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Selectivity of action of 8-alkylamino analogues of N^6-cyclopentyladenosine *in vivo*: haemodynamic *versus* anti-lipolytic responses in rats

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1 A_1 adenosine receptor agonists with reduced intrinsic activity may be therapeutically useful as result of an increased selectivity of action. In this study the tissue selectivity of three 8-alkylamino substituted analogues of N^6-cyclopentyladenosine (CPA) was investigated for haemodynamic and anti-lipolytic effects using an integrated pharmacokinetic-pharmacodynamic approach.

2 Chronically instrumented male Wistar rats received intravenous infusions of 4.0 mg kg^{-1} 8-methylaminoCPA (8MCPA), 12.0 mg kg^{-1} 8-ethylaminoCPA (8ECPA), 20.0 mg kg^{-1} 8-butylaminoCPA (8BCPA) or vehicle during 15 min. During experimentation, serial arterial blood samples were drawn for the determination of agonist concentrations and plasma non-esterified fatty acid (NEFA) levels. Blood pressure and heart rate were monitored continuously. In addition to the CPA analogues, each rat received a rapid bolus infusion of CPA to determine the maximal effects of the full agonist.

3 The concentration-time profiles of the CPA analogues could be described by a bi-exponential function. Values for clearance, volume of distribution at steady state and elimination half-life were 44 ± 5, 48 ± 6 and 39 ± 2 ml min^{-1} kg^{-1}, 0.97 ± 0.09, 0.84 ± 0.10 and 1.05 ± 0.07 l kg^{-1} and 25 ± 2, 28 ± 2 and 40 ± 2 min for 8MCPA, 8ECPA and 8BCPA, respectively (mean ± s.e.mean, $n = 6-8$).

4 Different models were used to derive the concentration-effect relationships for heart rate and NEFA, yielding estimates of potency (EC_{50}) and instrinsic activity (E_{max}) for both effects of the compounds *in vivo*. On heart rate the compounds acted as partial agonists, with E_{max} values of -173 ± 14, -131 ± 11 and -71 ± 6 beats min^{-1} for 8MCPA, 8ECPA and 8BCPA, respectively. These E_{max} values were significantly lower than the maximal effect of CPA (-208 ± 8 beats min^{-1}). With regard to the anti-lipolytic effect all three compounds were full agonists and lowered NEFA levels to the same extent as CPA (69%). The estimated E_{max} values were 63 ± 5, 63 ± 4 and 68 ± 2%, respectively.

5 Furthermore, the compounds were more potent in causing anti-lipolytic than cardiovascular effects. The EC_{50} values for the NEFA and heart rate lowering effects were 37 ± 15, 68 ± 22 and 659 ± 108 ng ml^{-1} and 164 ± 22, 341 ± 76 and 975 ± 190 ng ml^{-1} for 8MCPA, 8ECPA and 8BCPA, respectively (mean ± s.e.mean, $n = 6-8$).

6 This study demonstrates that partial agonists for the A_1 adenosine receptor have increased selectivity of action *in vivo*. The 8-alkylamino analogues of CPA may be useful anti-lipolytics with less pronounced haemodynamic side effects.

Keywords: A_1 adenosine receptor; partial agonists; CPA; pharmacokinetics; pharmacokinetic-pharmacodynamic modelling; tissue-selectivity; heart rate; lipolysis

Introduction

Adenosine receptor ligands may serve as new therapeutic agents for a variety of diseases (Jacobson *et al*., 1992; Williams, 1993). However, to date, no adenosine analogues have entered the clinic due to the difficulty of obtaining selectivity of action and the host of unwanted side effects associated with adenosine agonists. Selective A_1 adenosine agonists have been suggested to be useful as anti-lipolytic drugs in the treatment of non-insulin-dependent diabetes mellitus (NIDDM) (Hoffman *et al*., 1986; Strong *et al*., 1993; Foley, 1994). However, due to an ubiquitous peripheral distribution of the A_1 receptor, separation of the desirable effects from unwanted effects is difficult (Jacobson *et al*., 1992). The severe bradycardic and hypotensive action of A_1 adenosine receptor agonists (Olsson & Pearsson, 1990) have limited the use of these agonists for other therapeutic areas. For useful anti-lipolytic drugs, therefore, compounds need to be developed that selectively inhibit lipolysis with minimal or no effect on the cardiovascular system.

Low efficacy agonists for the A_1 adenosine receptor have gained interest due to their promiscuous behaviour to act either as full agonists, partial agonists or antagonists, depending on the tissue or physiological system. The intrinsic activity of low efficacy drugs *in vivo* depends both on properties of the compound (intrinsic efficacy) and properties of the system, e.g. receptor reserve and receptor effector coupling (Kenakin, 1993). Tissue differences in effects of A_1 receptors agonists may be expected, since the receptor is known to couple to a large variety of second messengers, such as adenylyl cyclase (Van Calker *et al*., 1978; Londos *et al*., 1981), K^+ channels (Kirsch *et al*., 1990), Ca^{2+} channels (Dolphin *et al*., 1986), and phospholipase C (Delahunty *et al*., 1988). Moreover, even within a single tissue, different mechanisms may be involved in the bradycardic response (activation of $I_{K(Ado)}$ and anti-β-adrenoceptor response). Between these two

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mechanisms a difference in receptor reserve was shown to exist (Srinivas *et al*., 1997). Interestingly, differences in receptor density and receptor reserve have been observed between adipose tissue and other tissues (Lohse *et al*., 1986; Dennis *et al*., 1992).

Recently, a series of N^6-cyclopentyladenosine (CPA) analogues has been synthesized with alkylamino substituents of various lengths at the 8-position (Roelen *et al*., 1996). In an *in vivo* cardiovascular study these compounds elicited a less pronounced decrease in heart rate and blood pressure than the full agonist CPA (Van Schaick *et al*., 1997b). Indeed, on the basis of quantification of the concentration-heart rate relationships, some of the compounds were found to be partial agonists at the cardiac A_1 receptor *in vivo*. The measure for intrinsic activity *in vivo* correlated highly to the measure for intrinsic activity *in vitro* (GTP shift in receptor binding experiments), which indicated that the effect on heart rate was a reflection of receptor activation. Furthermore, full agonists for the A_1 adenosine receptor have been found to be more potent agonists in adipose tissue than in cardiac tissue, indicating a difference in receptor-effector coupling efficiency (i.e. receptor reserve) between the two tissues (Gurden *et al*., 1993; Van Schaick *et al*., 1997a). These observations suggest that partial agonists may be more efficacious in reducing lipolysis and cause less cardiovascular side effects.

The present study was designed to investigate the selectivity of action of three of these CPA analogues (8-methylamino, 8-ethylamino and 8-butylamino-CPA) in rats (for structures see Figure 1). For both the anti-lipolytic and bradycardic effect, the relationships between agonist concentration and effect were quantified on the basis of integrated pharmacokinetic-pharmacodynamic models. These models provide estimates of potency and intrinsic activity of drugs *in vivo* (Mathôt *et al*., 1995b; Van Schaick *et al*., 1997b). Values of potency (EC_{50}) and intrinsic activity (E_{max}) of the agonists for the two pharmacological actions were compared in the same rats. A preliminary account of part of this work has been published in abstract form (Van Schaick *et al*., 1996).

Methods

Animals and surgical procedures

Animals Male, normotensive Wistar rats (Crl:(WI)WU BR, Broekman Instituut BV, Soomeren, The Netherlands), weighing 300–350 g, were used. The animals were housed individually in plastic cages with a normal 12 h light-dark cycle, had free access to laboratory chow (Standard Laboratory Rat, Mouse and Hamster Diets, SMR-A, Hope Farms, Woerden, The Netherlands) and tap water *ad libitum*.

Surgical procedure Two days before experimentation, indwelling cannulas were implanted as described previously (Mathôt *et al*., 1994). Briefly, cannulas were implanted into the right jugular vein (for drug administration) and both right and left femoral artery (for blood sample collection and recording of hemodynamic variables, respectively). The cannulas were tunnelled subcutaneously to the back of the neck and exteriorized. After the operation the cannulas were filled with a 25% (w/v) solution of polyvinylpyrrolidone (Brocacef, Maarssen, The Netherlands) in 0.9% (w/v) sodium chloride containing 50 i.u. ml^{-1} heparin (Hospital Pharmacy, Leiden, The Netherlands). This solution was removed from the cannula on the day of the experiment.

