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Modelling of the pharmacodynamic interaction of an A_1 adenosine receptor agonist and antagonist *in vivo*: N^6-cyclopentyladenosine and 8-cyclopentyltheophylline

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1 The purpose of this investigation was to develop a pharmacokinetic-pharmacodynamic model for the interaction between an adenosine A_1 receptor agonist and antagonist *in vivo*. The adenosine A_1 receptor agonist, N^6-cyclopentyladenosine (CPA) and the antagonist, 8-cyclopentyltheophylline (CPT) were used as model drugs. The CPA-induced reduction in mean arterial pressure and heart rate were used as measurements of effect.

2 Four groups of eight rats each received 200 $\mu g\,kg^{-1}$ of CPA i.v. in 5 min during a steady-state infusion of CPT at a rate of 0, 57, 114 or 228 $\mu g\,kg^{-1}\,h^{-1}$. The haemodynamic parameters were continuously measured and frequent blood samples were taken to determine the pharmacokinetics of the drugs.

3 CPT had no influence on the pharmacokinetics of CPA and the baseline values of the haemodynamic variables. Furthermore, no clear antagonism by CPT was observed of the CPA-induced reduction in mean arterial pressure. However, CPT antagonized the effect on heart rate, and with increasing CPT concentrations, a parallel shift of the CPA concentration-effect relationship to the right was observed.

4 An agonist-antagonist interaction model was used to characterize the interaction quantitatively. On the basis of this model, the pharmacodynamic parameters of both CPA and CPT could be estimated. For CPA the values were (mean ± s.e.): $E_{max} = 198 \pm 11$ b.p.m., $EC_{50} = 2.1 \pm 0.7$ ng ml^{-1}, Hill factor = 2.3 ± 0.6 and for CPT: $EC_{50} = 3.7 \pm 0.3$ ng ml^{-1} and Hill factor = 3.1 ± 0.1.

5 It is concluded that the competitive agonist-antagonist interaction model may be of value to characterize quantitatively the pharmacodynamic interactions between adenosine A_1 receptor ligands *in vivo*.

Keywords: N^6-cyclopentyladenosine; 8-cyclopentyltheophylline; pharmacokinetics; pharmacodynamics; interaction *in vivo*; mean arterial pressure; heart rate; modelling

Introduction

Adenosine shows a multiplicity of actions *in vivo*. The cardiovascular effects of adenosine have been associated with at least two types of adenosine receptors, the A_1 and A_2 subtypes respectively (Van Calker *et al.*, 1979; Londos *et al.*, 1980; Belardinelli & Lerman, 1991). Activation of the A_1 receptor has been reported to elicit bradycardia, depression of myocardial contractility and impulse conduction velocity (Belardinelli *et al.*, 1982; Evans *et al.*, 1982; Martens *et al.*, 1987) whereas activation at the A_2 receptor results in coronary and peripheral vasodilatation (Kusachi *et al.*, 1983; Webb *et al.*, 1990). For both receptor subtypes more or less selective and metabolically stable agonists and antagonists have been developed (Williams *et al.*, 1986; Bruns *et al.*, 1988; Hutchison *et al.*, 1989; Trivedi *et al.*, 1991). In principle these drugs may be of value in the treatment of disorders of the cardiovascular system (Daly, 1982).

The relative potency and intrinsic activity of receptor agonists and antagonists is typically determined in *in vitro* preparations (e.g. receptors, isolated organs). The potency and intrinsic activity of a drug *in vivo*, however, can be quite different. In the *in vivo* situation, many factors in the response generating system can modulate the primary receptor-mediated response (Kenakin, 1992). Especially for drugs acting on the cardiovascular system, homeostatic control mechanisms must be taken into consideration (Struyker Boudier, 1992). *In vivo* studies are therefore essential to characterize the pharmacodynamics of adenosine receptor agonists and antagonists, as was recently demonstrated for benzodiazepines (Mandema *et al.*, 1992).

Studies evaluating the potency and intrinsic activity of adenosine receptor ligands *in vivo*, are generally based on the determination of dose-response relationships. However, the dose-response relationship of a drug is not only determined by the affinity to and the intrinsic activity at the receptor, but also by pharmacokinetic factors like distribution to the site of action (protein binding) and the (inter)-activity of metabolites formed *in vivo* (Dingemanse *et al.*, 1988). Therefore, in studies which are designed to quantify pharmacological responses *in vivo*, experimental strategies must be used in which these potentially complicating pharmacokinetic factors are either excluded or accounted for (Levy, 1985). This requires focus on concentration-response rather than dose-response relationships.

