Respiratory stimulant effects of adenosine in man after caffeine and enprofylline

P. SMITS, J. SCHOUTEN & TH. THIEN

Division of General Internal medicine, Department of Internal Medicine, St Radboud Hospital, University of Nijmegen, Nijmegen, The Netherlands

In a double-blind and randomized study the respiratory stimulant effect of continuous intravenous adenosine infusion was studied after previous administration of caffeine, placebo and enprofylline in 10 healthy young volunteers. After placebo, adenosine induced an increase of minute ventilation (from 6.3 to 12.5 l min⁻¹), tidal volume (from 0.60 to 0.96 l), and breathing rate (from 11.0 to 14.8 min⁻¹). Venous *p*CO₂ fell and pH rose after adenosine. Caffeine significantly reduced the adenosine-induced changes of minute ventilation, tidal volume, venous *p*CO₂ and pH, whereas no changes occurred after enprofylline. Our results suggest that adenosine stimulates respiration in man by binding with specific P₁-purinoceptors, which can be blocked by caffeine, but not by enprofylline.

Keywords caffeine enprofylline adenosine respiration

Introduction

Apart from interesting effects on the cardiovascular system and the autonomic nervous system (Smits *et al.*, 1987; Fredholm & Sollevi, 1986), adenosine has been shown to stimulate respiration in man (Watt & Routledge, 1985). Several pharmacological effects of this endogenous nucleoside are thought to be brought about by binding with purinergic P₁-receptors (Burnstock, 1980). Methylxanthines, like caffeine (1,3,7-trimethylxanthine) and theophylline, are well known competitive antagonists of adenosine (Fredholm, 1985), whereas the bronchodilating drug enprofylline (3-propylxanthine) hardly shows any adenosine antagonism (Fredholm & Persson, 1982). Although it has been suggested that the respiratory stimulant effect of adenosine results from excitation of carotid body chemoreceptors (Watt *et al.*, 1987), it is not known whether these effects are mediated by specific P₁-purinoceptors in man. In this study we tested the hypothesis that the respiratory effects of adenosine in man are mediated by purinoceptors,

and consequently can be inhibited by caffeine, but not by enprofylline.

Methods

After approval of the local hospital ethics committee, 10 healthy volunteers (nine men, one woman) gave their informed consent for this study. Age, weight, height, and Quetelet-index (mean $\pm$ s.d.) of the subjects numbered respectively 25.1 $\pm$ 2.9 years, 72.3 $\pm$ 5.4 kg, 1.82 $\pm$ 0.06 m and 21.9 $\pm$ 2.0 kg m⁻². In random sequence all subjects underwent three double-blind tests to register the respiratory stimulant effects of increasing doses of adenosine after previous administration of placebo, caffeine or enprofylline. Before the start of each test the subjects abstained from caffeine for 36 h and from smoking for 12 h at least. All tests were performed in the afternoon from 13.30 to 16.30 h, and they were separated from each other by 2

days at least. During each test basal values of minute ventilation (MV), tidal volume (TV) and breathing rate (BR) were registered after a supine rest period of 30 min. After taking a blood sample from an indwelling catheter in the left antecubital vein for determination of xanthine concentrations, a loading dose of 3.0 mg kg^{-1} caffeine, 1.7 mg kg^{-1} enprofylline or placebo was infused for 20 min into an antecubital vein of the right arm. Since we aimed for steady state concentrations of caffeine and enprofylline of 4 to 5 mg l^{-1} during the tests, the loading dose was followed by a maintenance dose for caffeine and enprofylline of 0.6 and $1.1 \text{ mg kg}^{-1} \text{ h}^{-1}$ respectively. After the loading dose the respiratory parameters and plasma xanthine concentrations were again measured. Subsequently, a continuous infusion of adenosine was given with increasing infusion rates of 0.04, 0.08 and $0.12 \text{ mg kg}^{-1} \text{ min}^{-1}$, each for 15 min.