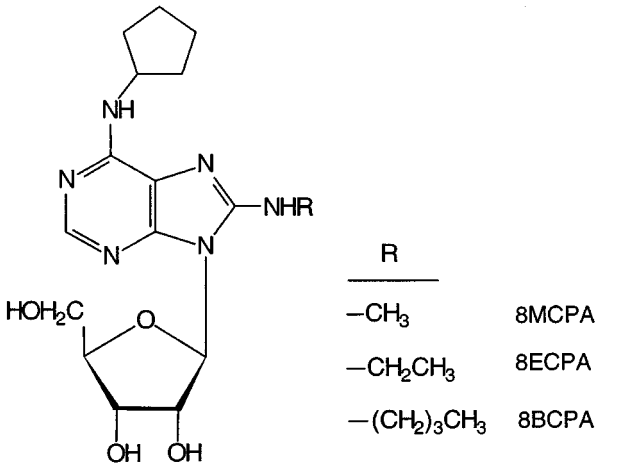

Figure 1 Chemical structures of the 8-methylamino-(8MCPA), 8-ethylamino- (8ECPA), and 8-butylamino-(8BCPA) derivatives of N^6-cyclopentyladenosine (CPA).

Experimental design

Dosage regimen Animals were fasted for 24 h before experimentation, with free access to water being allowed throughout this period. Fasted rats were allocated randomly to four groups of 6–8 rats and were given intravenous infusions of 1.0 mg 8-methylamino-CPA, 3.0 mg 8-ethylamino-CPA, 5.0 mg 8-butylamino-CPA or vehicle (20% DMSO in 0.9% saline) in 15 min. The doses of the compounds were not adjusted for the individual weights of the rats. The compounds were dissolved in 20% DMSO-saline (v/v) and administered in a volume of 765 μl using a syringe infusion pump (Braun, Melsungen, Germany). At the end of the experiments (4 h after drug infusion), each rat was given a bolus infusion of 20 μg CPA in 5 min (dissolved in saline).

All experiments were started between 9 h 00 min and 10 h 00 min to minimize disturbances of diurnal rhythms in the measurements. The rats were allowed to habituate to the experimental conditions for 1 h before drug administration. Cardiovascular recordings were started at least half an hour before drug administration and lasted approximately five hours. During the experiment the animals were conscious, freely moving and were allowed free access to water.

Cardiovascular measurements Arterial blood pressure was measured from the cannula in the left femoral artery using a miniature strain gauge P10EZ transducer, connected to a plastic diaphragm dome (TA1017, Disposable Critiflo Dome) (both Viggo-Spectramed B.V., Bilthoven, The Netherlands). The pressure transducer was connected to a polygraph amplifier console (RMP6018, Nihon Kohden Corporation, Tokyo, Japan). A tachograph, triggered by the blood pressure signal, provided measures for heart rate. Heart rate, blood pressure and mean arterial pressure signals were passed through a CED 1401 interface (Cambridge Electronic Design LTD, Cambridge, U.K.) into a 80486 computer and the Spike 2 programme (Spike 2 Software, Version 3.1, Cambridge, England) was used for data acquisition and off line data reduction. During the experiments, the cannula connected to the pressure transducer was flushed continuously with heparin-treated saline (20 iu ml^{-1}) at a flow rate of 500 μl per hour (Syringe infusion pump 22, Harvard apparatus, Plato B.V., Diemen, The Netherlands) to prevent disturbances of the

blood pressure by obstruction of the cannula.

Blood sampling Arterial blood samples for the determination of blood concentrations were drawn at predefined time-points (15 blood samples (20–100 μl) over an interval of 0–125 min for 8MCPA and 0–150 min for 8ECPA and 8BCPA). The samples wee haemolyzed immediately in glass tubes containing 400 μl millipore water at 0°C, to prevent degradation (Mathôt *et al*., 1993), and stored at −20°C until analysis. In the vehicle-treated group, blood samples were drawn according to the schedule for 8MCPA. The arterial line was kept patent by the injection of a small volume (100 μl) of saline containing 20 iu ml^{-1} heparin directly after sampling.

Blood samples (50 μl) for the non-esterified fatty acids (NEFA) measurements were drawn from the arterial line at specific time intervals. A total number of 24 samples (4 pre-dose and 20 post-dose) for characterization of the effect of the CPA analogues were drawn over a period of 4 h. After the bolus dose of CPA three 50 μl blood samples were collected at 35, 40 and 45 min. Aliquots of 50 μl blood were added to 50 μl ice cold EDTA/saline solution (1% (w/v) EDTA in 0.9% sodium chloride solution). After centrifugation 75–110 μl of plasma was separated and stored at −20°C until analysis. After sampling the arterial line was flushed with heparin-treated saline.

Analytical procedures

Drug assay The blood concentrations of the CPA analogues were assayed by reversed phase high pressure liquid chromatography (h.p.l.c.) based on the method described previously (Van Schaick *et al*., 1997b).

Calibration standards were prepared by addition of aqueous solutions of the compounds to a mixture of 100 μl blood and 400 μl water, resulting in blood concentrations of 0–2000 ng ml^{-1} 8MCPA and 0–3000 ng ml^{-1} (8ECPA and 8BCPA). After addition of 50 μl internal standard (DCCA for 8MCPA and 8ECPA, and 1-deaza-2-chloro-2'dCHA for the other compounds, respectively), the blood samples were subjected to liquid-liquid extraction using 5 ml ethyl acetate and shaking on a vortex. After centrifugation for 10 min at 2000 *g* the organic layer was transferred to a clean tube and 500 μl water and 50 μl 3 M sodium hydroxide were added. The samples were extracted for the second time and the aqueous layer was removed from underneath. The remaining organic layer was evaporated to dryness under reduced pressure at 40°C. the residue was dissolved in 150 μl water and a volume of 50 μl was injected into the h.p.l.c. system.

The liquid chromatographic system consisted of a Waters 510 solvent delivery pump, a WISP 712B automatic sample injector (both from Millipore-Waters, Milford, MA, U.S.A.) and a Spectroflow 757 u.v. detector (Applied Biosystems, Ramsey, NJ, U.S.A.) adjusted to a wavelength of 285 nm. A stainless-steel Microsphere C-18 cartridge column (100 mm × 4.6 mm i.d.; 3 mm particle size) (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 C-18 material (Chrompack Pellicular, particle size 20–40 mm, Chrompack Nederland BV) was used. Data processing was performed with a Chromatopack C-R3A reporting integrator (Shimadzu, Kyoto, Japan).

The mobile phases consisted of various mixtures of 0.05 M sodium acetate buffer of pH 4.0 and acetonitrile in the ratios 76/24, 69/31 and 63/37 (v/v) for 8MCPA, 8ECPA and 8BCPA, respectively. An amount of 100 μl (per litre) triethylamine (TEA) was added to the mobile phases of 8MCPA and 8BCPA. The flow rate was 0.50 ml min^{-1} and retention times were between 5–8 min for the CPA analogues.

The blood concentrations were calculated using the peak-height ratio of the CPA analogues and internal standard in the calibration curve. The calibration curves were analysed using weighted linear regression (weight factor: $1/y^2$). The limits of detection were approximately 10 ng ml^{-1}. Within-day and between-day coefficients of variation were determined in a concentration range of 25–500 ng ml^{-1} and were less than 8% and 10% (8MCPA), 4% and 7% (8ECPA), and 4% and 16% (8BCPA).

NEFA assay Plasma non-esterified fatty acid (NEFA) concentrations were determined using the Wako NEFA C-kit (Wako Chemicals GmbH, Neuss, Germany). The sensitivity of the assay was improved by using small volumes (50 μl of sample and 50 and 100 μl of reagent) on a 96-wells microtitre plate. The assay was linear in a concentration range of 0.025 to 0.3 mM. Within this concentration range the within-day and between-day coefficients of variation were less than 5% and 6%, respectively.