Recently we developed a pharmacokinetic-pharmacodynamic model for the quantitative characterization of the cardiovascular actions of the A_1 receptor agonist, cyclopentyladenosine (CPA), in normotensive rats. It was shown that in individual rats the CPA-evoked reduction in heart rate can be directly related to the CPA concentration in blood on the basis of the sigmoidal E_{max} pharmacodynamic model. In contrast, modelling of the CPA-induced reduction in mean arterial blood pressure was found to be more complex, presumably as a result of homeostatic control mechanisms

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(Mathôt *et al.*, 1994). The results of this previous study showed that the potency and intrinsic activity of A_1 receptor agonists *in vivo* can be determined quantitatively by pharmacokinetic-pharmacodynamic modelling, using the reduction in heart rate as a pharmacodynamic endpoint. With respect to antagonists however, the situation is more complex since administration of these compounds does not result in measurable haemodynamic effects. Therefore the interaction with an adenosine A_1 receptor agonist must be studied to determine the antagonist's pharmacodynamic parameters.

The present study was designed to develop a pharmacokinetic-pharmacodynamic model for the interaction between an adenosine A_1 receptor agonist and antagonist *in vivo*. The adenosine A_1 receptor agonist, N^6-cyclopentyladenosine (CPA), and the antagonist, 8-cyclopentyltheophylline (CPT), were used as model drugs.

Methods

Surgical procedures

Adult male normotensive SPF rats of Wistar descent (Sylvius Laboratory Breeding Facility, Leiden, The Netherlands) weighing 200–250 g were used. The animals were housed individually in plastic cages under a normal 12 h light (07 h 00 min–19 h 00 min)-dark cycle. Laboratory chow (Standard Laboratory Rat, Mouse and Hamster Diets, RMH-TH, Hope Farms, Woerden, The Netherlands) and tap water were available *ad libitum*.

Three days before experimentation, cannulae were implanted under light ether anaesthesia. For monitoring of arterial blood pressure and the collection of serial blood samples, the abdominal aorta was cannulated by approach through the left and right femoral artery. For that purpose cannulae consisting of 4.5 or 4.0 cm of polythene tubing (i.d. 0.28 mm; Portex, Medica BV, 's Hertogenbosch, The Netherlands), heat-sealed to 18 cm (i.d. 0.58 cm) polythene tubing (Portex) were used. For the simultaneous administration of CPA and CPT infusion solutions, the right jugular vein was cannulated with a cannula consisting of two separate tubings (PE45, Media BV, 's Hertogenbosch, The Netherlands). All cannulae were guided subcutaneously to the neck where they were exteriorized and anchored in place. To prevent clotting, cannulae were filled with a 25% (g v^{-1}) polyvinylpyrrolidone (PVP, Brocacef, Maarssen, The Netherlands) solution in physiological saline containing 50 iu ml^{-1} of heparin (Pharmacy, Academic Hospital, Leiden, The Netherlands) which was renewed daily.

Cardiovascular measurement

Arterial blood pressure was measured from the left femoral catheter in the abdominal aorta by use of a miniature strain gauge P10EZ transducer, equipped with a TA1017 CritiFlo diaphragm dome (both Viggo-Spectramed BV Bilthoven, The Netherlands). This dome allowed a continuous flushing of the cannula with heparinized saline (20 iu ml^{-1}) at a rate of 500 $\mu l\ h^{-1}$ (Harvard infusion pump 22, Plato, Diemen, The Netherlands). The pressure transducer was placed at the level of the animal's heart, when in normal position and connected to a polygraph amplifier console (RMP6018, Nihon Kohden Corp., Tokyo, Japan). Heart rate was captured from the pressure signal which was used to trigger a tachograph. Signals were recorded on a polygraph and concurrently converted in a CED 1401 interface (Cambridge Electronics Design Ltd., Cambridge, England) and tranferred to a 80387 computer (Philips, Eindhoven, The Netherlands). The data were stored on hard disk for off-line analysis. Data acquisition and reduction were performed with the Spike2 computer software (Cambridge Electronics Design Ltd., Cambridge, England).