Respiratory variables were measured during each adenosine infusion rate and also 5 min after stopping the highest infusion rate. At the end of the adenosine infusion a third blood sample was taken for determination of plasma xanthine concentrations. In five out of 10 subjects we also measured $p\text{CO}_2$ and pH of venous blood in all tests before and during adenosine infusion.

Respiratory parameters were measured by a 2 min-registration of MV and BR, using a hot-wire anemometer (Calculair®, Hospel), which was calibrated regularly with a 3 l syringe. TV was calculated from MV and BR ($\text{MV} = \text{TV} \times \text{BR}$). Plasma caffeine and enprofylline concentrations were determined by a h.p.l.c.-method. (Jonkman *et al.*, 1980; Edholm & Heintz, 1982). The data were analysed by paired Student's t-test (two-tailed), and all results are presented as mean values $\pm$ s.e.mean.

Results

In two tests with enprofylline the side effects during the highest adenosine infusion rate were too strong to register the respiratory parameters confidently, and so these missing data could not be included in the statistical analysis.

In the placebo test basal MV, TV and BR numbered $6.3 \pm 0.8 \text{ l min}^{-1}$, $0.60 \pm 0.10 \text{ l}$ and $11.0 \pm 1.0 \text{ min}^{-1}$ respectively. After caffeine MV rose from 6.7 ± 1.1 to $7.1 \pm 1.1 \text{ l min}^{-1}$, TV from 0.61 ± 0.12 to $0.67 \pm 0.11 \text{ l}$, and BR fell from 11.8 ± 1.0 to $11.0 \pm 0.9 \text{ min}^{-1}$. After enprofylline MV decreased from 6.9 ± 1.2 to $6.6 \pm 0.9 \text{ l min}^{-1}$, TV rose from 0.61 ± 0.12 to $0.64 \pm 0.12 \text{ l}$, and BR decreased from 12.0 ± 1.1 to $11.3 \pm 0.8 \text{ min}^{-1}$. Neither the baseline values nor

the xanthine-induced changes of these variables revealed significant differences between the three tests.

After placebo, infusion of adenosine induced a dose dependent respiratory stimulant effect: MV rose from 6.3 ± 0.8 to ultimately $12.5 \pm 1.7 \text{ l min}^{-1}$ ($P < 0.005$), TV from 0.60 ± 0.10 to $0.96 \pm 0.14 \text{ l}$ ($P = 0.001$) and BR from 11.0 ± 1.0 to $14.8 \pm 2.3 \text{ min}^{-1}$ (not significant). After stopping the adenosine infusion all parameters returned to basal values within 5 min. During the placebo test adenosine infusion induced a mean fall in $p\text{CO}_2$ from $6.4 \pm 0.5 \text{ kPa}$ to $5.7 \pm 0.6 \text{ kPa}$ ($n = 5$, $P < 0.03$), and a rise in pH from 7.36 ± 0.03 to 7.42 ± 0.03 units ($n = 5$, $P < 0.005$).

In Figure 1 the adenosine-induced changes of MV, TV and BR after placebo, caffeine and enprofylline are presented for each adenosine infusion rate. Statistical analysis was performed, using the mean changes over the three infusion rates. The results of this analysis are presented in Table 1. Caffeine significantly reduced the adenosine-induced rise in MV and TV. After enprofylline no significant differences were

Figure 1 Mean ($\pm$ s.e.mean) values of adenosine-induced changes (Δ) of minute ventilation, tidal volume and breathing rate after placebo (■), caffeine (□) and enprofylline (▨) are given for each adenosine infusion rate (0.04, 0.08 or $0.12 \text{ mg kg}^{-1} \text{ min}^{-1}$ adenosine).