Materials

The 8-alkylamino derivatives of N^6-cyclopentyladenosine (8-(methylamino)-CPA (8MCPA), 8-(ethylamino)-CPA (8ECPA) and 8-(butylamino)-CPA (8BCPA) were synthesized as described previously (Roelen *et al*., 1996). N^6-cyclopentyladenosine (CPA) and N^6-cyclohexyladenosine were obtained from RBI (Research Biochemicals Inc., Natick, MA). 1-Deaza-2-chloro-CPA and 1-deaza-2-chloro-2′deoxy-N^6-cyclohexyladenosine were kindly provided by Dr G. Cristalli (Camerino, Italy). Ethyl acetate was purchased from Baker Chemicals (Deventer, The Netherlands) and distilled before use. Acetonitrile (h.p.l.c. grade) was obtained from Westburg (Leusden, The Netherlands). All other chemicals were of analytical grade (Baker, Deventer, The Netherlands). Water was used from a Milli-Q system (Millipore SA, Molsheim, France).

Data analysis

Pharmacokinetic analysis In individual animals the blood concentration-time profiles of the 8-alkylamino derivates of CPA were fitted to a poly-exponential equation for intravenous infusion (Gibaldi & Perrier, 1982):

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

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

In this equation C_t is the concentration at time t, T is the infusion duration, C_i and λ_i are the coefficients and exponents of the equation, respectively. Various 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 concentration-time curve (AUC), the systemic clearance (*Cl*), the elimination half-life ($t_{1/2,n}$) and the volume of distribution at steady state (V_{dss}) were calculated following standard equations (Gibaldi & Perrier, 1982). The

pharmacokinetic functions were fitted to the data with weight $1/y^2$ using the non-linear least squares regression programme Siphar (Simed SA, Creteil, France). In each individual rat the fitted function of the concentration-time profile was used to calculate the concentrations at the measured effect-time points.

Modelling of heart rate The relationship between the blood agonist concentrations and heart rate was described on the basis of the sigmoidal E_{max} model (Holford & Sheiner, 1982) (equation 2) as reported recently (Mathôt *et al*., 1995b; Van Schaick *et al*., 1997b).

$$E(C) = E_0 + \frac{E_{max} \cdot C^n}{EC_{50}^n + C^n} \quad (2)$$

where E(C) is the observed effect at blood concentration C, E_0 is the baseline heart rate, E_{max} is the maximal effect, EC_{50} is the blood concentration at half maximal effect and n is a constant expressing the sigmoidicity of the concentration-effect relationship.

Modelling of the anti-lipolytic effect The relationship between SPA blood concentration and the anti-lipolytic effect was quantified by a physiological pharmacokinetic-pharmacodynamic model, which describes the change in NEFA concentrations as being an indirect response to the inhibition of the factors controlling it (Dayneka *et al*., 1993; Jusko & Ko, 1994). In previous investigations, this indirect response model for the effect on plasma NEFA concentrations was thoroughly validated. The model was shown to provide consistent pharmacodynamic parameter estimates for the A_1 receptor agonist N^6-(p-sulphophenyl)adenosine (SPA) independent of dosage regimen or utilized marker of lipolysis (fatty acids or glycerol) (Van Schaick *et al*., 1997a; 1998). The rate of change of the NEFA concentrations in this model is described by:

$$\frac{dN}{dt} = k_s \cdot f(C) - k_{out} \cdot N \quad (3)$$

where k_s represents the zero-order rate constant for the synthesis of NEFAs, k_{out} the first-order rate constant for the elimination of NEFAs and N the plasma NEFA concentration. The function f(C) represents the fractional inhibitory effect according to the sigmoidal E_{max} model:

$$f(C) = 1 - \frac{E_{max} \cdot C^n}{EC_{50}^n + C^n} \quad (4)$$

where C is the SPA concentration, E_{max} is the maximal inhibition of lipolysis, EC_{50} is the SPA concentration at half-maximal inhibition and n_H is the Hill-factor expressing the steepness of the curve. The differential equation (equation 3) was transformed into equation 5 to which the NEFA data were fitted:

$$N(t) = N_0 \cdot (1 - f(C)) \cdot e^{-k_{out} \cdot t} + N_0 \cdot f(C) \quad (5)$$

where N_0 is the baseline NEFA concentration. The equations were fitted to the data using the non-linear least squares regression program Siphar (Simed SA, Creteil, France).

Statistical analysis The pharmacokinetic and pharmacodynamic parameter estimates of the different groups were statistically compared using the parametric one-way analysis of variance (ANOVA) or a non-parametric Kruskall-Wallis test, if more appropriate. Parameters for heart rate and NEFA, that have been obtained in the same individual rat, were compared using a paired *t* test. A significance level of 5% was selected. All data are presented as mean ± s.e., unless indicated otherwise.

Results

Pharmacokinetic profiles

The averaged blood concentration-time profiles after intravenous administration of 8MCPA, 8ECPA and 8BCPA for 15 min are shown in Figure 2. Upon cessation of the infusion, the profiles were characterized by a rapid distribution phase followed by a slower elimination phase. A bi-exponential pharmacokinetic function adequately described the time profile of the concentration in individual rats. Pharmacokinetic parameters are summarized in Table 1. The 8-butylaminoCPA analogue had the largest volume of distribution and half-life.

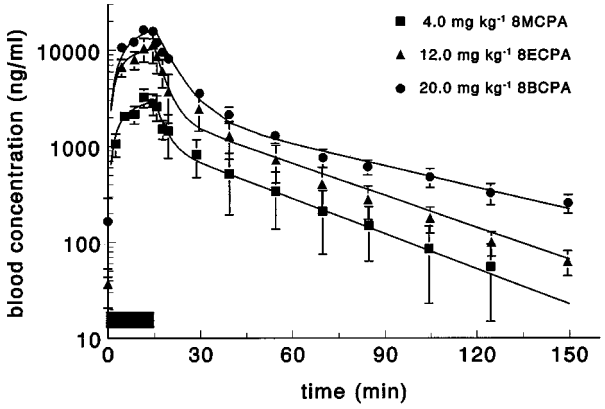

Figure 2 Concentration time profiles after intravenous infusions of 4.0 mg kg^{-1} 8MCPA ($n=7$), 12.0 mg kg^{-1} 8ECPA ($n=8$), and 20.0 mg kg^{-1} 8BCPA ($n=6$) for 15 min to rats. Data are presented as mean and the fitted lines represent the best fits on the basis of averaged pharmacokinetic parameter estimates; vertical lines show s.d.

Table 1 Pharmacokinetic parameter estimates obtained after i.v. infusion of the three 8-alkylamino derivatives of CPA for 15 min to conscious normotensive rats

	n	*Dose* (mg kg^{-1})	$t_{½,n}$ (min)	Cl (ml min^{-1} kg^{-1})	V_{dss} (lkg^{-1})	*MRT* (min)
CPA[a]		0.2	6.9 ± 1.1	76 ± 3	0.32 ± 0.04	4.1 ± 0.4
8MCPA	7	4.0	25 ± 2	44 ± 5	0.97 ± 0.09	23 ± 2
8ECPA	8	12.0	28 ± 2	48 ± 6	0.84 ± 0.10	18 ± 1
8BCPA	6	20.0	40 ± 2***	39 ± 2	1.05 ± 0.07	27 ± 1**

The values presented are means ± s.e. [a]Data from Mathôt *et al*. (1994). *n*: number of observations; $t_{½,n}$: terminal half-life; *Cl*; clearance; V_{dss}: volume of distribution at steady state; MRT: mean residence time. **MRT of 8BCPA significantly different from the value for 8ECPA ($P<0.01$, ANOVA). ***Terminal half-life of 8BCPA significantly different from the other compounds ($P<0.001$, ANOVA).

