was calculated as follows:

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

in which  $A_0$  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

Draflazine or placebo solutions in a formulation with 5% hydroxypropyl- $\beta$ -cyclodextrine (Janssen Pharmaceutica; Beerse, Belgium) were prepared with sodium chloride (NaCl 0.9%) by a specially trained research nurse who was not otherwise involved in the practical performance of the trial.

### Statistics

For each three-minute registration period, the thoracic excursions were averaged to one value. Effects of placebo or drug infusion were expressed as percentage change from baseline. When an overall analysis by Friedman two-way nonparametric analysis of variance (ANOVA) showed significant differences in responses ( $P < .05$ , chi-square test), the paired Wilcoxon signed rank test was used to find out which

draflazine dosages were different from placebo. All results are expressed as median (95% confidence interval).

### RESULTS

Figure 2 shows the effect of draflazine on breathing rate and tidal volume. Draflazine did not significantly affect breathing rate. Tidal volume changed by  $-7.2\%$  ( $-36.5$ – $49.1\%$ ) after placebo, and  $-8.7\%$  ( $-35.4$ – $47.8\%$ ),  $38.4\%$  ( $0.0$ – $121.3\%$ ),  $35.0\%$  ( $-10.5$ – $70.4\%$ ), and  $114.0\%$  ( $38.3$ – $184.8\%$ ) after 1, 2, 4, and 6 mg of draflazine, respectively ( $P < .05$  versus placebo for 2, 4, and 6 mg of draflazine). Nucleoside transport was inhibited by 0% ( $0$ – $1\%$ ) after placebo, and 70% ( $59$ – $74\%$ ), 81% ( $76$ – $85\%$ ), 90% ( $86$ – $93\%$ ), and 93% ( $90$ – $96\%$ ) after the 4 increasing draflazine dosages, respectively ( $P < .05$  for each draflazine dosage versus placebo). Plasma adenosine concentrations did not significantly change: 0.0  $\mu\text{mol/L}$  ( $-0.06$ – $0.03$   $\mu\text{mol/L}$ ) after placebo, and 0.0  $\mu\text{mol/L}$  ( $-0.02$ – $0.0$   $\mu\text{mol/L}$ ), 0.02  $\mu\text{mol/L}$  ( $0.0$ – $0.11$   $\mu\text{mol/L}$ ), 0.02  $\mu\text{mol/L}$  ( $0.0$ – $0.11$   $\mu\text{mol/L}$ ), and 0.0  $\mu\text{mol/L}$  ( $-0.06$ – $0.06$   $\mu\text{mol/L}$ ) after 1, 2, 4, and 6 mg of draflazine (Friedman:  $P = .12$ ).

### DISCUSSION

These results show that ventilation is increased by high-grade nucleoside transport inhibition with draflazine. Until now, nucleoside transport inhibition has been the only known action of this drug.<sup>13,17-19</sup> Recently, we have shown that the hemodynamic and neurohumoral effects of high-grade nucleoside transport inhibition with draflazine in humans can be antagonized with the adenosine receptor antagonist caffeine.<sup>20</sup> This supports the assumption that the effects of draflazine are mediated by endogenous adenosine. Additionally, draflazine is able to potentiate the vasodilator effect of intra-arterially-infused adenosine in humans<sup>21</sup> confirming the *in vivo* action of draflazine as a nucleoside transport inhibitor. Therefore, the present results suggest that endogenously released adenosine can play a role in the regulation of ventilation. Plasma adenosine concentration was not significantly affected by draflazine. This finding contrasts with the observations of others<sup>22,23</sup> who reported a doubling of adenosine concentrations after dipyridamole administration.<sup>22,23</sup> However, in these dipyridamole studies adenosine formation, uptake, and breakdown after blood sampling were not optimally antagonized. Therefore, in these studies baseline plasma adenosine concentrations may have been underestimated, and the administered dipyridamole may have affected adenosine uptake by