Pharmacokinetic-pharmacodynamic experiments

To investigate the effect of different CPT steady-state concentrations on the concentration versus effect relationship of CPA, 32 rats were randomly assigned to four groups receiving a continuous intravenous infusion of CPT at a rate of 0, 57, 114 or 228 $\mu g\ kg^{-1}\ h^{-1}$. CPT was dissolved in ethylenediamine (0.2 M) before dilution with 0.9% physiological saline to provide isotonic infusion solutions. After 75 min, as steady-state was reached, the animals received an intravenous infusion of CPA (200 $\mu g\ kg^{-1}$) in 5 min. CPA was dissolved in 638 μl of physiological saline. Arterial blood samples of 100 or 200 μl were collected at −1, 1, 2, 3, 4, 5, 6, 7, 9, 12, 15, 20, 25, 30, 35, 60 and 120 min relative to the beginning of the CPA infusion, for simultaneous analysis of both CPA and CPT concentrations. Blood samples were immediately haemolyzed in 400 or 300 μl of ice-cold water and stored at −30°C pending h.p.l.c. analysis.

During the experiments arterial blood pressure and heart rate were monitored continuously. After connection of the arterial catheter to the recording equipment, the rat was allowed to habituate to the experimental conditions for 30 min. The recording of the haemodynamic values was started at 30 min before the beginning of the CPT infusion, for baseline determination and continued up to 3 h after CPA dosing.

For the pharmacokinetic-pharmacodynamic modelling procedure, time-effect points were obtained by averaging 60 to 300 s of consecutive heart rate or blood pressure data. Data were collected more frequently at the time of rapid change in drug concentration.

Drug analysis

In each blood sample, concentrations of both CPA and CPT were determined simultaneously by reversed phase h.p.l.c. with u.v. detection according to the method described earlier by Mathôt *et al.* (1993). The between-day coefficients of variation of three different quality control samples of CPA (7.5, 19 and 560 ng ml^{-1}) and CPT (12.5, 25 and 50 ng ml^{-1}) were 12, 5.7 and 2.2% ($n = 20$) and 17, 14 and 12% ($n = 15$), respectively.

Protein binding

The plasma to blood concentration ratio (P/B) and the extent of plasma protein binding (free fraction, f_u) was determined in three separate groups of 6 rats each. These rats received an intravenous bolus infusion of 200 $\mu g\ kg^{-1}$ of CPA in 5 min during an infusion with physiological saline or two different infusions of CPT (infusion rates 912 and 1824 $\mu g\ kg^{-1}\ h^{-1}$). From each rat, blood samples with a volume of 1.6 ml were drawn at 5 and 7 min after the start of the CPA infusion and transferred directly into heparinized tubes on ice. An aliquot of 50 μl was haemolyzed in 400 μl of ice-cold water. This sample was used for determination of the whole blood concentration. The remaining blood was centrifuged at 4°C and the plasma separated. A 50 μl sample of plasma was stored for determination of the CPA and CPT concentration. 500 μl of the plasma sample was subjected to ultrafiltration through a YMT ultrafiltration membrane at 1090 *g* for 10 min at 37°C (Amicon Micropartition System, Amicon Division, Danvers, MA, U.S.A.). The concentrations of CPA and CPT were determined in 75 μl of the ultrafiltrate. The concentrations of CPA and CPT were determined in duplicate using the analytical procedures described in the drug analysis section.

Data analysis

The values of the pharmacokinetic and pharmacodynamic parameters of CPA were quantified for the individual rats by the non-linear least squares regression program Siphar

(Simed SA, Creteil, France). The blood concentration-time profiles of CPA after intravenous infusion were described by a poly-exponential function:

$$C_t = R_0 \cdot \sum_{i=1}^{n} \frac{C_i}{\lambda_i} \cdot (e^{-\lambda_i \cdot (t-T)} - e^{-\lambda_i \cdot t}) \quad (1)$$

with:

$$(t-T) = \begin{cases} t-T \text{ for } t>T \\ 0 \text{ otherwise} \end{cases}$$

In this equation C_t is the blood concentration of CPA at time t, T is the infusion duration of rate R_0 and C_i is the coefficient associated with the i^{th} exponent λ_i. Different exponential models were investigated and the most suitable model was chosen on the basis of the Akaike information criterion (Akaike, 1974). Basic pharmacokinetic parameters as area under the curve (AUC), total blood clearance (Cl), volume of distribution at steady state (V_{ss}) and terminal half-life ($t_{\frac{1}{2}},\lambda_i$) were calculated from the coefficients and exponents of the fitted functions according to standard procedures (Gibaldi & Perrier, 1982).