Haemodynamic effects

The effects of the i.v. infusions of the three CPA analogues on heart rate and mean arterial pressure are depicted in Figure 3. Intravenous administration of the compounds elicited a direct and rapid reduction in both HR and MAP. During the infusion the reductions reached a maximum and returned to baseline values after termination of the infusion. The CPA analogues elicited less pronounced reductions in heart rate and blood pressure than CPA (Figure 4). In each group CPA caused a maximal reduction in HR and MAP of -201 ± 8 beats min^{-1} (reduction of 54%) and -48 ± 3 mmHg (reduction of 46%), respectively. Administration of the vehicle (20% DMSO in saline) did not have an effect on haemodynamics (Figure 3).

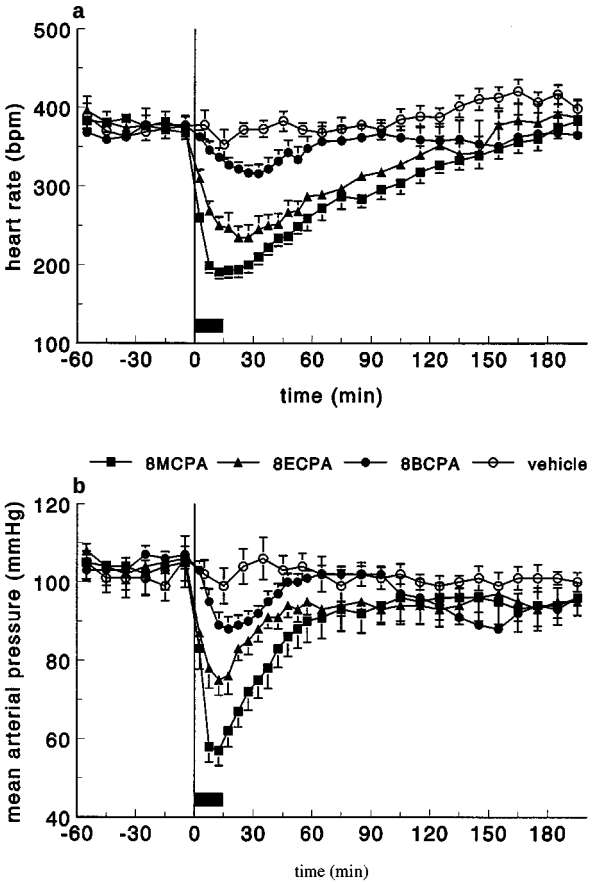

Figure 3 Effect on heart rate (a) and mean arterial pressure (b) for rats that were given an i.v. infusion of 4.0 mg kg^{-1} 8MCPA ($n=7$), 12.0 mg kg^{-1} 8ECPA ($n=8$) 20.0 mg kg^{-1} 8BCPA ($n=6$) or vehicle (20% DMSO/saline) ($n=6$) for 15 min (solid bar). Data are presented as mean and vertical lines show s.e.mean.

The pharmacokinetic equations derived from the concentration-time profiles were used to calculate blood agonist concentrations at the time points of the heart rate measurements. The reductions in heart rate were directly related to the blood concentrations on the basis of the sigmoidal E_{max} model, since no hysteresis was observed between concentrations and effect. The no-drug effect values (E_0) were obtained from averaging the heart rate during the last hour of the experiment (over the period 135–195 min post-dose) and were fixed in the modelling procedure (see Mathôt *et al.*, 1994). Figure 5 displays the observed heart rate effect *vs* time in combination with the concentration *vs* time profiles of three individual rats that were given 8MCPA, 8ECPA and 8BCPA intravenously in 15 min. In each individual animal the sigmoidal E_{max} model adequately described the relationship between the adenosine agonist concentrations and the bradycardic effect (lower graphs). The pharmacodynamic parameter estimates obtained from the pharmacokinetic-pharmacodynamic modelling procedure are summarized in Table 2. The difference in intrinsic activity of the CPA analogues is expressed in the E_{max} value, which decreased with increasing chain length at the 8-position. Furthermore, the EC_{50} values of 8MCPA, 8ECPA, and 8BCPA were 164 ± 22, 341 ± 76, and 975 ± 190 ng ml^{-1}, respectively. No significant differences were observed between the no-drug response values (E_0) of the treatment groups. The 8BCPA concentration heart rate relationships were significantly steeper than the curves of the other two analogues, which was reflected in a larger value of the Hill-factor for 8BCPA.

Anti-lipolytic effects

The time course of the plasma NEFA concentration, after intravenous administration of the CPA analogues and vehicle to fasted rats, is shown in Figure 6. The baseline NEFA concentrations before administration were similar in each group (Table 3). The plasma NEFA concentrations in the vehicle-group fluctuated slightly, resulting in somewhat higher NEFA concentrations during the experiment.

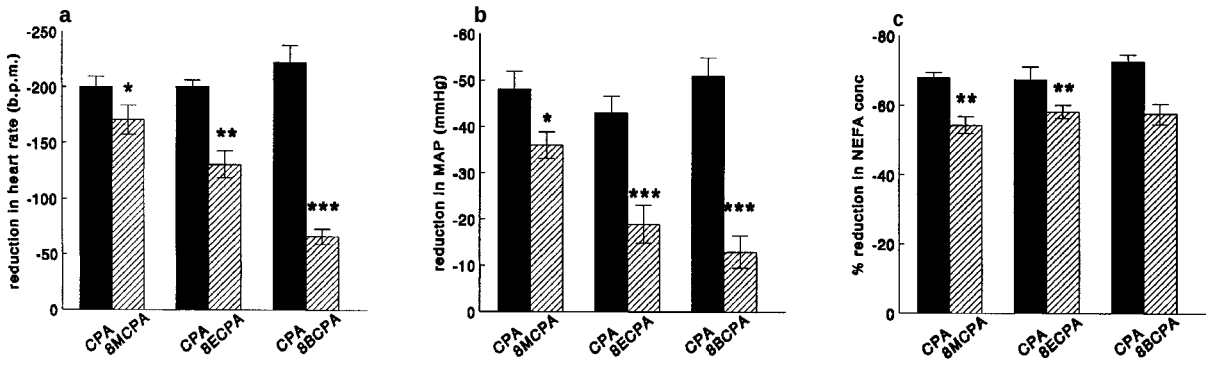

Figure 4 Comparison of the maximal reduction in heart rate (a), mean arterial presure (b) and NEFA concentrations (c) mediated by CPA to the reductions mediated by the derivatives of CPA; the maximal effects of HR and MAP were observed at the end of the infusions, whereas the maximal reduction in NEFAs was observed between 35–45 min post dose; data are mean ± s.e. Statistical significance: $*P<0.05$, $**P<0.01$, $***P<0.001$ *versus* the effect of CPA (paired *t* test).

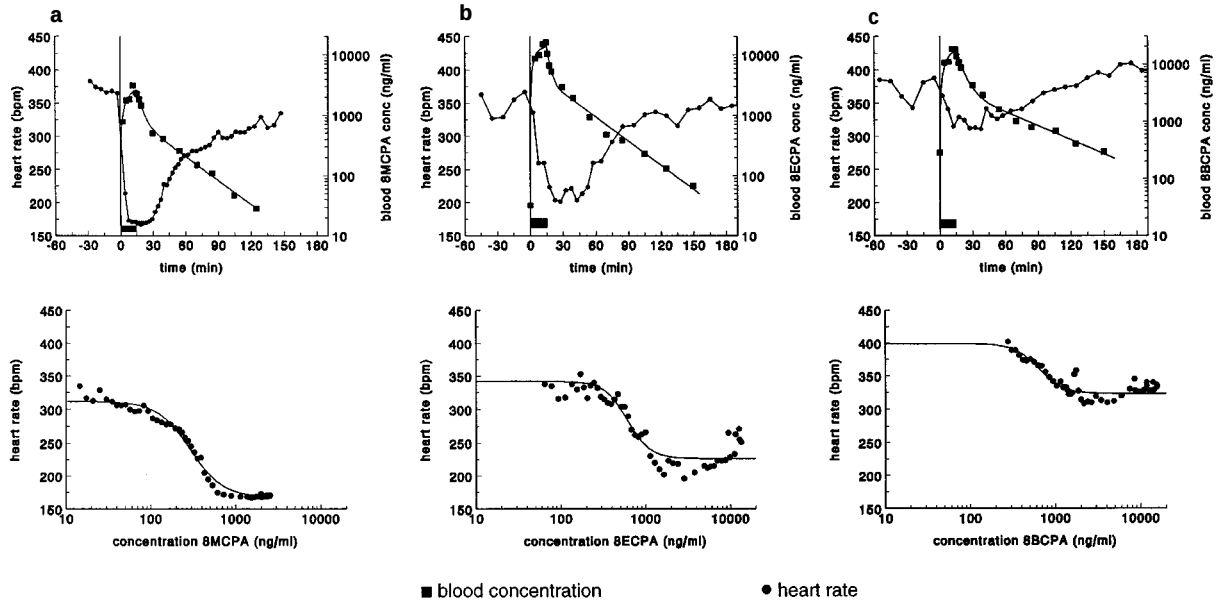

Figure 5 (Upper figures) Heart rate *vs* time and blood concentration *vs* time profiles of three individual rats that were dosed with 8MCPA (a), 8ECPA (b) and 8BCPA (c), respectively. (Lower figures) fitted concentration-heart rate relationships of the same rats on the basis of the sigmoidal E_{max} pharmacodynamic model (equation 5).

