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Deoxyribose analogues of N^6-cyclopentyladenosine (CPA): partial agonists at the adenosine A_1 receptor *in vivo*

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1 The purpose of the present study was to quantify the cardiovascular effects of the 2'-, 3'- and 5'-deoxyribose analogues of the selective adenosine A_1 receptor agonist, N^6-cyclopentyladenosine (CPA) *in vivo*. The blood concentration-effect relationships of the compounds were assessed in individual rats and correlated to their receptor binding characteristics.

2 The pharmacokinetics and pharmacodynamics of the compounds were determined after a single intravenous infusion of 0.80 mg kg^{-1} (2.5 μmol kg^{-1}) of 5'dCPA, 1.2 mg kg^{-1} (3.8 μmol kg^{-1}) of 3'dCPA or 20 mg kg^{-1} (63 μmol kg^{-1}) of 2' dCPA. The heart rate (HR) and mean arterial blood pressure (MAP) were monitored continuously during the experiment and serial arterial blood samples were taken for analysis of drug concentration.

3 The relationship between blood concentrations and the reductions in both heart rate and blood pressure were described according to the sigmoidal E_{max} model. For the bradycardiac effect, the potencies based on free drug concentrations ($EC_{50,u}$) of 5'dCPA, 3'dCPA and 2'dCPA in blood were 5.9 ± 1.7, 18 ± 4 and 260 ± 70 ng ml^{-1} (19 ± 6, 56 ± 11 and 830 ± 210 nM), respectively, and correlated well with the adenosine A_1 receptor affinity *in vitro*. The E_{max} value of 2'dCPA was significantly less than those of the other compounds, suggesting that this compound may be regarded as a partial agonist when compared to the other analogues. The rank order of the maximal reduction in heart rate of the compounds corresponded well with the order of the GTP-shifts, as determined *in vitro*.

4 It is concluded that deoxyribose derivatives of CPA may be partial agonists for the adenosine A_1 receptor and may serve as tools for further investigation of adenosine receptor partial agonism *in vivo*.

Keywords: Adenosine A_1 receptor; cardiovascular effects; N^6-cyclopentyladenosine (CPA); pharmacokinetic-pharmacodynamic modelling; partial agonism

Introduction

The purine nucleoside, adenosine, is known to exert its pharmacological effects via membrane-bound receptors, which have been divided into three major subclasses, A_1, A_2 and A_3 (Van Galen *et al.*, 1994). Activation of adenosine receptors has been shown to produce a wide variety of effects, such as cardiac depression, hypotension, antilipolytic activity and depression of neuronal excitability in the central nervous system (Jacobson *et al.*, 1991). The ubiquity of the adenosine receptor in the body may limit the development of novel purinergic therapeutics, since drugs targeted to the adenosine receptor are likely to exert a host of unwanted side effects. The major drawbacks to the potential therapeutic use of adenosine receptor agonists are the profound hypotensive and cardiac depressant effects. The development of agonists with reduced intrinsic activity may be beneficial, since this may result in less pronounced haemodynamic actions, a potential increase in selectivity of effects and a possible circumvention of the development of tolerance (IJzerman *et al.*, 1994).

A series of deoxyribose analogues of N^6-substituted adenosines has been synthesized recently and tested in radioligand binding studies on rat brain membranes (Van der Wenden, 1994). It was shown that the removal of the 2'-, 3'- or 5'-hydroxyl group decreased the affinity for the adenosine A_1 receptor. The intrinsic activity of these compounds was evaluated by the assessment of the influence of GTP on the adenosine A_1 receptor affinity. In comparison with the parent compounds, smaller GTP-induced reductions in receptor affinity were observed for the 2'- and 3'-deoxy-derivatives, indicating that these compounds may be partial agonists for the adenosine A_1 receptor. The removal of the 5'-hydroxyl group showed no effect on the GTP-shift.

The purpose of the present study was to characterize the cardiovascular effects of the 2'-, 3'- and 5'-deoxyribose analogues of the selective adenosine A_1 receptor agonist N^6-cyclopentyladenosine (CPA) (Figure 1) *in vivo* by use of an integrated pharmacokinetic-pharmacodynamic model. This approach has been developed recently for the quantification of the haemodynamic effects of CPA in normotensive rats (Mathôt *et al.*, 1994). In that study, estimates of the *in vivo* potency and intrinsic activity of CPA were obtained in individual rats by relating the reduction in heart rate to the CPA blood concentration according to the sigmoidal E_{max} model. This approach has recently been validated by its application in the quantitative characterization of the cardiovascular effects of the diastereomers of N^6-phenylisopropyladenosine (PIA), and the pharmacodynamic interaction of CPA and the adenosine A_1 antagonist, 8-cyclopentyltheophylline (CPT) Mathôt *et al.*, 1995; Appel *et al.*, 1995).

In the present study, the concentration-effect relationships of the deoxyribose analogues were determined in individual rats. The observed *in vivo* potency and intrinsic activity were correlated to the affinity for the adenosine A_1 receptor and the GTP-shift *in vitro*, respectively.

Methods

Animals and surgery

Male, normotensive SPF rats of Wistar descent (200-250 g) were used throughout the study. The animals were obtained from the Sylvius Laboratory Breeding Facility, Leiden, The

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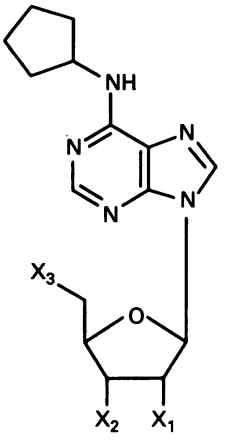

	X_1	X_2	X_3
2'dCPA	H	OH	OH
3'dCPA	OH	H	OH
5'dCPA	OH	OH	H
CPA	OH	OH	OH

Figure 1 Chemical structure of deoxyribose analogues of N^6-cyclopentyladenosine (CPA).

