Mechanism-Based Pharmacokinetic-Pharmacodynamic Modeling of Antilipolytic Effects of Adenosine A₁ Receptor Agonists in Rats: Prediction of Tissue-Dependent Efficacy In Vivo


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ABSTRACT

In this study, we analyzed the antilipolytic effects of six \(N^6\)-cyclopentyladenosine analogs in rats and developed a mechanistic pharmacokinetic-pharmacodynamic model to quantify and predict the tissue-selective action of adenosine A₁ receptor agonists in vivo. Freely moving rats received an i.v. infusion of vehicle or compound over 15 min. Arterial blood samples were taken at regular time intervals for the determination of concentrations of drugs using HPLC analysis and of nonesterified fatty acids (NEFAs). All \(N^6\)-cyclopentyladenosine analogs that were investigated produced a significant decrease in the NEFA plasma concentration after i.v. infusion. The pharmacokinetic behavior of each ligand was described by a standard two-compartment model. The pharmacokinetic parameter estimates were then used to simultaneously fit the individual \((n = 6–8)\) time-NEFA concentration profiles for each agonist to a physiological indirect response model in combination with the Hill equation to obtain estimates of the NEFA elimination rate constant \((k_e)\) and upper asymptote (fractional inhibition), midpoint location, and midpoint slope parameter \((\alpha, \text{pEC}_{50}, \text{and} n_H)\), respectively, of the concentration-effect relationship. Subsequently, the data were analyzed with the operational model of agonism to obtain estimates of in vivo affinity and efficacy. It was estimated that the in vivo density and/or coupling of adenosine A₁ receptors mediating antilipolytic effects is \(\sim 38\) times higher compared with the receptors mediating bradycardia. The model predicts that it is possible to design ligands that produce significant inhibition of lipolysis and are completely devoid of cardiovascular effects in vivo.

Adenosine exerts its physiological effects via at least four receptor subtypes: A₁, A₂A, A₂B, and A₃ (Fredholm et al., 1998; Ralevic and Burnstock, 1998). It has been suggested that agonists for adenosine A₁ receptors on adipocytes may be used as antilipolytic drugs in the treatment of noninsulin-dependent diabetes mellitus (Foley et al., 1997; Donnelly and Qu, 1998). To date, however, the pronounced cardiodepressant effects mediated by adenosine A₁ receptors in the heart (see Ralevic and Burnstock, 1998) have been a major impediment for the development of selective adenosine A₁ agonists into potential antilipolytic drugs (Cox et al., 1997; Donnelly and Qu, 1998; Ishikawa et al., 1998). One of the possible strategies to overcome this problem is based on the idea that low-efficacy agonists may display greater tissue selectivity compared with high-efficacy ligands (see Kenakin, 1993; IJzerman et al., 1996). In the search for ligands with reduced intrinsic efficacy, we have identified deoxyribose and 8-alkylamino analogs of \(N^6\)-cyclopentyladenosine (CPA; Van der Wenden et al., 1995; Roelen et al., 1996) that behave as partial agonists for the adenosine A₁ receptor-mediated effect on heart rate in rats (Mathôt et al., 1995; IJzerman et al., 1996; Van der Graaf et al., 1997; Van Schaick et al., 1997). Very recently, we have shown that despite their limited cardiovascular action, 8-alkylamino CPA analogs still produce near-maximal antilipolytic effects in rats, suggesting that reducing intrinsic efficacy may indeed be a feasible strategy to enhance in vivo tissue selectivity of adenosine A₁ receptor agonists (Van Schaick et al., 1998). The aims of the present study were to obtain “proof of concept” for this approach by studying the antilipolytic effects of deoxyribose CPA analogs and to develop a model that can be used for the optimization of the design of tissue-selective adenosine A₁ re-
receptor agonists. The commonly used, empirical Hill equation has only limited applicability as a model to predict tissue-selective expression of agonism because intrinsic activity, potency, and steepness of the concentration-effect relationship are dependent not only on drug-specific properties (i.e., affinity for the receptor and intrinsic efficacy) but also on characteristics of the biological system (see Van der Graaf and Danhof, 1997a).