Table 2 Pharmacodynamic parameter estimates for the reduction in heart rate after i.v. administration of the 8-alkyamino derivatives of CPA to conscious normotensive rats

	n	E_0 (beats min^{-1})	E_{max}[a] (beats min^{-1})	EC_{50} (ng ml^{-1})	*Hill-factor*
CPA[a]	6	355±13	−208±0.19	1.8±0.5	1.4±0.4
8MCPA	7	371±14	−173±14	164±22	1.3±0.1
8ECPA	8	376±14	−131±11	341±76	3.3±0.5
8BCPA	5	380±19	−71±6	975±190**	5.1±1.6**

The values are presented as mean±s.e. [a]Data from Mathôt *et al.* (1994). *n*: number of observations; E_0: baseline hart rate; E_{max}: maximal effect; EC_{50} agonist concentrations at 50% of maximal effect. [a]E_{max} values of the compounds were significantly different (8MCPA *vs* 8ECPA ($P<0.05$); 8BCPA *vs* 8ECPA ($P<0.01$), ANOVA). **EC_{50} value and Hill factor of 8BCPA significantly different from other compounds ($P<0.01$, Kruskall-Wallis).

After administration of the agonists, plasma NEFA concentrations decreased slowly and reached a maximal reduction after approximately 40–50 min. All three CPA-analogues produced a similar reduction in plasma NEFA levels, despite their differing intrinsic activity for heart rate. NEFA concentrations did return, albeit that the original baseline values were not reached within the time span of the experiment. The reduction in NEFA levels observed after administration of the short bolus of CPA, was used to compare the intrinsic activities (maximal effects) of the CPA analogues with CPA within the same animal. The maximal suppression of NEFA levels was slightly smaller for the CPA analogues than for CPA (Figure 4c). However, all three analogues produced the same maximal effect despite the length of the alkylamino side chain.

In contrast to the bradycardic effect, the reduction in NEFA concentrations is not a direct effect but the result of the inhibition of the synthesis of NEFAs. Therefore, this effect should be quantified on the basis of a physiological indirect effect model (Jusko & Ko, 1994). The delay between agonist concentrations and effect (the reduction in

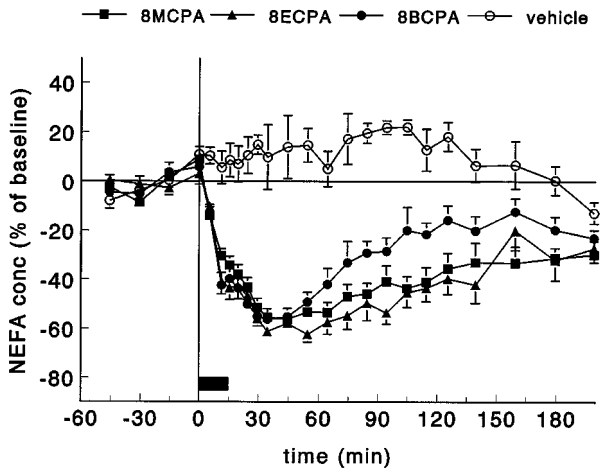

Figure 6 Time profiles of the plasma NEFA concentrations after intravenous infusions of 4.0 mg kg^{-1} 8MCPA ($n=7$), 12.0 mg kg^{-1} 8ECPA ($n=8$), 20.0 mg kg^{-1} 8BCPA ($n=6$) for 15 min (solid bar) to conscious, fasted rats. The vehicle-treated group received 765 μl of 20% (v/v) DMSO/saline during 15 min ($n=6$). Data are illustrated as mean with vertical lines showing s.e.

Table 3 Pharmacodynamic parameter estimates for the reduction in plasma NEFA concentrations after i.v. administration of the three 8-alkylamino derivatives of CPA to conscious normotensive rats

	n	N_0 (mM)	E_{max} (% reduction from baseline)	EC_{50} (ng ml^{-1})	*Hill factor*	K_{out} (min^{-1})
8MCPA	7	0.38±0.01	63±5	37±15	0.9±0.07	0.07±0.01
8ECPA	8	0.36±0.02	63±4	68±22	1.2±0.12	0.08±0.01
8BCPA	6	0.36±0.01	68±2	659±108**	1.9±0.7	0.07±0.01

The values are presented as mean±s.e. *n*: number of observations; N_0 baseline NEFA concentrations; E_{max}: maximal effect; EC_{50}: agonist concentrations at 50% of maximal effect; K_{out}; elimination rate constant. **EC_{50} value of 8BCPA significantly different from other two compounds ($P<0.01$, Kruskall-Wallis); other parameters not significantly different.

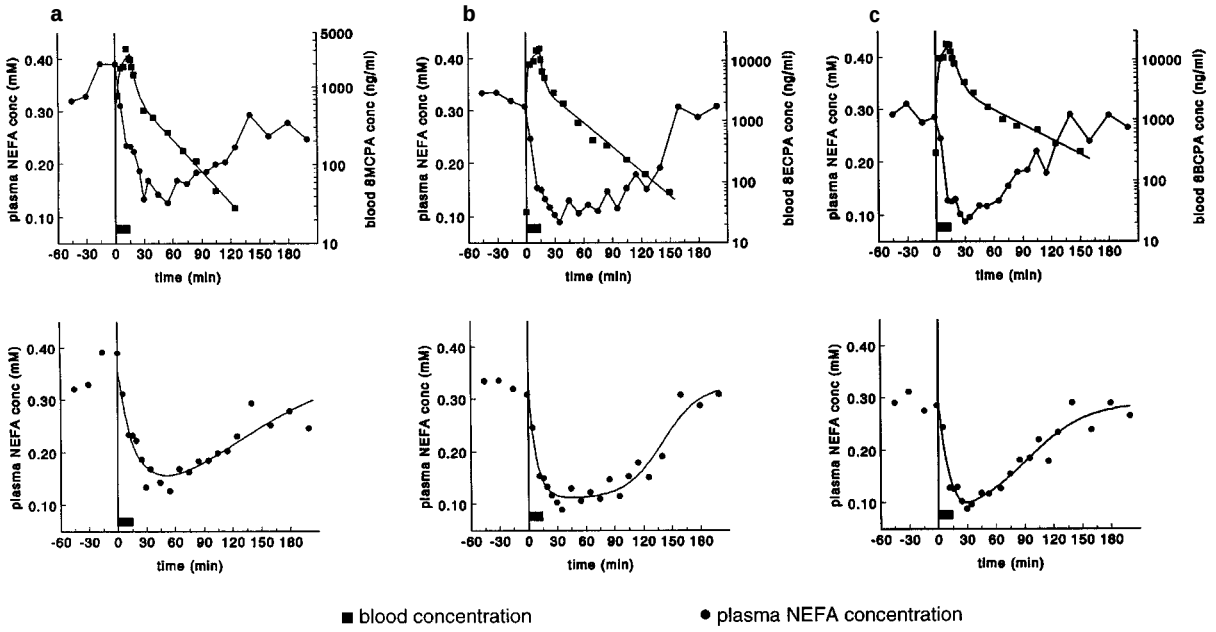

Figure 7 (Upper figures) Plasma NEFA concentrations *vs* time and blood concentration *vs* time profiles of three individual rats that were dosed with 8MCPA (a), 8ECPA (b) and 8BCPA (c), respectively. (Lower figures) predicted time profile of plasma NEFA concentrations of the same rats on the basis of the indirect suppression model (equation 4).

ambient NEFA concentrations) is characterized by the slow elimination rate (k_{out}) of the fatty acids from the circulation. As such the slow decrease in NEFA concentrations is dependent on the elimination rate of NEFAs from the circulation and independent of the adenosine agonist used (Figure 6).