Netherlands. They were housed individually in plastic cages under a normal 12 h light (07 h 00 min–19 h 00 min)-dark cycle and had free access to standard lab food (Standard Laboratory Rat, Mouse and Hamster Diets, RMH-TH, Hope Farms, Woerden, The Netherlands) and tap water.

Two days before experimentation, in-dwelling cannulae were implanted under light ether anaesthesia as described by Mathôt *et al.* (1994). The abdominal aorta was cannulated by an approach through the left and the right femoral arteries for the measurement of the arterial blood pressure and the collection of serial blood samples, respectively. The right jugular vein was cannulated for administration of the compound. The cannulae were manoeuvred subcutaneously to emerge on the nape of the neck.

Experimental protocol

On the day of the experiment, the cannulae were connected between 09 h 00 min and 10 h 00 min. Arterial blood pressure was measured from the left femoral catheter in the abdominal aorta, with a miniature strain gauge P10EZ transducer equipped with a TA1017 CritiFlo diaphragm dome (Viggo-Spectramed BV, Bilthoven, The Netherlands). During the experiment the diaphragm dome was flushed with heparinized saline (20 iu ml^{-1}) at a rate of 500 µl h^{-1} (Harvard infusion pump 22, Plato, Diemen, The Netherlands) to avoid clotting in the cannula. The pressure transducer was placed at the level of the animal's heart and connected to a polygraph amplifier console (RMP6018, Nihon Kohden Corp., Tokyo, Japan). The heart rate was determined from the pressure signal, which was coupled to a tachograph. Signals were recorded on a polygraph and concurrently converted in a CED1401 interface (Cambridge Electronics Design Ltd., Cambridge, England) and transferred into a 80486 computer (Inteb, Sassenheim, The Netherlands). The data were stored on hard disk for off-line analysis. Data acquisition and reduction were performed with Spike2 computer software (Cambridge Electronics Design Ltd.).

The rats were allowed to acclimatize to the experimental conditions for 1 h. All administrations were started between 10 h 00 min and 11 h 00 min in order to exclude diurnal variation in base-line cardiovascular responses. Recording started 30 min prior to the beginning of the administration and was continued for 5 h.

The rats were assigned randomly to four treatment groups that received 0.80 mg kg^{-1} (2.5 µmol kg^{-1}) of 5'dCPA, 1.2 mg kg^{-1} (3.8 µmol kg^{-1}) of 3'dCPA, 20 mg kg^{-1} (63 µmol kg^{-1}) of 2'dCPA, or the vehicle intravenously over 15 min. The compounds were administered in a volume of 765 µl of 20% dimethylsulphoxide (DMSO)-water (v/v) using a motor-driven syringe pump (Braun, Melsungen, Germany). Serial arterial blood samples with varying volumes were drawn over a period of 0–100 min (5'dCPA), 0–105 min (3'dCPA) or 0–240 min (2'dCPA) for the determination of blood concentrations. The samples were haemolysed immediately in glass centrifuge tubes, containing 400 µl of distilled water at 0°C, to prevent possible degradation as has been described previously for CPA (Mathôt *et al.*, 1993). The samples were stored at –35°C until h.p.l.c. analysis. A blood sample with a volume of 350 µl was taken at $t = 14.5$ min and directly transferred to a heparinized tube on ice. This sample was used for the determination of binding to blood cells and plasma proteins (see below). In the placebo treated group, blood samples were drawn according to the sampling schedule of the administration of 3'dCPA. For all the treatment groups, the maximal total volume of blood withdrawn did not exceed 1500 µl.

Binding to blood cells and plasma proteins

The plasma-to-blood ratio (P/B) and the fraction unbound in plasma (f_u) were determined for each rat 14.5 min after administration of the compound was started. The total blood concentration was determined in a 20 µl aliquot obtained from the 350 µl blood sample. The rest of the blood sample was centrifuged for 10 min at 10,000 *g* at 4°C to separate blood cells. The plasma was separated and a sample of 20 µl was retained for analysis. A volume of 120 µl of the supernatant was subjected to ultrafiltration for 10 min at 1090 *g* at 37°C to separate free compound from plasma protein-bound compound using the Amicon Micropartition System with an YMT ultrafiltration membrane (Amicon Division, Danvers, MA, U.S.A.). The unbound concentration was determined in 50 µl of the ultrafiltrate. The samples were stored at –35°C until analysis.

Drug analysis

The deoxyribose derivatives of CPA were assayed in blood, plasma and ultrafiltrate by reversed-phase high-performance liquid chromatography, based on the method described earlier by Mathôt *et al.* (1993). The calibration standards were prepared by adding 100 µl of aqueous solutions of the compounds to 100 µl blood haemolyzed in 400 µl water, yielding blood concentration ranges of 0.010 to 1.0 µg ml^{-1}, 0.015 to 1.0 µg ml^{-1} and 0.050 to 10 µg ml^{-1} for 5'dCPA, 3'dCPA and 2'dCPA, respectively. R-PIA (0.1 µg in 50 µl water) was added to the samples as internal standard. Following the addition of 50 µl 3.0 M NaOH, the samples were extracted for 60 s with 5 ml of ethyl acetate by using a vortex mixer. After centrifugation for 15 min at 2000 *g* the organic layer was transferred to another tube and evaporated under reduced pressure at 40°C. The residue was dissolved in 150 µl of the mobile phase and 100 µl were injected into the chromatographic system.