To characterize the competitive pharmacodynamic interaction between two drugs acting on a common receptor, the following equation has been proposed (Holford & Sheiner, 1981):

$$E = E_0 + \frac{E_{max,1} \cdot \left(\frac{C_1}{EC_{50,1}}\right)^{n,1} + E_{max,2} \cdot \left(\frac{C_2}{EC_{50,2}}\right)^{n,2}}{1 + \left(\frac{C_1}{EC_{50,1}}\right)^{n,1} + \left(\frac{C_2}{EC_{50,2}}\right)^{n,2}} \quad (2)$$

where E_0 is the baseline effect value, E_{max} is the maximum effect, EC_{50} is the concentration of half maximal effect, n is a constant expressing the sigmoidicity of the concentration-effect relationship and 1 and 2 are subscripts referring to the two interacting drugs. Thus, in the situation of the interaction between CPA and CPT, $EC_{50,1}$ and $EC_{50,2}$ are the EC_{50} values of CPA and CPT, respectively. These EC_{50} values provide a measure of the affinity of CPA and CPT to the biological system *in vivo*. *In vivo* an EC_{50} value has a meaning analogous to a pK_B value *in vitro*. However, due to drug distribution and metabolism processes *in vivo*, the relationship between the two parameters cannot be uniquely defined. If one of the drugs has no intrinsic activity, i.e. $E_{max,2}$ is zero, equation 2 can be rewritten as:

$$E = E_0 + \frac{E_{max,1} \cdot C_1^{n,1}}{EC_{50,app}^{n,1} + C_1^{n,1}} \quad (3)$$

with:

$$EC_{50,app} = EC_{50,1} \cdot \left(1 + \left(\frac{C_2}{EC_{50,2}}\right)^{n,2}\right)^{\frac{1}{n,1}} \quad (4)$$

The concentration-effect relationships of CPA during the steady-state infusions of CPT were quantified by equation 3. The relationship between the steady-state CPT concentration and the apparent EC_{50} of CPA was fitted to equation 4 by non-linear least squares regression analysis (Siphar, Simed SA, Creteil, France), to derive the pharmacodynamic parameters of CPT.

Drugs

N^6-cyclopentyladenosine (CPA) was purchased from Boehringer Mannheim GmbH (Mannheim, Germany), 8-cyclopentyltheophylline (CPT) and N^6-cyclohexyladenosine (CHA) were obtained from Research Biochemicals Inc. (Natick, MA, U.S.A.).

Statistical analysis

Statistical analysis was conducted by analysis of variance (ANOVA) or, if more appropriate as determined by Bartlett's test for non-homogeneity of variances, the non-parametric Kruskal-Wallis test. A one-way ANOVA was applied to investigate the effect of different concentrations of CPT on the pharmacokinetic and pharmacodynamic characteristics of CPA and the concentration-dependency and the mutual interaction of the protein binding of CPA and CPT. A two-way ANOVA was applied to compare the values of the haemodynamic parameters during the CPT infusion to the baseline values. All values are reported as mean ± s.e. unless stated otherwise. In the statistical analysis a significance level of 5% ($P<0.05$) was selected.

Results

Pharmacokinetics

The time profiles of the CPA blood concentrations are shown in Figure 1 for four typical rats, one out of each group. For all rats the blood concentration versus time profile of CPA was best described by a bi-exponential equation. The effect of the different steady-state concentrations of CPT on the pharmacokinetic parameters of CPA is summarized in Table 1. In the rats receiving CPT at the rate of 57 $\mu g\,kg^{-1}\,h^{-1}$ the value of the clearance of CPA was slightly, but statistically significantly higher than in the other groups. No other significant differences were found between the four different treatment groups, indicating the absence of a major effect of CPT on the concentration-time profile of CPA. The clearance of CPT was calculated on the basis of the steady-state concentrations during the zero-order infusion, both before and after the administration of CPA. CPT clearance after the administration of CPA was 91 ± 5 ml $min^{-1}\,kg^{-1}$ and significantly lower when compared to the value before CPA administration of 113 ± 5 ml $min^{-1}\,kg^{-1}$ ($P<0.001$).