Figure 7 depicts the anti-lipolytic effects of the CPA analogues in three individual rats in combination with the individual concentration-time profiles. These graphs more clearly illustrate the delay between agonist concentrations and effect. After termination of the infusion the agonist concentrations are declining, while the effect is still progressing and reaches its maximal reduction after 40–50 min. The indirect suppression model adequately described the time course of the anti-lipolytic effect in each animal (Figure 7: lower graphs). The baseline NEFA concentrations (N_0) were calculated from the NEFA measurements before administration and fixed in the model. The estimated pharmacodynamic parameters for the NEFA-lowering effect are summarized in Table 3. All analogues induced a similar maximal suppression of plasma NEFA concentrations of approximately 64%. No significant differences between the parameters E_{max}, k_{out}, N_0 and Hill-factor were detected. The compounds only differed significantly in potency for the anti-lipolytic effect. The EC_{50} values were 37±15, 68±22 and 659±108 ng ml^{-1} for 8MCPA, 8ECPA and 8BCPA, respectively.

Tissue selectivity

Selectivity of the adenosine agonists for the two pharmacological actions was investigated by comparison of the concentration-effect relationships for both the bradycardic and anti-lipolytic effect within the same individual animal. The relative inhibitory effects of the agonists on both heart rate and lipolysis are depicted in Figure 8. Substitution of the 8-alkylamino group resulted in partial agonism (reduced maximal effect) for the effect on heart rate, whereas the compounds were nearly full agonists for the anti-lipolytic effect (Table 4). Furthermore the concentration-effect relationships for the anti-lipolytic effect are slighly shifted to the left, indicating the higher potency of the compounds for this effect. Figure 9 shows the difference in EC_{50} for the two effects within the individual rats. For 8BCPA this difference was less pronounced and not statistically significant. However, in most rats the EC_{50} value for the anti-lipolytic effect was lower than

for the bradycardic effect. Calculation of the ratio between receptor affinity in the presence of GTP ($K_{i,+GTP}$) (Roelen *et al*., 1996) and the EC_{50} based on free drug concentrations ($EC_{50,u}$), yielded a measure of the receptor reserve between adipose and cardiac tissue *in vivo* (Table 4). The $EC_{50,u}$ values were obtained by correction of the EC_{50} values for binding to plasma proteins as described previously (Van Schaick *et al*., 1997b).

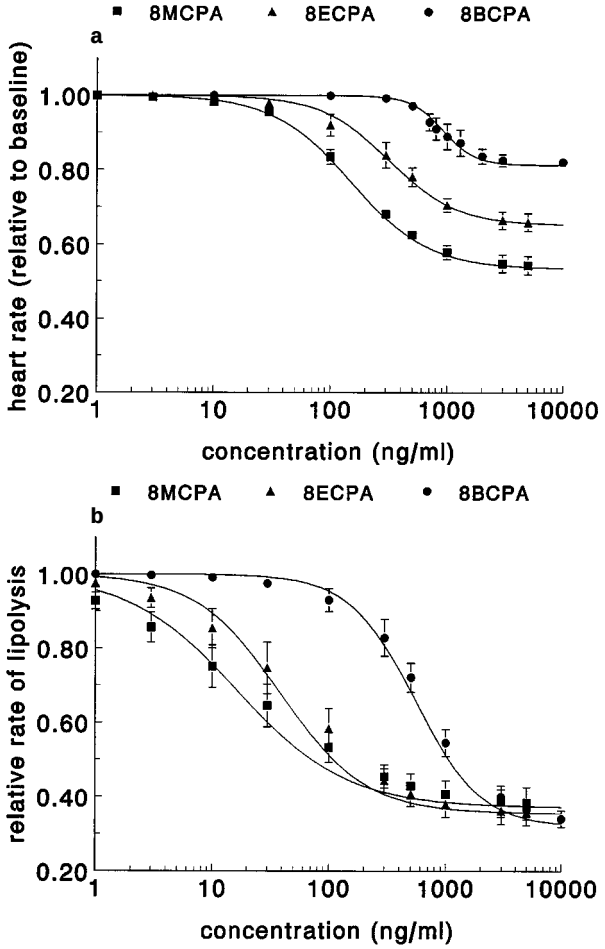

Figure 8 Relationships between blood concentration and the relative effects on heart rate (a) and lipolysis (b) for the 8-alkylamino analogues of CPA; the estimated pharmacodynamic parameters (Tables 2 and 3) were used to construct the curves according to the sigmoidal E_{max} model. The data are mean values of 6–8 curves; vertical lines show s.e.

Discussion

The 8-alkylamino analogues of CPA that were investigated in this study were recently developed as part of a series of N^6,C8-disubstituted adenosines (Roelen *et al*., 1996). In this series of compounds, the 8-alkylamino-derivates of the prototypic agonst N^6-cyclopentyladenosine (CPA) were identified as potent and selective partial agonists for the A_1 adenosine receptors *in vivo*. Functionally, the partial agonistic behaviour of these compounds was demonstrated for cardiac A_1 adenosine receptors in conscious rats (Van Schaick *et al*., 1997b). In that study, an integrated pharmacokinetic-pharmacodynamic approach was used to derive quantitative estimates of A_1 adenosine receptor activation *in vivo*. Quantification of the blood concentration-heart rate relationships revealed that the intrinsic activity of the compounds reduced with increasing length of the alkyl-group at the 8-position. Although it is conceivable that the observed reduced bradycardic activity of these compounds results from a differential potency in inhibiting a (potential) sympathetic reflex, this is not a very likely explanation for the following reasons. The compounds 8MCPA and 8ECPA have a similar affinity to the A_1 adenosine receptor but the maximum effect on heart rate is different. This difference between the two compounds can therefore not be explained by a difference in potency with respect to the inhibition of a sympathetic reflex. Furthermore, in extensive investigations on the pharmacokinetic-pharmacodynamic correlations of selective adenosine A_1 receptor agonists, no evidence of the influence of a sympathetic reflex has been obtained (Mathôt *et al*., 1994; Van Schaick *et al*., 1997b). Only for the non-selective adenosine receptor agaonst 8-butylamino-adenosine, reflex tachycardia was observed. This effect appears to be related to adenosine A_{2a} receptor mediated vasodilatation rather than A_1 receptor adenosine activation (Mathôt *et al*., 1996). Thus it appears that the 8-alkylamino-derivates of CPA are true partial agonists at the A_1 adenosine receptor *in vivo*. These *in vivo* results are consistent with measures for intrinsic activity *in vitro*, such as the GTP-shift (the ratio between the receptor affinity in the presence and absence of 1 mM GTP) in receptor binding experiments (Roelen *et al*., 1996) and the [^{35}S]-GTPγS-binding in rat brain membranes (Lorenzen *et al*., 1996). The partial agonistic properties of these CPA derivatives has recently been confirmed in another series of *in vitro* studies, in

Table 4 Comparison of the A_1-receptor affinity *in vitro*, and the potency, receptor reserve and intrinsic activity of the 8-alkylamino derivatives of CPA for the effect on NEFA and heart rate *in vivo*.