The liquid chromatographic system consisted of a Spectroflow 400 solvent delivery system (Applied Biosystems, Ramsey, NJ, U.S.A.), a WISP-712B autosampler (Millipore-Waters, Milford, MA, U.S.A.) and a Spectroflow 757 variable-wavelength u.v. detector (λ 269 nm) (Applied Biosystems, Ramsey, NJ, U.S.A.). Chromatography was performed at 27°C using a stainless-steel Microsphere C18 3-µm cartridge column (100 mm × 4.6 mm i.d.) (Chrompack Nederland BV, Bergen Op Zoom, The Netherlands) equipped with a guard column (20 mm × 2 mm i.d.) (Upchurch Scientific, Oak Harbor, WA, U.S.A.) packed with C18 (particle size 20–40 µm) (Chrompack Nederland BV). Data processing was performed with a Chromatopack C-R3A reporting integrator (Shimadzu,

Kyoto, Japan). The mobile phase consisted of a ternary mixture of acetonitrile, methanol and 10 mM acetate buffer (pH 4.0) with a ratio of 4/47/49 (by volume) for the analysis of 5'dCPA and a ratio of 4/44/52 (by volume) for the analysis of 2'dCPA and 3'dCPA. The flow rate was 0.50 ml min^{-1} and the retention times of 5'dCPA, 3'dCPA and 2'dCPA were 8.7, 7.9 and 8.2 min, respectively. The retention times of R-PIA were 11 and 14 min with the mobile phase ratio of 4/47/49 and 4/44/52, respectively. The calibration curves were constructed by weighted linear regression (weight factor: 1 / (peak-height-ratio) and were linear ($r > 0.9991$). The within-day and between-day coefficients of variation of the calibration standards were less than 7.0 and 10% ($n = 6$), respectively, for all compounds. The extraction yields of 5'dCPA, 3'dCPA and 2'dCPA were greater than 88, 85 and 86%, respectively; the corresponding limits of detection were 0.004, 0.004 and 0.006 μg ml^{-1} for samples of 100 μl of blood as determined by a signal-to-noise ratio of 3.

Chemicals

2'-Deoxy-CPA (2'dCPA) and 3'-deoxy-CPA (3'dCPA) were synthesized by Dr E.M. Van der Wenden at the department of Medicinal Chemistry of the Leiden-Amsterdam Center for Drug Research. 5'-Deoxy-CPA (5'dCPA) was a gift from Parke Davis (Ann Arbor, MI, U.S.A.). R-N^6-phenylisopropyladenosine (R-PIA) was obtained from Sigma (St. Louis, MO, U.S.A.). Ethyl acetate was purchased from Baker Chemicals (Deventer, The Netherlands) and distilled prior to use. Acetonitrile (h.p.l.c. grade) was obtained from Westburg (Leusden, The Netherlands). Water was drawn from a Milli-Q system (Millipore SA, Molsheim, France). All the other chemicals used were of analytical grade (Baker, Deventer, The Netherlands).

Data analysis

Compartmental analysis of the blood concentration-time profiles was performed by fitting the following equation to the concentration-time data of individual rats:

$$C(t) = \sum_{i=1}^{n} \frac{C_i}{\lambda_i T} \cdot (1 - e^{-\lambda_i t}) \qquad t \leqslant T \qquad (1A)$$

$$C(t) = \sum_{i=1}^{n} \frac{C_i}{\lambda_i T} \cdot (e^{-\lambda_i (t-T)} - e^{-\lambda_i t}) \qquad t > T \qquad (1B)$$

In this equation $C(t)$ is the blood concentration at time t, C_i is the coefficient associated with the i^{th} exponent λ, and T is the infusion duration. Different exponential models were investigated and the most suitable model was chosen on the basis of the Akaike information criterion (Yamaoka *et al.*, 1978). The area under the blood concentration-time curve (AUC), the systemic clearance (Cl), the mean residence time (MRT), the volume of distribution at steady-state (V_{ss}) and the terminal half-life ($t_{1/2,n}$) were calculated following standard procedures using the coefficients and exponents of the fitted functions (Gibaldi & Perrier, 1982).

The poly-exponential pharmacokinetic fit was used to calculate the blood concentration at the time of the averaged cardiovascular effect measurement. Time-effect points were obtained by averaging 60–300 s of consecutive heart rate or blood pressure data. Data were collected more frequently at the time of rapid change in drug concentration. For each individual rat concentrations were correlated to cardiovascular responses according to the sigmoidal E_{max} pharmacodynamic model with a fixed no-drug value (Holford & Sheiner, 1981):

$$E = E_0 + \frac{E_{max} \times C^{n_H}}{EC_{50}{}^{n_H} + C^{n_H}} \qquad (2)$$

where E is the cardiovascular response, E_0 is the no-drug response, EC_{50} is the blood concentration that corresponds to 50% of the maximum effect (E_{max}) and n_H expresses the sigmoidicity of the curve (Hill factor). EC_{50} values based on free drug concentrations ($EC_{50,u}$) were obtained after correction for

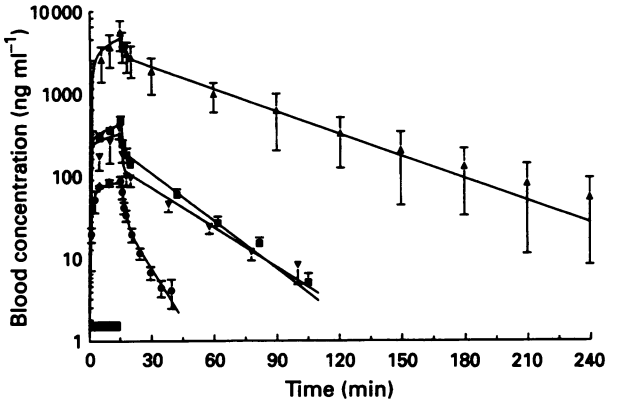

Figure 2 Blood concentration of rats after intravenous infusion of 20 mg kg^{-1} (63 μmol kg^{-1}, ▲) of 2'dCPA, 1.2 mg kg^{-1} (2.8 μmol kg^{-1}, ■) or 3'dCPA, 0.80 mg kg^{-1} (2.5 μmol kg^{-1}, ▼) of 5'dCPA and 0.20 mg kg^{-1} (0.60 μmol kg^{-1}; ●) of CPA over 15 min. The data are presented as mean ± s.d. and the fitted solid lines are the averages of 7 (2'dCPA, 3'dCPA and 5'dCPA) and 6 (CPA) curves. The CPA data have been reported earlier (Mathôt *et al.*, 1994).