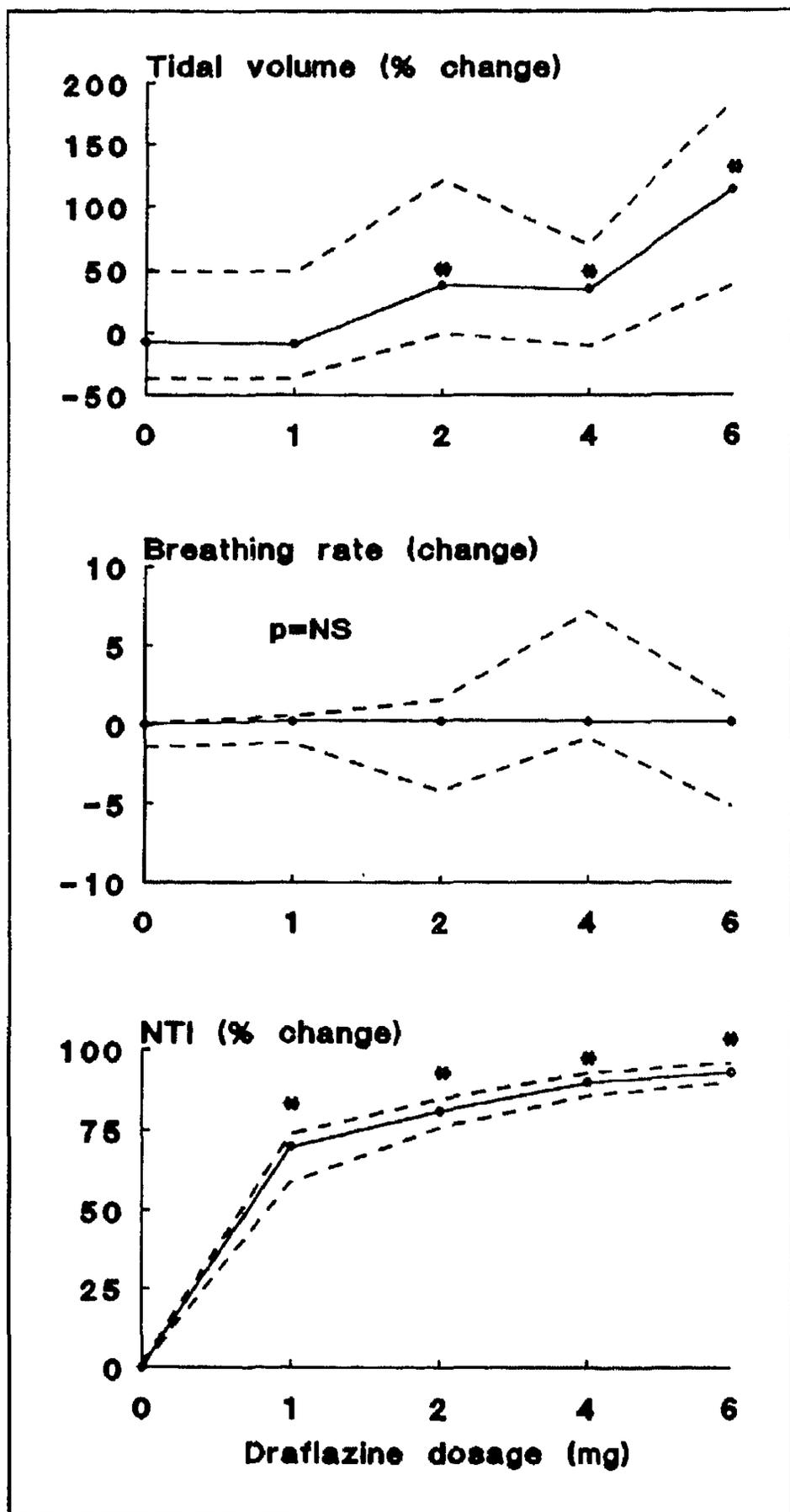


Figure 2. Effects of placebo (0 mg), 1, 2, 4, and 6 mg of draflazine on plethysmographically determined tidal volume (upper panel), breathing rate (middle panel), and ex vivo nucleoside transport inhibition (NTI, lower panel) as determined by standardized incubation of erythrocytes with adenosine are shown. Results are expressed as median with 95% confidence interval. \* indicates statistically significant differences from placebo.

erythrocytes after blood sampling. The present study does not rule out a small draflazine-induced increase in plasma adenosine levels, because intra-subject variability of the adenosine detection method is

high. Additionally, we cannot exclude a detectable increase in adenosine concentration during more sustained nucleoside transport inhibition. Alternatively, nucleoside transport inhibition may transform the endothelium to a functional barrier preventing adenosine from leaving the interstitial space and resulting in an increase of adenosine in the interstitial compartment only.

In conclusion, draflazine-induced high-grade nucleoside transport inhibition stimulates ventilation in humans. We suggest that this effect is mediated by an increased extracellular adenosine concentration in the interstitial compartment only.

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