	$K_{i,+GTP}$[a] (nM)	$EC_{50,u}$ (nM)	Receptor reserve[b]	Intrinsic activity relative E_{max}[c]
Inhibition of lipolysis				
8MCPA	980 (880–1070)	47±20	21	0.91
8ECPA	1330 (1100–1560)	55±17	24	0.92
8BCPA	1130±180	283±46	4	1.0
Reduction of heart rate				
8MCPA	980 (880–1070)	212±29	4.6	0.83
8ECPA	1330 (1100–1560)	277±54	4.8	0.63
8BCPA	1130±180	418±81	2.7	0.34

[a]K_i values in the presence of GTP; data are presented as median (range) or mean±s.e., $n=3$ (from Roelen *et al*., 1996). [b]Ratio K_{i+GTP}/$EC_{50,u}$ as indicator of receptor reserve. [c]Intrinsic activity relative to the full agonist CPA; E_{max} for heart rate: -208 beats min^{-1} (Mathôt *et al*., 1994); E_{max} for NEFA: 0.69 (averaged reduction after administration of the bolus infusion of CPA in the present study).

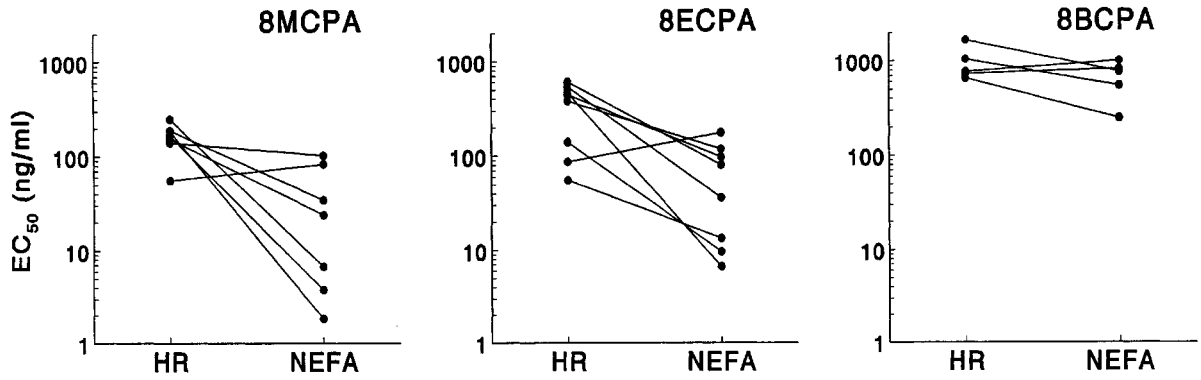

Figure 9 Individual estimates of the EC_{50} value for heart rate (HR) and plasma NEFA concentrations (NEFA); the values that were obtained within the same rat are connected by the solid lines.

which it was shown that these compounds have a reduced intrinsic activity on G-protein activation and the inhibition of adenylate cyclase in a rat cerebral cortex preparation. In addition it was shown that the CPA-derivatives may act as antagonists of CPA, which is consistent with their partial agonistic properties (Lorenzen *et al*., 1997).

Low efficacy agonists compounds such as those studied here may be therapeutically useful, because they can act either as agonists, partial agonists or antagonists depending on the physiological system or tissue (Kenakin, 1993). Thus, the 8-alkylamino analogues of CPA may have selective A_1 adenosine receptor mediated effects *in vivo*, because they are able to differentiate between tissues and organs on the basis of differences in receptor-effector coupling, receptor density or other characteristics of the biological system (e.g. the degree of homeostatic control). To test this hypothesis, the selectivity of action (bradycardia *vs* anti-lipolytic) of three agonists (8MCPA, 8ECPA and 8BCPA) with differences in intrinsic activity was investigated in the present study.

After intravenous administration, the compounds were shown to exert both anti-lipolytic effects, as observed from effects on plasma non-esterified fatty acids (NEFA) concentrations, and bradycardic and hypotensive effects in conscious rats. The observed effects were consistent with activation of A_1 adenosine receptors in adipose and cardiac tissue (Strong *et al*., 1993; Gardner *et al*., 1994). For each effect the relationship between agonist concentration and response was quantified on the basis of integrated pharmacokinetic-pharmacodynamic (PK-PD) models. This approach has the advantage that the effects could be compared quantitatively without the influence of pharmacokinetics or time-dependent factors such as the delayed onset of the lowering of the NEFA concentrations (Mathôt *et al*., 1994; Van Schaick *et al*., 1997a; 1998). Accordingly, two different PK-PD models were applied. The reduction in heart rate was quantified on the basis of a direct effect model, whereas for the NEFA lowering effect an indirect response model was most adequate. The validity has been demonstrated for both models (Mathôt *et al*., 1994; Appel *et al*., 1995; Van Schaick *et al*., 1997a; 1998). The underlying concentration-effect relationships were described by the sigmoidal E_{max} model yielding parameter estimates of EC_{50} (potency), E_{max} (intrinsic activity) and Hill-factor in both models.

Generally, estimates of potency and intrinsic activity are obtained in isolated systems or receptor binding *in vitro*, to avoid interference of complicating mechanisms that are present *in vivo* (Gerencer *et al*., 1992). However, it is important to realise that selectivity of action is not only determined by pharmacodynamic principles but is also influenced by pharmacokinetic factors. Thus, in order to investigate adequately selectivity *in vivo* these complicting factors need to be included. For this purpose, combined pharmacokinetic-pharmacodynamic approaches are useful (Kenakin, 1992). These approaches have been shown to provide an adequate quantification of the interaction between drug concentration and response, and include variation in phrmacokinetics (Breimer & Danhof, 1997).

The time courses of the agonist concentration in blood were best described on the basis of a bi-exponential equation for each CPA analogue. Pharmacokinetic parameters differed slightly between the three adenosine agonists. Only 8BCPA had a significantly longer terminal half-life due to a lower clearance and larger apparent volume of distribution (Table 1). The larger volume of distribution of 8BCPA may be caused by an increased lipophilicity as a result of the substition of the butyl-group. Some pharmacokinetic parameters deviated from our recently reported values. In the present study the clearance values were 2 fold lower, which may indicate a lower metabolic capacity of these animals in comparison to the previous ones. The volumes of distribution at steady state were not different between the studies. This parameter is mainly determined by the physico-chemical properties of the compounds. These differences resulted in longer terminal half-lives in the present experiment (25, 28, and 40 min) in comparison to previous half-lives (16, 17, and 24 min for 8MCPA, 8ECPA and 8BCPA, respectively) (Van Schaick *et al*., 1997b).

The reductions in HR and MAP after intravenous administration of the CPA analogues were consistent with activation of A_1 adenosine receptors in rats (Coffin & Spealman, 1987; Trivedi *et al*., 1991; Mathôt *et al*., 1994) and quite different from responses mediated by the selective A_{2A} agonist CGS21680 (Mathôt *et al*., 1995a) or the mixed A_1/A_{2A} agonist 8-butylaminoadenosine (Mathôt *et al*., 1996). The observed reduction in mean arterial pressure of the compounds is mainly caused by an A_1 receptor mediated reduction in cardiac output (Webb *et al*., 1990; Merkel *et al*., 1993). Additionally, blood pressure values were only reduced at concentrations that reduced heart rate and the reduction lasted shorter than the bradycardic effect. Similar observations were made for the highly selective A_1 receptor agonists CPA (Mathôt *et al*., 1994) and GR79236 (Gardner *et al*., 1994; Merkel *et al*., 1995), which were shown to have 5- to 10 fold higher potencies for the effect on heart rate than on blood pressure.

The relationship between blood agonist concentration and heart rate were characterized adequately on the basis of the sigmoidal E_{max} model in individual rats (Figure 5). In previous studies, this approach has been used successfully to obtain quantitative estimates of adenosine receptor ligands *in vivo*. The concentration-effect relationships were shown to be dose-independent (Mathôt *et al*., 1994), consistent for various A_1 adenosine receptor agonists (Mathôt *et al*., 1995b; Van Schaick *et al*., 1997b) and shifted to higher concentrations in the presence of steady state levels of the adenosine antagonist 8-cyclopentyltheophylline (Appel *et al*., 1995).