Table 1 Pharmacokinetic parameters obtained after intravenous infusion of CPA, 5'dCPA, 3'dCPA and 2'dCPA in 15 min to conscious normotensive rats

Compound	*Dose* (mg kg^{-1})	n	C_1 (μg ml^{-1})	λ_1 (min^{-1})	C_2 (μg ml^{-1})	λ_2 (min^{-1})	*AUC* (μg min ml^{-1})	*Cl* (ml min^{-1} ml^{-1})	*MRT* (min)	V_{ss} (ml kg^{-1})	$t_{1/2,n}$ (min)
CPA#	0.20	6	1.3 ± 0.2	0.69 ± 0.10	0.12 ± 0.03	0.11 ± 0.02	2.9 ± 0.2	76 ± 3	4.1 ± 0.4	320 ± 40	6.9 ± 1.1
5'dCPA	0.80	7	24 ± 6	2.6 ± 0.4	0.39 ± 0.03	0.043 ± 0.003	18 ± 2	55 ± 6	13 ± 2	740 ± 160	16 ± 1
3'dCPA	1.2	7	30 ± 3	2.8 ± 0.2	0.76 ± 0.10	0.052 ± 0.003	25 ± 1	58 ± 2	11 ± 1	660 ± 40	14 ± 1
2'dCPA	20	7	92 ± 19	0.66 ± 0.21	14 ± 1	0.024 ± 0.002	750 ± 70	33 ± 3	33 ± 2	1050 ± 70	29 ± 2

The individual concentration-time courses were described with a bi-exponential pharmacokinetic function with coefficients C_1 and C_2 and exponents λ_1 and λ_2 (equation 1). These coefficients and exponents were used to calculate the area under the blood concentration-time curve (*AUC*), the systemic clearance (*Cl*), the mean residence time (*MRT*), the volume of distribution at steady-state (V_{ss}) and the terminal half-life ($t_{1/2,n}$) (Gibaldi & Perrier, 1982). Values are reported as mean ± s.e. Data were analyzed statistically by a one-way ANOVA or a Kruskal-Wallis test, if more appropriate.

[1] *Cl*, *MRT*, V_{ss}, and $t_{1/2,n}$ of 3'dCPA and 5'dCPA were significantly different from those of both 2'dCPA and CPA.

Data from Mathôt *et al.* (1994).

binding of the compound to blood cells and plasma proteins. In the modelling a fixed value of E_0 was used, since this value is precisely defined by the data. It has been demonstrated that incorporation of a fixed value of E_0 greatly improves the precision of the pharmacodynamic parameter estimates (Mathôt *et al.*, 1994).

In order to compare the cardiovascular responses and the pharmacokinetic and pharmacodynamic parameter estimates obtained after the administration of different doses, a one-way analysis of variance (ANOVA) or a non-parametric Kruskal-Wallis test, if more appropriate, was performed. Unless indicated otherwise, all data are reported as the mean ± s.e.

Results

Pharmacokinetics

The averaged concentration-time profiles after the administration of 0.80 mg kg^{-1} (2.5 μmol kg^{-1}) of 5'dCPA, 1.2 mg kg^{-1} (3.8 μmol kg^{-1}) of 3'dCPA and 20 mg kg^{-1} (63 μmol kg^{-1}) of 2'dCPA are shown in Figure 2. For reasons of comparison, Figure 2 also displays the concentration profile as obtained after intravenous administration of 0.20 mg kg^{-1} (0.60 μmol kg^{-1}) of CPA over 15 min using data reported earlier (Mathôt *et al.*, 1994) and which were obtained under identical experimental conditions. For all the compounds a bi-exponential function adequately described the time course of blood concentration. The pharmacokinetic parameters are summarized in Table 1. The parameters Cl, MRT, V_{ss} and $t_{1/2n}$ of 3'dCPA and 5'dCPA were significantly different from both 2'dCPA and CPA.

The plasma-to-blood ratio (P/B) was assessed *in vivo* as 0.55 ± 0.03, 0.54 ± 0.02 and 0.64 ± 0.01 ($n = 7$) for 5'dCPA, 3'dCPA and 2'dCPA, respectively, with corresponding values for the fraction unbound in plasma (f_u) of 61 ± 2%, 68 ± 2% and 63 ± 2% ($n = 7$). P/B and f_u of CPA have been reported as 0.41 ± 0.01 and 72 ± 1% ($n = 14$) (Mathôt *et al.*, 1994).

Pharmacokinetic-pharmacodynamic relationship

Figures 3 and 4 depict the time course of heart rate and mean arterial pressure, respectively, after administration of the compounds. Both figures also show the effect-time profiles as have been observed following intravenous administration of 0.20 mg kg^{-1} of CPA over 15 min (Mathôt *et al.*, 1994). After the infusion was initiated, the bradycardic and hypotensive effect rapidly reached a maximum and maintained that level throughout the infusion. The maximal reduction in heart rate and blood pressure after administration of 2'dCPA was less than after administration of the other compounds. After the infusion was finished, the haemodynamic parameters gradually returned to normal values. Following the infusion of 5'dCPA, 3'dCPA and 2'dCPA, the heart rate was not significantly different from the placebo-treated group from 160, 100 and 190 min onwards, respectively. The bradycardia was re-