Table 1 Mean ($\pm$ s.e. mean) values of adenosine-induced changes (Δ) of minute ventilation (MV), tidal volume (TV) and breathing rate (BR) after placebo, caffeine and enprofylline: the changes during each adenosine infusion rate were averaged to one mean value

	Placebo	P ₁	Caffeine	P ₂	Enprofylline	P ₃
Δ MV (1 min ⁻¹)	2.9 $\pm$ 1.0	<0.05	1.0 $\pm$ 0.5	<0.01	4.4 $\pm$ 1.0	NS
Δ TV (1)	0.20 $\pm$ 0.05	<0.05	0.06 $\pm$ 0.03	NS	0.18 $\pm$ 0.08	NS
Δ BR (min ⁻¹)	1.4 $\pm$ 1.0	NS	0.6 $\pm$ 0.6	NS	3.5 $\pm$ 1.4	NS

P₁:placebo vs caffeine; P₂:caffeine vs enprofylline; P₃:placebo vs enprofylline;
NS: not significant.

observed when compared with placebo. After placebo, adenosine-induced fall in venous $p\text{CO}_2$ averaged -0.8 ± 0.2 kPa and pH rose with 0.06 ± 0.01 units. After caffeine these changes were attenuated numbering respectively -0.1 ± 0.02 kPa ($n = 5$, $P < 0.005$ vs placebo) and 0.01 ± 0.01 units ($n = 5$, $P < 0.01$ vs placebo). After enprofylline these changes numbered respectively -1.1 ± 0.3 kPa and 0.06 ± 0.02 units, being not significantly different from the placebo test.

Basal plasma caffeine concentrations were comparable in the three tests and never exceeded 0.6 mg l^{-1} . After the loading dose of caffeine and enprofylline plasma concentrations numbered respectively $5.4 \pm 0.1 \text{ mg l}^{-1}$ and $3.2 \pm 0.2 \text{ mg l}^{-1}$, and at the end of the highest adenosine infusion rate respectively $5.4 \pm 0.2 \text{ mg l}^{-1}$ and $4.4 \pm 0.4 \text{ mg l}^{-1}$.

In all tests the subjects experienced typical side effects of adenosine (Watt & Routledge, 1985; Smits *et al.*, 1987), including hyperventilation, a discomfort in the neck, chest and upper abdomen, and palpitations. In nine subjects these side effects were less pronounced after caffeine. Enprofylline enhanced the adenosine-induced complaints of nausea.

Discussion

The placebo test of this study shows the respiratory stimulant effect of continuous infusion of adenosine in man, with increases of MV, TV and BR (Figure 1). This confirms earlier observations after bolus injections of adenosine (Watt & Routledge, 1985). Our results of lowered $p\text{CO}_2$ and increased pH also suggest hyperventilation, although this was not arterial but venous blood.

In a former study a relatively low dose of caffeine significantly inhibited some adenosine-induced circulatory changes in man (Smits *et al.*, 1987). We now used similar doses to study the effects of caffeine on the respiratory stimulant effect of adenosine. With respect to enprofylline

we aimed equimolar plasma concentrations to be sure that observed differences between the caffeine and enprofylline test can not be explained by large differences in plasma concentrations. As presented plasma concentrations of caffeine and enprofylline at the end of the adenosine infusion indeed were in the range of 4 to 5 mg l^{-1} .

Experimental data in cats, as well as in man, suggest that the respiratory stimulant effect of exogenous adenosine is mediated by carotid body chemoreceptors (McQueen & Ribeiro, 1986; Watt *et al.*, 1987). Our current results show that caffeine, but not enprofylline, inhibits this effect of exogenous adenosine in man, suggesting that adenosine effects on the carotid body are mediated by specific P₁-purinoceptors. Although we used a relatively low dose, caffeine itself did not induce changes in respiratory parameters. So, under the experimental conditions of our study, endogenous adenosine seems not to contribute to the regulation of respiration. However, during ischaemia, hypoxia, hypotension or shock the release of purines is increased (Berne *et al.*, 1983; Wooliscroft & Fox, 1986), and besides changes in $p\text{O}_2$, pH and $p\text{CO}_2$ the increase in plasma adenosine concentrations might contribute to the regulation of ventilation during such events. Whether caffeine inhibits this physiological response is unknown, but according to our experimental data this may be of importance. In addition, the lack of effect of enprofylline on the putative stimulation of respiration by adenosine during such events, might be an advantage when compared with bronchodilating drugs that exhibit adenosine antagonism.