Blood to plasma distribution and plasma protein binding

The plasma to blood concentration-ratio and the free fraction in plasma of CPA and CPT were determined in separate experiments *in vivo*. For CPA, the plasma to blood concentration-ratio was on average 0.52 ± 0.02 and the free fraction in plasma 0.63 ± 0.01 ($n = 36$). These values were independent of the concentration in the concentration range of 100–780 ng ml^{-1} in blood. Neither parameter was influenced by co-administration of CPT. For CPT the value

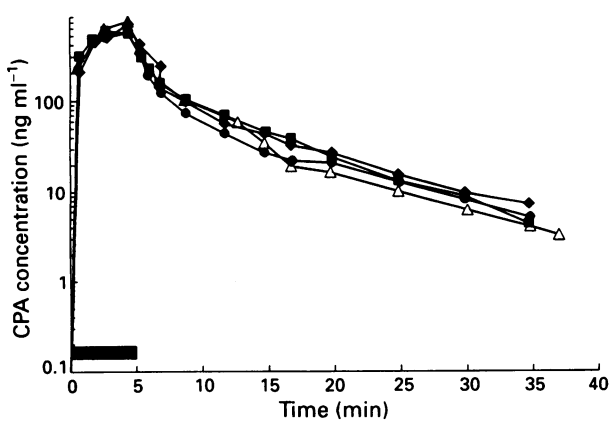

Figure 1 Time course of the N^6-cyclopentyladenosine (CPA) blood concentration for 4 individual rats which received 200 $\mu g\,kg^{-1}$ of CPA in 5 min during an i.v. infusion of physiological saline (Δ), 8-cyclopentyltheophylline (CPT) 57 $\mu g\,kg^{-1}\,h^{-1}$ (■), CPT 114 $\mu g\,kg^{-1}\,h^{-1}$ (●) and CPT 228 $\mu g\,kg^{-1}\,h^{-1}$ (◆).

of the plasma to blood concentration-ratio was 0.54 ± 0.02 and of the free fraction in plasma 0.26 ± 0.01 ($n = 24$). These values were used for calculation of the $EC_{50app,u}$ and the $EC_{50,u}$ of CPA and CPT, respectively. $EC_{50,u}$ is the value of the EC_{50} calculated on the basis of free (= unbound) plasma concentrations and provides therefore the most realistic estimate of the affinity of the drug to the biological system *in vivo*.

Pharmacodynamics and pharmacodynamic interaction

The potential effect of CPT on mean arterial blood pressure and heart rate was evaluated during the steady-state infusion before the administration of CPA. At all three levels of CPT, a slight decrease in blood pressure and heart rate relative to baseline occurred. A similar change was observed during saline (= placebo) infusion. In Figure 2 the effect of CPT on the CPA-induced reduction in mean arterial pressure is shown for the same four rats as in Figure 1. In the absence of CPT, the infusion of CPA resulted in a rapid decrease of the mean arterial pressure to values which were approximately 40–50% of the preinfusion levels. After termination of the CPA infusion, mean arterial blood pressure augmented gradually to plateau levels. The plateau levels at the end of the experiment were lower than the preinfusion values. No clear antagonism by CPT of the effect on mean arterial blood pressure was observed. In Figure 3 the effect of CPT is shown for the CPA-induced reduction in heart rate. In contrast to the effect of CPA on mean arterial pressure, the effect of CPA on heart rate was fully reversible within the time frame of the experiment. In the presence of CPT the effect of CPA on heart rate returned to preadministration values more rapidly. The influence of CPT on the concentration-effect relationship of CPA for heart rate is shown in Figure 4. As no hysteresis was observed between CPA blood concentrations and the effect on heart rate, the two values appeared to be directly related to each other. The relationship was quantified by the sigmoidal E_{max} pharmacodynamic model according to equation 3. The effect of the different steady-state levels of CPT on the pharmacodynamic parameter estimates of CPA is shown in Table 2. CPT did not have a significant effect on the maximal effect of CPA (E_{max}) and the shape of the curve (n). However, the different CPT concentrations markedly increased the apparent EC_{50} of CPA. The parallel shift to the right suggests a competitive interaction between the two drugs. Figure 5 shows the relationship between the CPT concentration and the apparent EC_{50} of CPA. This relationship was quantified by equation 4 in order to derive the pharmacodynamic parameters of CPT. The introduction of a sigmoidicity factor n_2 for CPT resulted in an improvement of the fit according to the Akaike Information Criterion. The EC_{50} of CPT was 3.7 ± 0.3 ng ml^{-1} when calculated on the basis of whole blood concentrations. After correction for the plasma to blood concentration-ratio and plasma protein binding, a value of the $EC_{50,u}$ of 0.5 ± 0.1 ng ml^{-1} was obtained. The value of the slope factor n of CPT was 3.1 ± 0.1.