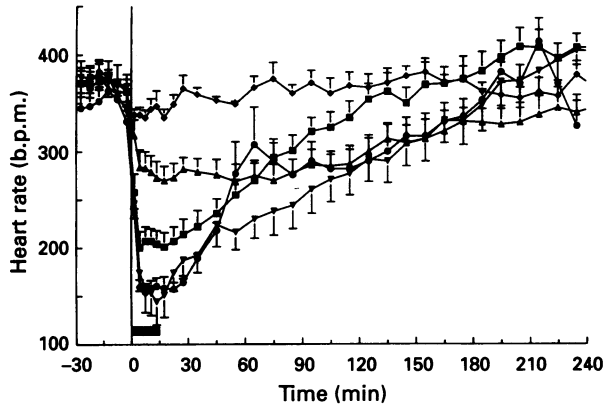

Figure 3 Average time profiles of heart rate for rats given an intravenous infusion of 20 mg kg^{-1} of 2'dCPA (▲, $n = 7$), 1.2 mg kg^{-1} of 3'dCPA (■, $n = 7$), 0.80 mg kg^{-1} of 5'dCPA (▼, $n = 7$) and 0.20 mg kg^{-1} of CPA (●, $n = 7$) over 15 min. Control rats had received the vehicle (◆, $n = 7$). Data are presented as mean ± s.e. For each time point data were analysed by one-way ANOVA to test for differences between the treatment groups and the control group.

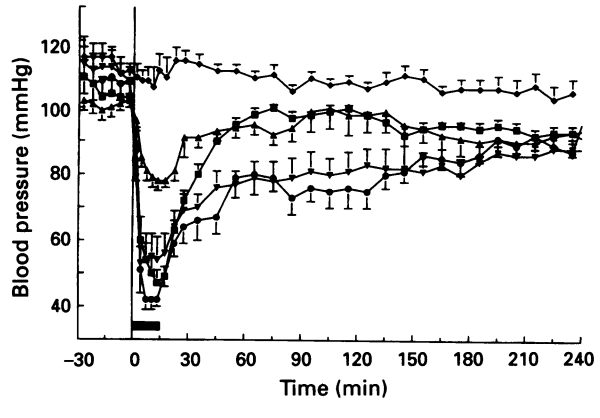

Figure 4 Average time profiles of mean arterial blood pressure for rats given an intravenous infusion of 20 mg kg^{-1} of 2'dCPA (▲, $n = 7$), 1.2 mg kg^{-1} of 3'dCPA (■, $n = 7$), 0.80 mg kg^{-1} of 5'dCPA (▼, $n = 7$) and 0.20 mg kg^{-1} of CPA (●, $n = 7$) over 15 min. Control rats had received the vehicle (◆, $n = 7$). Data are presented as mean ± s.e. For each time point data were analysed by one-way ANOVA to test for differences between the treatment groups and the control group.

Table 2 Pharmacodynamic parameter estimates for the reduction in heart rate after intravenous infusion of CPA, 5'dCPA, 3'dCPA and 2'dCPA to conscious normotensive rats

Compound	n	E_0 (b.p.m.)	E_{max} (b.p.m.)	EC_{50} (ng ml^{-1})	$EC_{50,u}$ (ng ml^{-1})	n_H
CPA#	6	355 ± 13	−208 ± 19	1.8 ± 0.5	0.54 ± 0.15	1.4 ± 0.4
5'dCPA	7	368 ± 14	−215 ± 31	19 ± 6	5.9 ± 1.7	1.4 ± 0.3
3'dCPA	7	349 ± 17	−167 ± 16	48 ± 8	18 ± 4	1.4 ± 0.3
2'dCPA	7	338 ± 18	−65 ± 12	650 ± 160	260 ± 70	2.3 ± 0.6

For each rat the blood concentration-heart rate relationship was fitted to the sigmoidal E_{max} model (equation 1), yielding estimates for the baseline response (E_0), intrinsic activity (E_{max}), potency (EC_{50}), Hill factor (n_H) and potency, based on free concentrations ($EC_{50,u}$). Values are reported as mean ± s.e. Data were analyzed statistically by a one-way ANOVA or a Kruskal-Wallis test, if more appropriate.
[1] The E_{max} of 2'dCPA was significantly lower than the corresponding values of the other compounds.
[2] The EC_{50} and $EC_{50,u}$ were significantly different between the four compounds.
Data from Mathôt *et al.* (1994).

versible, whereas blood pressure values gradually increased to plateau levels, which were significantly lower than those of placebo-treated rats. The volume of the infusion and the volume of blood withdrawn had no effect on the base-line levels as shown in Figures 3 and 4.

The individual pharmacokinetic functions were used to calculate the blood concentration at the time points of cardiovascular measurement. Hysteresis was not observed between blood concentrations and cardiovascular effects for any of the compounds, and the values were correlated directly.

For each individual rat the relationship between blood concentration and heart rate was quantified according to the sigmoidal E_{max} model. The no-drug response values (E_0) were fixed to values which were obtained by averaging 30 min of consecutive heart rate data, starting at the time point at which the heart rate of the drug-treated rats was not significantly different from those of placebo-treated rats. Figure 5 shows the concentration-heart rate relationships of four individual rats which had received different administrations. The pharmacodynamic parameters, as obtained by the kinetic-dynamic

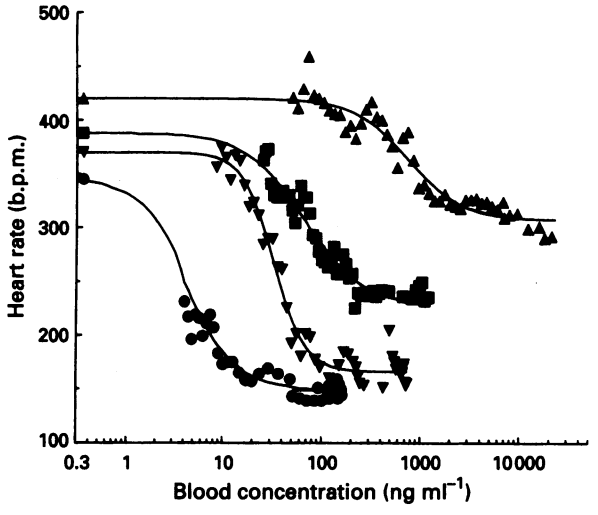

Figure 5 Blood concentration-heart rate relationship of four individual rats which had received an intravenous infusion of 20 mg kg^{-1} of 2'dCPA (▲), 1.2 mg kg^{-1} of 3'dCPA (■), 0.80 mg kg^{-1} of 5'dCPA (▼) and 0.20 mg kg^{-1} of CPA (●) over 15 min. The solid line represents the sigmoidal E_{max} model fitted to the concentration-effect data (equation 2). The no-drug response (E_0) values were fixed.