The adenosine receptors of the carotid body of the cat are of the A₂-subtype (McQueen & Ribeiro, 1986), and effects via such receptors are often mediated by an increase in cAMP (Fredholm, 1985). Consequently, inhibition of phosphodiesterase theoretically can enhance this rise of intracellular cAMP. Since enprofylline has strong phosphodiesterase-inhibiting, but no adenosine-antagonistic properties (Fredholm,

1985; Fredholm & Persson, 1982), this drug could even enhance adenosine mediated effects. Apart from the potentiation of adenosine-induced side effects after enprofylline no further evidence was found for this suggestion in our study.

In conclusion, caffeine significantly inhibits the respiratory stimulant effect of adenosine. Enprofylline in an equimolar plasma concentra-

tion did not affect these adenosine effects. Our observations suggest that adenosine stimulates respiration in man via specific P_1 -purinoceptors. Further studies are needed to evaluate the clinical relevance of these observations.

The preparation of this manuscript by Anky Verweijen was greatly appreciated, and we gratefully acknowledged the technical assistance of Joeke Reijenga during all tests.

References

- Berne, R. M., Knabb, R. M., Ely, S. W. & Rubio, R. (1983). Adenosine in the local regulation of blood flow: a brief overview. *Fed. Proc.*, **42**, 3136–3142.
- Burnstock, G. (1980). Purinergic receptors in the heart. *Circ. Res.*, **46**, I175–I182.
- Edholm, L. E. & Heintz, L. (1982). Column liquid chromatographic analysis of enprofylline. *Internal report 844-RD-0077*, Astra Pharmaceuticals, Lund, Sweden.
- Fredholm, B. B. (1985). On the mechanism of action of theophylline and caffeine. *Acta med. Scand.*, **217**, 149–153.
- Fredholm, B. B. & Persson, C. G. A. (1982). Xanthine derivatives as adenosine receptor antagonists. *Eur. J. Pharmac.*, **81**, 673–676.
- Fredholm, B. B. & Sollevi, A. (1986). Cardiovascular effects of adenosine. *Clin. Pharmac.*, **6**, 1–21.
- Jonkman, J. H. G., Schoenmaker, R., Greving, J. E. & de Zeeuw, R. A. (1980). Rapid and selective theophylline serum and saliva assay by means of high pressure liquid chromatography. *Pharm. Weekbl.*, **2**, 557–561.
- McQueen, D. S. & Ribeiro, J. A. (1986). Pharmacological characterization of the receptor involved in chemoexcitation induced by adenosine. *Br. J. Pharmac.*, **88**, 615–620.
- Smits, P., Boekema, P., Thien, Th., Abreu de, R. & van 't Laar, A. (1987). Evidence for an antagonism between caffeine and adenosine in the human cardiovascular system. *J. cardiovasc. Pharmac.*, **10**, 136–143.
- Watt, A. H. & Routledge, P. A. (1985). Adenosine stimulates respiration in man. *Br. J. clin. Pharmac.*, **20**, 503–506.
- Watt, A. H., Reid, P. G., Stephens, M. R. & Routledge, P. A. (1987). Adenosine-induced respiratory stimulation in man depends on site of infusion. Evidence for an action on the carotid body? *Br. J. clin. Pharmac.*, **23**, 486–490.
- Woolliscroft, J. O. & Fox, J. H. (1986). Increased body fluid purine levels during hypotensive events. *Am.J.Med.*, **81**, 472–478.

(Received 27 March 1987,
accepted 25 August 1987)