Table 1 Effect of different steady-state 8-cyclopentyltheophylline (CPT) concentrations on the pharmacokinetic parameter estimates of N^6-cyclopentyladenosine (CPA)

CPT conc (ng ml^{-1})	*Cl* (ml min^{-1} kg^{-1})	V_{SS} (ml kg^{-1})	$t_{\frac{1}{2}}$ (min)	*Number of animals*
0	63 ± 2	269 ± 16	6.4 ± 0.2	7
14 ± 1	76 ± 4*	264 ± 6	5.9 ± 0.4	7
32 ± 1	61 ± 4	268 ± 6	7.0 ± 0.2	6
59 ± 5	63 ± 3	313 ± 26	7.1 ± 0.3	8

The values are mean ± s.e.
*Significantly different from the values at other CPT concentrations in one-way ANOVA ($P < 0.05$).

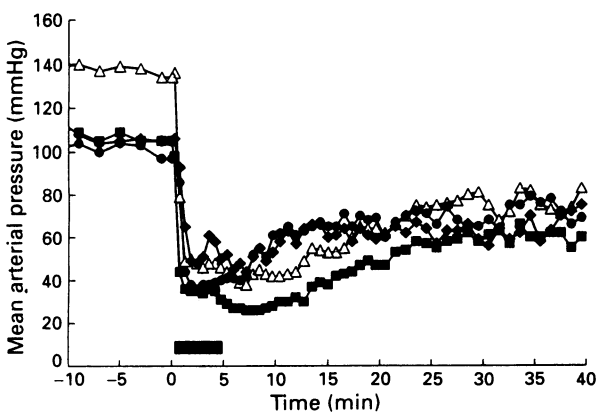

Figure 2 Time course of the mean arterial pressure for 4 individual rats which received 200 μg kg^{-1} of N^6-cyclopentyladenosine (CPA) in 5 min during an i.v. infusion of physiological saline (Δ), 8-cyclopentyltheophylline (CPT) 57 μg kg^{-1} h^{-1} (■), CPT 114 μg kg^{-1} h^{-1} (●) and CPT 228 μg kg^{-1} h^{-1} (◆).

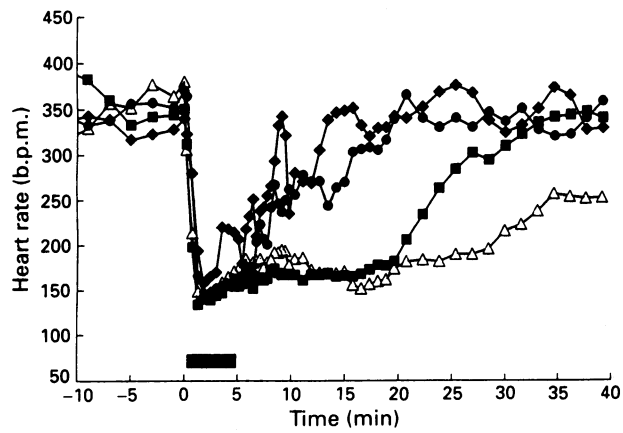

Figure 3 Time course of the heart rate for 4 individual rats which received 200 μg kg^{-1} of N^6-cyclopentyladenosine (CPA) in 5 min during an i.v. infusion of physiological saline (Δ), 8-cyclopentyltheophylline (CPT) 57 μg kg^{-1} h^{-1} (■), CPT 114 μg kg^{-1} h^{-1} (●) and CPT 228 μg kg^{-1} h^{-1} (◆).

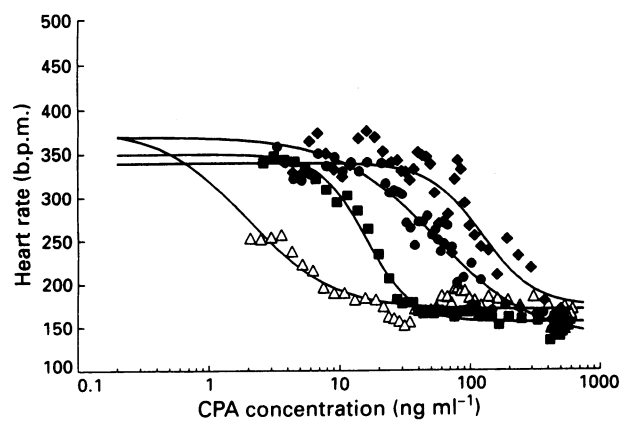

Figure 4 N^6-cyclopentyladenosine (CPA) blood concentration versus heart rate relationship for 4 individual rats which received 200 μg kg^{-1} of CPA in 5 min during an i.v. infusion of physiological saline (Δ), 8-cyclopentyltheophylline (CPT) 57 μg kg^{-1} h^{-1} (■), CPT 114 μg kg^{-1} h^{-1} (●) and CPT 228 μg kg^{-1} h^{-1} (◆).