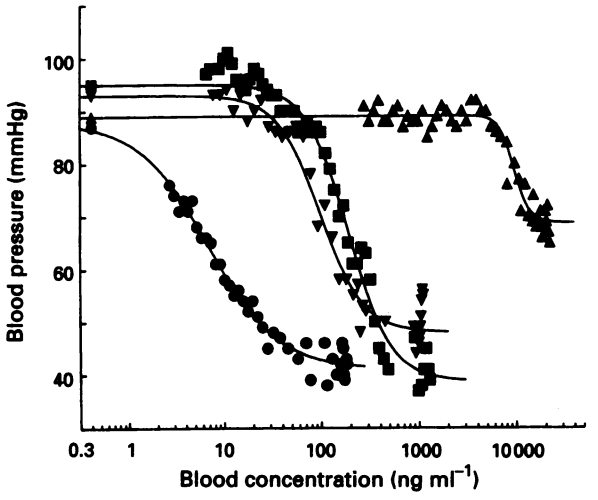

Figure 6 Blood concentration-mean arterial pressure relationship of four individual rats which had received an intravenous infusion of 20 mg kg^{-1} of 2'dCPA (▲), 1.2 mg kg^{-1} of 3'dCPA (■), 0.80 mg kg^{-1} of 5'dCPA (▼) and 0.20 mg kg^{-1} of CPA (●) over 15 min. The solid line represents the sigmoidal E_{max} model fitted to the concentration-effect data (equation 2). The no-drug response (E_0) values were fixed.

Table 3 Pharmacodynamic parameter estimates for the reduction in mean arterial pressure after intravenous infusion of CPA, 5'dCPA, 3'dCPA and 2'dCPA to conscious normotensive rats

Compound	n	E_0 (mmHg)	E_{max} (mmHg)	EC_{50} (ng ml^{-1})	EC_{50} (ng ml^{-1})	n_H
CPA#	6	88±3	−47±3	6.8±2.0	2.0±0.6	0.84±0.13
5'dCPA	7	89±4	−40±8	110±20	38±5	3.2±2.0
3'dCPA	7	94±2	−44±4	290±40	100±10	3.9±1.1
2'dCPA	7	97±3	−14±2	11000±1100	4500±500	9.6±2.4

For each rat the blood concentration-heart rate relationship was fitted to the sigmoidal E_{max} model (equation 2), yielding estimates for the baseline response (E_0), intrinsic activity (E_{max}), potency (EC_{50}), Hill factor (n_H) and potency, based on free concentrations ($EC_{50,u}$). Values are reported as mean±s.e. Data were analyzed statistically by a one-way ANOVA or a Kruskal-Wallis test, if more appropriate.
[1]The E_{max} of 2'dCPA was significantly lower than the corresponding values of the other compounds.
[2]The EC_{50} and $EC_{50,u}$ were significantly different between the four compounds.
[3]The n_H was significantly different between the compounds, except for CPA and 5'dCPA.
#Data from Mathôt *et al.* (1994).

Table 4 Correlation of the adenosine A_1 receptor affinity (apparent K_i) and the *in vivo* potency ($EC_{50,u}$) for the reduction in heart rate and mean arterial pressure

		Heart rate		*Mean arterial pressure*	
Compound	K_i# (nM)	$EC_{50,u}$ (nM)	$EC_{50,u}/K_i$	$EC_{50,u}$ (nM)	$EC_{50,u}/K_i$
CPA	5.9±0.1	1.6±0.4	0.27	6.0±1.8	1.0
5'dCPA	70±10	19±6	0.26	120±20	1.1
3'dCPA	110±20	56±11	0.51	320±40	2.9
2'dCPA	1900±500	830±210	0.44	14000±1000	7.4

Values are reported as mean±s.e.
#The apparent K_i values were determined in the absence of GTP and are the means of three independent experiments performed in duplicate (Van der Wenden, 1994).

Table 5 Comparison of the *in vitro* GTP-shift and the intrinsic activity (E_{max}) for the reduction in heart rate and mean arterial pressure

			Heart rate		*mean arterial pressure*	
Compound	*GTP-shift*#	*GTP-shift*†	E_{max} (b.p.m.)	E_{max}†	E_{max} (mmHg)	E_{max}†
CPA	6.4±0.9	1	−2.15±31	1	−47±3	1
5'dCPA	6.0±1.3	0.93	−208±19	0.97	−40±8	0.85
3'dCPA	4.3±0.9	0.67	−167±16	0.78	−44±4	0.94
2'dCPA	4.1±0.8	0.64	−65±12	0.30	−14±2	0.30

Values are reported as mean±s.e.
GTP-shift from Van der Wenden (1994).
† Data were normalized with respect to CPA.

modelling procedure, are given in Table 2. The no-drug response (E_0) and the Hill factor (n_H) were not significantly different for the different treatments. The E_{max} of 2'dCPA was significantly lower than the values of the other compounds. The $EC_{50,u}$ values of the compounds were significantly different, ranging from 0.54 ng ml^{-1} (CPA) to 260 ng ml^{-1} (2'dCPA).