Therefore, to be able to predict the intrinsic activity and potency of a ligand for a particular pharmacological effect, a model is required that explicitly separates drug- and system-specific properties. It has been demonstrated that the operational model of agonism (Black and Leff, 1983) is a particularly useful tool to explain and predict differential expression of agonism across tissues in in vitro studies (Black and Leff, 1983; Leff and Giles, 1992; Black, 1996; Taberno et al., 1996; Van der Graaf et al., 1996; Wilson et al., 1996; Vivas et al., 1997; Shankley et al., 1998). Recently, we have shown that the operational model of agonism, in combination with an integrated pharmacokinetic-pharmacodynamic approach, can provide estimates of in vivo affinity and efficacy of CPA analogs for the heart rate effect in rats that are highly consistent with in vitro data obtained in radioligand-binding studies of adenosine A1 receptors (Van der Graaf et al., 1997). In the present study, we applied the operational model of agonism in combination with a physiological indirect response model (Daynega et al., 1993; Jusko and Ko, 1994) to analyze the antilipolytic effects of 8-alkylamino and deoxyribose CPA analogs in vivo. The analysis of the 8-alkylamino CPA analogs is based on original experimental data that have been published recently in another study (Van Schaick et al., 1998). The outcomes of our new mechanism-based pharmacokinetic-pharmacodynamic modeling approach demonstrate that the antilipolytic and bradycardiac effects of CPA analogs are indeed consistent with expectations for the involvement of a homogeneous adenosine A1 receptor population in vivo and provide a measure for the difference in functional adenosine A1 receptor expression/coupling between adipose and cardiac tissues. Furthermore, it is shown that the degree of separation between adenosine A1 receptor-mediated antilipolytic and bradycardiac effects in vivo can be predicted accurately on the basis of in vitro radioligand-binding data.

**Materials and Methods**

**In Vivo Pharmacological Experiments.** Details of the methods of the pharmacokinetic-pharmacodynamic experiments have been published previously (Van Schaick et al., 1998). Briefly, 2 days before experimentation, the abdominal aortas of male Wistar rats (200–250 g) was cannulated by an approach through the left and right femoral arteries for the measurement of arterial blood pressure and the collection of serial blood samples, respectively, and the right jugular vein was cannulated for administration of drugs. Animals were fasted for 24 h before experimentation, with free access to water. Conscious, freely moving rats received an i.v. infusion of vehicle (765 µl of 20% dimethyl sulfoxide (DMSO) in 0.9% saline) or compound over 15 min using a Braun (Melsungen, Germany) syringe pump. Continuous hemodynamic recordings were started 30 min before the start of the infusion and were continued for at least 5 h. Serial arterial blood samples (~15) were taken at regular time intervals for the determination of concentration of drugs. The samples (20–200 µl) were hemolyzed immediately and stored at −20°C until HPLC analysis based on the methods described in detail by Mathôt et al. (1995) and Van Schaick et al. (1997a, 1998). For the determination of plasma concentrations of nonesterified fatty acids (NEFAs), 24 blood samples of 50 µl each were taken over a period of 4 h. The total volume of blood taken from each rat never exceeded 2 ml (~10% of the total blood volume). Previously, we have shown that the experimental procedure itself has no significant effect on NEFA concentrations and heart rate (Van Schaick et al., 1997b, 1998). To each blood sample, 50 µl of ice-cold EDTA/saline solution was added, and after centrifugation, plasma was stored at −20°C until analysis. Plasma NEFA concentration was determined using the NEFA C-kit (Wako Chemicals GmbH, Neuss, Germany) with modifications described by Van Schaick et al. (1997b).