Table 2 Effect of different steady-state 8-cyclopentyltheophylline (CPT) concentrations on the pharmacodynamic parameter estimates of N^6-cyclopentyladenosine (CPA)

CPT conc (ng ml^{-1})	E_0 (b.p.m.)	E_{max} (b.p.m.)	$EC_{50app,u}$ (ng ml^{-1})	$EC_{50app,u}$ (ng ml^{-1})	*Hill factor*
0	351 ± 13	198 ± 11	2.1 ± 0.7	0.7 ± 0.2	2.3 ± 0.6
14 ± 1	343 ± 5	203 ± 17	15 ± 2*	4.9 ± 0.7*	2.1 ± 0.5
32 ± 1	356 ± 7	203 ± 12	38 ± 9*	13 ± 3*	3.5 ± 1.3
59 ± 5	348 ± 6	205 ± 11	91 ± 12*	30 ± 4*	2.3 ± 0.6

Heart rate was used as the measure of the pharmacodynamic response. The values are reported as mean ± s.e.
*Significantly different from the value in the control group (CPT concentration = 0) in one-way ANOVA ($P<0.001$).

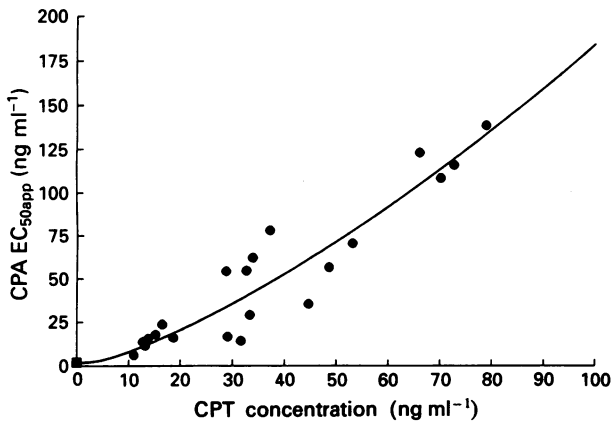

Figure 5 Relationship between 8-cyclopentyltheophylline (CPT) steady-state blood concentrations and apparent EC_{50} of N^6-cyclopentyladenosine (CPA). The solid line was obtained by fitting the agonist-antagonist interaction model (equation 4) to individual data.

Discussion

Of the various adenosine receptor antagonists, 8-cyclopentyltheophylline (CPT) has been demonstrated to be the most selective with respect to binding to the A_1 receptor subtype (Bruns *et al.*, 1988). Based on its favourable physicochemical properties CPT is also particularly suitable for *in vivo* pharmacological studies (Bruns *et al.*, 1987). So far however, quantitative studies characterizing the interaction between an adenosine receptor agonist and antagonist *in vivo* are lacking. In the present study a quantitative model was derived to characterize the pharmacokinetic and pharmacodynamic interaction of CPT and CPA *in vivo* in the rat.

CPT did not affect the pharmacokinetic parameters of CPA (Table 1). In contrast, there appears to be a significant effect of CPA on the clearance of CPT: a lower value of the clearance of CPT was observed after the bolus infusion of CPA in all treatment groups. This decrease in clearance of CPT may be explained by a redistribution of renal blood flow and a decrease of glomerular filtration rate (Spielman & Thompson, 1982). In this respect it is of interest that the renal clearance of the CPT analogue, theophylline, has been demonstrated to be highly dependent on urinary flow rate (Tang-Liu *et al.*, 1982).

Both the mean arterial blood pressure and heart rate were used as measures of drug effect in this study. As was the case in a previous study (Mathôt *et al.*, 1994) the effect on mean arterial pressure was rather complex. After the start of the CPA infusion a maximum reduction in mean arterial pressure was reached within 2 min, indicating rapid equilibration between blood and the site of action. After termination of the CPA infusion however, mean arterial pressure did not return to baseline values but to a new consistently lower value. In addition no significant antagonism of the effect on mean arterial blood pressure by CPT was observed (Figure 2). These findings show that CPA-induced reduction in mean arterial pressure is under a rather complex homeostatic control, which makes modelling of the pharmacokinetic/pharmacodynamic relationship and the interaction with CPT difficult. In contrast, the effect on heart rate was found to be more straightforward: a significant reduction was observed which, after the termination of the CPA infusion, returned to the baseline value. Furthermore, a clear antagonism of this effect by CPT was observed (Figure 3). These findings support previous observations that heart rate provides an especially useful parameter to study adenosine A_1 receptor mediated responses *in vivo* (Webb *et al.*, 1990; Milavec-Krizman *et al.*, 1991; Mathôt *et al.*, 1994). In this respect it is important that the decline in heart rate has been associated with activation of the adenosine A_1 receptor (Oei *et al.*, 1988; Trivedi *et al.*, 1991; Ueeda *et al.*, 1991), whereas both A_1 but mainly A_2 receptors have been reported to mediate the decrease in mean arterial pressure (Daval *et al.*, 1991; Trivedi *et al.*, 1991).