No-drug response values of mean arterial pressure were obtained by calculating the average level over the last 30 min of the experiment. At that time MAP had not returned to the pre-administration value. However, it appears that when using the value of MAP in the final 30 min of the experiment, valid pharmacodynamic parameter estimates are obtained which are independent of the rate of administration (Mathôt *et al.*, 1994). The concentration-hypotensive effect relationship was adequately described according to the sigmoidal E_{max} model. Figure 6 shows the concentration-mean arterial blood pressure relationships of four individual rats which had received different administrations. The average pharmacodynamic parameters are given in Table 3. No significant differences were detected among the no-drug responses of the compounds. The E_{max} of 2'dCPA was significantly lower than the values of the other compounds, whereas the Hill factor was significantly higher. The $EC_{50,u}$ values of the compounds were significantly different, ranging from 2.0 ng ml^{-1} (CPA) to 4500 ng ml^{-1} (2'dCPA).

Correlation of in vivo *and* in vitro *data*

The affinities and the GTP-shifts of the compounds have been determined in radioligand binding studies on rat brain homogenates and have been reported earlier by Van der Wenden (1994). GTP-shifts had been obtained by dividing the apparent K_i value of the ligand in the presence of GTP by the apparent K_i value in the absence of GTP. The *in vivo* potency ($EC_{50,u}$) of the compounds correlated well with the apparent adenosine A_1 receptor affinity as determined in the absence of GTP (Table 4). The $EC_{50,u}/K_i$ ratio ranged rom 0.26 to 0.51 and from 1.0 to 7.4 for the reduction in heart rate and blood pressure, respectively. The rank order of the compounds for the maximal reduction in heart rate (E_{max}) was CPA ≥ 5'dCPA > 3'dCPA > 2'dCPA, and corresponded well with the order for the GTP-shifts (CPA ≥ 5'dCPA > 3'dCPA ≥ 2'dCPA) as shown in Table 5. The E_{max} for the hypotensive effect of 3'dCPA was slightly higher than the corresponding value for 5'dCPA, resulting in the rank order CPA ≥ 3'dCPA ≥ 5'dCPA > 2'dCPA.

Discussion

In dose-response studies of adenosine receptor agonists, correlations have been reported between the receptor affinity and the cardiovascular activity *in vitro* and *in vivo* (Hamilton *et al.*, 1987; Coffin & Spealman, 1987; Oei *et al.*, 1988; Abiru *et al.*, 1991). However, discrepancies between the rank orders of receptor affinity and *in vivo* potency have been reported as well, and were suggested to be generated by differences in pharmacokinetic properties of the ligands (Webb *et al.*, 1991). When evaluating potency and activity *in vivo*, the relationship between the dose and the pharmacological effect is determined by both pharmacokinetic processes (absorption, distribution, metabolism and excretion) and pharmacodynamic processes (drug receptor interaction, second messengers, etc). In the present study, significant differences were found between the pharmacokinetics of a series of deoxyribose analogues of CPA (Table 1). In comparison with CPA, the deoxyribose analogues exhibited a two to three fold larger volume of distribution at steady state, which can be explained by the more lipophilic character of these compounds. The increased lipophilicity of the deoxyribose derivatives of CPA was also indicated by the following order of the retention times on the reversed-phase h.p.l.c. systems: CPA < 3'dCPA < 2'dCPA < 5'dCPA (data not shown). The increased volume of distribution and the reduced blood clearance resulted in an increase of the terminal half-lifes of the deoxy analogues as compared with CPA. In dose-response studies these pharmacokinetic differences would bias the comparison of the observed potencies. Due to different volumes of distribution, the observed potency of the deoxyribose analogues of CPA would decrease in comparison with the parent compound, thereby reducing the correlation with receptor affinity. The correction of the EC_{50} values for the differences in binding to blood cells and plasma proteins had merely a marginal effect on the *in vivo-in vitro* correlation because of the small difference in $[(P/B) \times f_u]$ values, which ranged from 30% (CPA) to 40% (2'dCPA).

Binding of agonists to the A_1 receptor has been reported to elicit bradycardia and depressions in myocardial contractility and impulse conduction velocity (Evans *et al.*, 1982; Belardinelli *et al.*, 1982; Martens *et al.*, 1987). The induced hypotension after administration of selective adenosine A_1 receptor agonists has been suggested to be the result of a reduction in cardiac output (Webb *et al.*, 1990). The time course of bradycardia and hypotension as observed after the administration of the deoxy analogues of CPA corresponded qualitatively with the cardiovascular responses of the parent compound (Figures 3 and 4). After the infusion was started, a maximum reduction in both heart rate and mean arterial pressure was reached within 2 min for all compounds. Both parameters increased gradually after the termination of the infusion. The reduction in heart rate was reversible, whereas mean arterial pressure levels returned to levels lower than those of placebo-treated rats (Figure 4). Heart rate levels of drug-treated rats were not significantly different from vehicle-treated rats after 190 min (Figure 3). The no-drug responses (E_0) were fixed to post-dose values, the advantage of which is that possible baseline changes, caused by diurnal variation, can be taken into account. Blood pressure values were still significantly reduced after 190 min, indicating that the hypotensive effect is under a rather complex homeostatic control and is not merely related to the direct activation of the adenosine A_1 receptor. These findings are further supported by observations from Appel *et al.* (1995), who have found that the selective adenosine A_1 receptor antagonist, 8-cyclopentyltheophylline (CPT)

did not competitively block the hypotensive effects elicited by CPA *in vivo*. Therefore, it was decided to eliminate the confounding influence of the prolonged hypotension on the concentration-blood pressure relationship in the present experiments by fixing the no-drug response to post-dose values.