**Drugs.** The 8-alkylamino CPA analogs 8-(methylamino)-CPA (8MCPA), 8-(ethylenamino)-CPA (8ECAPA), and 8-(butylamino)-CPA (8BCPA), and the deoxyribose CPA analogs 2’,3’-deoxy-CPA (2’3’DCPA) and 3’-deoxy-CPA (3’DCPA) were synthesized at the Division of Medicinal Chemistry of the Leiden/Amsterdam Center for Drug Research as described previously (Van der Wenden et al., 1995; Roelen et al., 1996). 5’-deoxy-CPA (5’DCPA) was a gift from Parke Davis (Ann Arbor, MI). All drugs were dissolved in 20% DMSO in 0.9% saline and administered in a volume of 765 µl.

**Data Analysis.** Pharmacokinetic analysis was performed by fitting the blood concentration-time profiles to a standard two-compartment model (see Rowland and Tozer, 1995) by use of the ADVAN6 module within the nonlinear mixed-effect modeling software package NONMEM (see below). The estimates of the pharmacokinetic model parameters $k_{10}$ (rate constant of elimination), $k_{21}$ (rate constant for transfer from central to peripheral compartment), $k_{31}$ (rate constant for transfer from peripheral to central compartment), and $V_C$ (volume of central compartment) were then used to calculate individual agonist blood concentrations at the times of the NEFA measurements. These data were used to quantify the relationship between agonist blood concentration and time course of the antilipolytic effect. For this purpose, the data for each individual rat were fitted simultaneously to the physiological pharmacokinetic-pharmacodynamic model that was proposed and validated recently by Van Schaick et al. (1997b,c, 1998). In this model, which is based on original work by Jusko and coworkers (Daynega et al., 1993; Jusko and Ko, 1994), the rate of change of concentration of NEFAs in blood over time is described as:

$$\frac{d[\text{NEFAs}]}{dt} = k_s(1 - f([A])) - k_r[\text{NEFAs}]$$

(1)

where $k_s$ and $k_r$ are the zero order rate constant for synthesis and the first order rate constant for elimination of NEFAs, respectively. The effect of adenosine A1 receptor agonists (A) on the synthesis of NEFAs is mediated via a function f in the model, which describes the relationship between [A] and fractional inhibition of $k_s$. Initially, $f([A])$ was formulated as the empirical Hill equation, as described previously (Van Schaick et al., 1997b,c, 1998):

$$f([A]) = \frac{\alpha [A]^{n_E}}{EC_{50}^{n_E} + [A]^{n_E}}$$

(2)

where $\alpha$, $EC_{50}$, and $n_E$ are the upper asymptote, midpoint location, and midpoint slope parameters of the agonist concentration-effect relationship. Previously, we demonstrated that this model provides parameter estimates that are independent of dose and rate of infusion (Van Schaick et al., 1997b).

Subsequently, the Hill equation was replaced by the operational model of agonism (Black and Leff, 1983):

$$f([A]) = \frac{E_m \cdot \tau^*[A]^n}{(K_A + [A]^n)^r + \tau^*[A]^n}$$

(3)

where $E_m$ is the maximum effect achievable in the system, $K_A$ is the agonist dissociation equilibrium constant, $n$ is the slope index for the occupancy-effect relation, and $\tau$ is the efficacy parameter, which is defined by the ratio of total receptor concentration and the concentration of agonist-receptor complex required to produce half-maxi-
mal effect. The Hill equation parameters $\alpha$ and $EC_{50}$ can be expressed in terms of the operational model of agonism as follows (Black and Leff, 1983):

$$
\alpha = \frac{E_m \cdot \tau^n}{1 + \tau^n} 
$$

$$
EC_{50} = \frac{K_A}{(2 + \tau^{(21/3)})^{1/3} - 1} 
$$

(4)

(5)

Inspection of eq. 5 shows that $EC_{50}/K_A \rightarrow 1/(2^{21/3} - 1)$ when $\tau \rightarrow 0$ (Fig. 1). Furthermore, with high-efficiency values, eq. 5 approximates to a simple linear relationship, $EC_{50}/K_A = 1/\tau$, regardless of the value of $n$ (Fig. 1).