In order to derive the quantitative model of the interaction between CPT and CPA, it is essential to establish whether CPT possesses some intrinsic cardiovascular activity of its own. CPT at the different steady-state levels did not produce any relevant changes in baseline heart rate and mean arterial pressure. In all experiments (both during infusion of CPT and placebo) a slight decrease in the baseline value of heart rate was observed. This effect was attributed to the fact that the experimental animals became familiar with the experimental environment.

CPA caused heart rate to be maximally decreased within 30 s after the initiation of the infusion. The absence of a significant time delay between CPA blood concentration and pharmacological effect indicates that blood concentrations reflect concentrations of CPA at the site of action. Therefore blood concentrations were directly related to the heart rate on basis of equation 3. The competitive nature of the interaction between the two drugs was investigated by studying the effects of different steady-state concentrations of CPT. Figure 4 and Table 2 clearly show the parallel shift to the right of the CPA concentration-effect relationship, induced by CPT. With increasing CPT concentrations the apparent EC_{50} of CPA increases; however, no changes occur in the shape of the curve (n) and the maximal effect E_{max} of CPA. This indicates that *in vivo*, CPT competitively antagonizes the effect of CPA on heart rate, which is in accordance with the competitive nature of the interaction between the two drugs at the A_1 receptor *in vitro* (Bruns *et al.*, 1988). Equations 3 and 4 describe the competitive interaction of an agonist and antagonist acting at the same receptor (Holford & Sheiner, 1981; 1982). The difference between these equations and the commonly used sigmoidal E_{max} pharmacodynamic model is the term $EC_{50,app}$, which expresses the parallel shift of the concentration-effect relationship of the agonist by the antagonist. Figure 5 shows that the model can describe the relationship between the CPT concentrations and the $EC_{50,app}$ successfully, but also that this relationship is non-linear. Therefore a shape factor for CPT (n_2) with a value larger

than 1 is required. The interaction model was used in a slightly modified version by Jonkers *et al.* (1989) to quantify the interaction between β-adrenoceptor agonists and antagonists *in vivo*. In that study the relationship between $EC_{50,app}$ and antagonist concentration was assumed to be linear ($n_2 = 1$), which is to be expected in terms of drug-receptor interaction dynamics *in vitro*. However, in *in vivo* studies values of n larger than one are often found as was recently demonstrated in a study on the pharmacodynamic interaction between the benzodiazepine agonist, midazolam and its competitive antagonist, flumazenil (Mandema *et al.*, 1992). It is emphasized that pharmacodynamic models are often derived on empirical grounds. This does not distract however, from their value in quantifying relationships between drug concentration and effect (Holford & Sheiner, 1981; 1982). In the present pharmacokinetic-pharmacodynamic modelling study, estimates of the potency and intrinsic activity of adenosine receptor agonists and antagonists were obtained *in vivo*. For CPA the mean value of the EC_{50} based on free concentrations was 0.7 ± 0.1 ng ml^{-1}. This is very similar to the value of EC_{50} based on free concentrations of 0.8 ± 0.1 ng ml^{-1} reported previously. These estimates of the EC_{50} are very similar to the values of the binding constant K_i of CPA to the high affinity state of the adenosine receptor *in vitro* (Mathôt *et al.*, 1994). The value EC_{50} of CPT, based on free plasma concentrations as derived from the interaction model was 0.5 ng ml^{-1}. This value is in the same concentration-range as the binding constant K_i to the adenosine receptor *in vitro* of 2.5 ng ml^{-1} (Bruns *et al.*, 1987).

In conclusion, a competitive interaction model appears to characterize adequately the pharmacodynamic interaction between an adenosine A_1 receptor agonist and a competitive antagonist *in vivo*. In principle this model may be generally applicable to characterize the pharmacodynamic interactions between a wide range of adenosine receptor ligands with different intrinsic activity.

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