At the end of each experiment, CPA was administered in a short bolus as internal control of the maximal effect of the prototypic full agonist CPA. The administered dose of CPA (20 μg CPA in 5 min) was shown to result in blood concentrations that were over a 100 fold higher than the EC_{50} of CPA for HR *in vivo* (1.8 ng ml^{-1}) and thus sufficiently high to mediate a maximal reduction in HR (Mathôt *et al*., 1994). As compared to CPA as a full agonist, the analogues produced less pronounced reductions in HR and MAP (Figure 4). The maximal effect elicited by the short bolus of CPA was similar in all treatment groups (approximately -210 beats min^{-1} and -48 mmHg for HR and MAP, respectively) and consistent with maximal effects in previous studies. Apparently, the maximal reduction of CPA was not influenced by previous administration of one of the analogues or vehicle, indicating no development of acute tolerance towards the agonists.

The difference in intrinsic efficacy of the compounds was reflected in the concentration-HR relationship (Figure 8, Table 2). The averaged E_{max} values ranged between -173 ± 14 and -71 ± 6 beats min^{-1} for 8MCPA and 8BCPA, respectively, and were consistent with previous results (Van Schaick *et al*., 1997b). Furthermore, the compounds were moderately potent in lowering HR. Potencies *in vivo* were 164, 341 and 975 ng ml^{-1} for 8MCPA, 8ECPA, and 8BCPA, which corresponded to over 100 fold lower potency than CPA (Roelen *et al*., 1996; Van Schaick *et al*., 1997b). The averaged Hill-factor in the 8BCPA treated group was larger than the Hill-factor in the other treatment groups. For 8BCPA the Hill-factor had large interindividual variation and was extremely steep in two individual animals. This large variation is caused by the low intrinsic activity of 8BCPA, which resulted in a small reduction in heart rate that is biased by the relatively large variation in baseline HR values (Figure 3).

The lowering of the NEFA concentrations was not directly related to blood concentrations, as result of a delay between concentrations and effect. This delay between drug concentrtions and effect was characterized by a rate-limiting elimination of the physiological pharmacodynamic parameter (fatty acids) from blood (Dayneka *et al*., 1993; Jusko & Ko, 1994). Previously, the indirect response model was shown to describe adequately the time course of the NEFA concentrations after i.v. administration of the A_1 adenosine receptor agonist N^6-(*p*-sulphophenyl)adenosine (SPA) to rats (Van Schaick *et al*., 1997a). The slow decrease in plasma NEFA concentrations, after inhibition of lipolysis by SPA, was shown to be independent of the rate of drug input. Moreover, the model was shown to be consistent for different infusion regimens and applicable to both NEFA and glycerol lowering effects (Van Schaick *et al*., 1998). These findings justify the conclusion that the NEFA lowering effect of adenosine A_1 receptor ligands is directly related to the concentration and not indirectly to the haemodynamic response (i.e. effect on blood pressure, heart rate).

The indirect response model adequately predicted the time course of the NEFA concentrations in individual rats (Figure 7). The parameter k_{out} characterized the elimination rate of NEFAs and was not different between the groups, which demonstrates that the delay was not caused by a slow distribution of the agonists to the site of action, but the result of the elimination of NEFAs (Van Schaick *et al*., 1997a). The average elimination-rate constant was 0.07 ± 0.001 min^{-1}, which corresponded to a half-life for NEFAs of approximately 10 min.

The maximal suppression of NEFA concentrations was not different between the agonists. In contrast to what was observed for heart rate, all compounds produced an equal reduction in NEFA concentrations that was almost as large as the reduction by CPA (69%) (Figure 4). The maximal decrease was similar to the observed reduction in ambient NEFA concentrations after administration of other A_1 adenosine agonists *in vivo* (Strong *et al*., 1993; Gardner *et al*., 1994; Wagner *et al*., 1994). The maximal reductions in NEFA concentrtions of 8MCPA and 8BCPA were slighly less than after administration of CPA. This difference is probably not physiologically relevant, because it was not observed for 8ECPA. Furthermore, this maximal effect was based on average NEFA concentrations of 2 to 3 blood samples rather than the maximal effect obtained on the basis of a PK-PD modelling procedure. The model-estimated intrinsic activities (E_{max}) were not different between the agonists. All three analogues acted as full agonists with intrinsic activities similar to CPA.

The most important objective of this study was to investigate the tissue (and response) selectivity of these low efficacy A_1 adenosine receptor agonists. Comparison of the *in vivo* concentration-response relationships revealed that the selectivity between the bradycardic and anti-lipolytic effect was caused by differences in both potency and intrinsic activity of the compounds for the two effects (Figure 8). The concentrations of the CPA analogues responsible for 50% of the maximal effect were approximately 5 fold lower for NEFA than for heart rate. Furthermore, the compounds had reduced intrinsic activity for the cardiovascular effect, whereas they acted as full agonistic inhibitors of lipolysis.

Differences in potencies of A_1 agonists between anti-lipolytic and haemodynamic effects have been shown previously (Gardner *et al*., 1994; Wagner *et al*., 1994; Merkel *et al*., 1995). However, since most observations were made on the basis of dose, no exact mechanism for the selectivity *in vivo* could be identified (Gardner *et al*., 1994). Recently, on the basis PK-PD modelling, the A_1 receptor agonist SPA was shown to elicit anti-lipolytic effects at lower blood concentrations (lower EC_{50}) than for heart rate (Van Schaick *et al*., 1997a). This observation was in line with the observed EC_{50} difference of the non-selective agonist NECA *in vitro* between rat right atria and isolated adipocytes (Gurden *et al*., 1993). The difference in sensitivity is the result of a difference in efficiency of receptor coupling (receptor reserve) between adipose and cardiac tissues (Lohse *et al*., 1986; Dennis *et al*., 1992). In tissues with a large amount of receptor reserve, compounds may be more potent (EC_{50}) than in other tissues irrespective of their affinity (K_i) for the receptors (Kenakin, 1993). Thus, for 8MCPA and 8ECPA the ratio $K_{i,+GTP}/EC_{50,u}$ was larger for the anti-lypolytic effect (21 and 24) than for heart rate (4.6 and 4.8) (Table 4). For 8BCPA this reserve for both effects was smaller, because the efficiency of receptor coupling is also dependent on the intrinsic efficacy of the agonist. Due to its lower intrinsic efficacy, 8BCPA less effectively promotes the signal transduction between occupied receptors and effect, which results in the observation of a lower potency *in vivo*.

Interestingly, the CPA analogues were full agonists for the anti-lipolytic effect with only limited effect at the cardiovascular system. At concentrations up to 20 μg ml^{-1} (49 μM), 8BCPA is only able to cause 34% of the bradycardic effect that is normally observed for a full agonist. To our knowledge, such observations have not been obtained previously for adenosine agonists. Most A_1 adenosine receptor agonists, that have been developed as potential anti-lipolytic drugs so far, have been high efficacy agonists and as such full agonists for both the effect on lipolysis and heart rate (Gardner *et al*., 1993; Wagner *et al*., 1994; Merkel *et al*., 1995). Thus partial A_1 adenosine receptor agonists exhibit an improved selectivity of action of the antilipolytic *versus* the bradycardic response. However, the possibility that this selectivity may to a certain extent be influenced by the sympathetic tone should be considered. In this respect it is important that in a recent study it was shown that two separate mechanisms may be involved in the bradycardic response to adenosine A_1 receptor agonists (i.e. activation of $I_{K(Ado)}$ and an anti-β-adrenoceptor effect) (Srinivas *et al*., 1997).

In conclusion, this study demonstrates that development of low efficacy agonists for the A_1 adenosine receptor does result in compounds with improved selectivity of action *in vivo*. On the basis of their pharmacological profile these compounds may be useful in diabetes (Foley, 1994) or other disorders of lipid metabolism (Vanotti *et al*., 1994). Additionally, these compounds may induce less receptor down regulation or desensitization (IJzerman *et al*., 1994).

The 8-alkylamino derivatives of CPA are potent inhibitors of lipolysis and have less severe cardiovascular side effects. To our knowledge this is the first study that clearly demonstrates a separation between anti-lipolytic and cardiovascular effects of systemically administered A_1 adenosine agonists. Although 8BCPA had only moderate affinity at A_1 adenosine receptors (Roelen *et al*., 1996), its usefulness as an anti-lipolytic agent is promising due to limited cardiovascular side efects.

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