Adenosine receptor agonists may also cause hypotension by activation of peripheral adenosine A_2 and A_3 receptors (Kusachi *et al.*, 1983; Webb *et al.*, 1990; Fozard & Carruthers, 1993). The activation of A_2 receptors has been reported to be accompanied by tachycardia (Webb *et al.*, 1991). Although deoxyadenosines have been reported to bind at the A_3 receptor, it is not clear yet whether they actually activate or block the receptor (Van Galen *et al.*, 1994). Due to the high A_1 receptor selectivity of the deoxy analogues of CPA ($K_i(A_2)/K_i(A_1) > 100$; Van der Wenden, 1994), possible activation of the A_2 receptor would be expected to be found only at high blood concentrations at the end of the infusion. In the present experiments, however, a maximum decrease in heart rate and blood pressure was reached almost immediately after the start of the infusion. No changes were observed during the rest of the infusion, suggesting that the adenosine A_2 receptor was not activated.

The determination of the relationship between concentration and cardiovascular effect can be complicated by several factors, which may include an equilibrium delay between concentrations of the drug in blood and those at the receptor site, the formation of active metabolites, the occurrence of acute tolerance/sensitization, and the involvement of homeostatic control mechanisms (Dingemanse *et al.*, 1988; Struyker Boudier, 1992). The role of functional adaptation and the formation of active metabolites has been studied extensively for CPA (Mathôt *et al.*, 1994). By varying the rate of administration of CPA, it could be demonstrated that functional adaptation does not occur within the time frame of the experiment; the concentration-effect curves were consistent and did not show hysteresis or proteresis. Furthermore, by using radio receptor assays it was shown that active metabolites did not interfere with the pharmacological response. In the present study, no hysteresis or proteresis was observed for any of the effects of the deoxy analogues. In addition, the maximal bradycardia and hypotension were reached within 2 min after the start of the administration, indicating rapid equilibrium between blood and the site of action. These findings indicate that the time course of concentration in blood reflects the change of concentration at the site of action. It is therefore justified to compare the *in vivo* concentration-effect relationships of the compounds with observations from receptor binding studies.

Free plasma concentrations of the agonists determine receptor activation *in vivo*. Thus, EC_{50} values were corrected for binding to blood cells and plasma proteins in order to compare the relative potencies for the different compounds. Comparison of the $EC_{50,u}$ values indicates significant differences between the *in vivo* potency of the four compounds. The unbound concentrations of the compounds required to produce 50% of the maximal reduction in heart rate are in the same order of magnitude as the K_i values as determined in the radioligand binding studies (Table 4). The fairly constant $EC_{50,u}/K_i$ ratio demonstrates that the reduction in heart rate is a relevant measure of the effects of adenosine A_1 receptor agonists *in vivo*, originating directly from interaction with the receptor. It has been known that agonist potency depends on both affinity and efficacy, i.e. with a constant receptor affinity, a reduction in efficacy will induce a decrease in potency (Kenakin, 1993). In the present study, the slightly increased $EC_{50,u}K_i$ ratios of 2'dCPA and 3'dCPA may therefore be explained by the reduced efficacy of the compounds in comparison with 5'dCPA and CPA.

It has been shown that selective adenosine A_1 receptor agonists induce bradycardia at doses which have minimal effects on blood pressure (Webb *et al.*, 1990). The greater sensitivity of heart rate of adenosine A_1 receptor activation is demonstrated in the present study by the $EC_{50,u}$ values for the reduction in heart rate of the compounds, which are four to fourteen fold lower than the corresponding values for the hypotensive effect. The correlation between the $EC_{50,u}$ for the reduction in blood pressure and the binding affinity for the adenosine A_1 receptor is in accordance with the hypothesis of Webb *et al.* (1990), who proposed that the hypotensive response is at least partially produced by an A_1 receptor-mediated reduction in cardiac output. For the hypotensive effect, significant differences were detected between the Hill factors of the compounds, whereas no differences were observed for the reduction in heart rate. The mechanism behind these differences remains unclear and has yet to be addressed specifically.

Agonists, but not antagonists, differentiate between two states of the receptor: the high affinity (K_H) and the low affinity K_L) state. In the absence of GTP the receptor exists in a mixture of low and high affinity states. The addition of GTP in radioligand binding assays produces a transition to the low affinity state, which is presumably caused by uncoupling the G-protein from the receptor (Stiles, 1988). GTP-induced reductions in receptor affinity can be found for agonists, while no influence is observed on the affinity of antagonists. The GTP-shift of β-adrenoceptor agonists has been reported to correlate strongly with the intrinsic activity in activating adenylate cyclase (Kent *et al.*, 1980). A high correlation was also shown to exist between the K_L/K_H ratio of agonists for the dopamine D_2 receptor and intrinsic activity *in vitro* and *in vivo* (Lahti *et al.*, 1992). On the basis of the GTP-shifts (as shown in Table 5), 2'dCPA and 3'dCPA can be considered as partial agonists for the adenosine A_1 receptor, whereas 5'dCPA and CPA are full agonists. The rank order of the GTP-shifts was similar to the order of the E_{max} values for the bradycardic effect. However, 3'dCPA exhibited a greater maximal reduction in heart rate and blood pressure than 2'dCPA, despite their similarity in GTP-shifts. For the adenosine A_1 receptor, the GTP-shift therefore appeared only indicative for the *in vivo* intrinsic activity.

In conclusion, this study has shown that estimates of the *in vivo* potency and intrinsic activity of adenosine A_1 receptor agonists can be obtained in individual rats by relating blood concentration to heart rate. The *in vivo* potency for bradycardia corresponded well with the receptor affinity, whereas the GTP-shift appeared only indicative for *in vivo* partial agonism. Due to its reduced maximal response *in vivo*, 2'dCPA may be regarded as a partial agonist when compared to the other compounds. Therefore, deoxyribose analogues of CPA may be interesting leads in the search for selective adenosine A_1 receptor agonists which exert their effect either by depression of cardiac performance, by modulation of CNS function, or by regulation of lipid metabolism.

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