Leff et al. (1990) have shown that the operational model can be used to obtain estimates of affinity and efficacy of a partial agonist by comparison with a full agonist. This “comparative method” (originally proposed by Barlow et al., 1967; Leff et al., 1990) is based on the idea that per definition, the intrinsic activity of a full agonist is identical to the maximum system response. Therefore, when $E_m$ is constrained to the estimate of the Hill equation parameter, $\alpha$, for a full agonist, $K_A$ and $\tau$ for a partial agonist can be estimated by directly fitting the concentration-effect data to the operational model of agonism. However, Van der Graaf and Danhof (1997b) recently demonstrated that ignoring interindividual variation in $E_m$ may result in erroneous estimates of affinity and efficacy. Therefore, the data were fitted to the pharmacodynamic models using nonlinear mixed-effect modeling with the NONMEM software package (Beal and Sheiner, 1992; see also Schoemaker and Cohen, 1996) according to the method described recently, which takes into account interindividual variability in the model parameters (see Van der Graaf et al., 1997, for details). Briefly, with this approach, no individual parameters are estimated for each concentration-effect relationship curve. Instead, the model parameters for each individual animal are assumed to originate from a common distribution, and only the mean and interindividual variability are estimated. This means that regardless of the number of individual concentration-effect curves in a data set, only six and eight estimates (i.e., the mean parameters and associated interindividual variabilities) are obtained for the Hill equation and operational model of agonism, respectively (see Van der Graaf et al., 1997, for details).

$EC_{50}$, $K_A$, and $\tau$ were estimated as $pEC_{50}$ ($-\log EC_{50}$), $pK_A$ ($-\log K_A$), and $\log \tau$, respectively, because these parameters are assumed to be log-normally distributed (Leff et al., 1990; Van der Graaf et al., 1997). All fitting procedures were performed by use of the ADVAN6 module within the software package NONMEM (NONMEM project group, University of California, San Francisco). An IBM-compatible personal computer (Pentium 133 MHz) running under Windows 95 and Visual-NM 2.2.2 (RDPP, Montpellier, France) was used with the Microsoft FORTRAN PowerStation 4.0 compiler and NONMEM version IV, level 2.0 (double precision). Parameters and associated S.E. values were estimated using the first order method, and additive intraindividual and multiplicative interindividual residual error models were assumed (for details, see Schoemaker and Cohen, 1996; Van der Graaf et al., 1997). Estimates of the interindividual variability were expressed as coefficient of variation (CV). Interindividual variability of $\log \tau$ was assumed to be the same for different ligands because in the model, it depends on differences in receptor density and coupling between animals, which is independent of the agonist used. Interindividual variability of $K_A$ was assumed to be insignificant because receptor affinity is generally considered to be constant across animals from the same strain. Individual parameter estimates for each subject were calculated using the first order Bayesian estimation method implemented in the NONMEM software (see Schoemaker and Cohen, 1996). (The NONMEM syntax for the analysis described in this report is available on request.)

**Results**

**Descriptive Pharmacokinetic-Pharmacodynamic Modeling.** All CPA analogs that were investigated produced a significant decrease in the NEFA plasma concentration after i.v. infusion. NEFA concentration started to decrease shortly after the start of the administration and reached a minimum 30 to 60 min after the infusion was stopped (Fig. 2). The pharmacokinetic behavior of each adenosine A1 analog could be described adequately by the two-compartment model (Table 1). From these population fits, estimates of clearance (Cl) and volume of distribution at steady state ($V_{SS}$) were calculated (Cl = 35, 48, 83, 44, 46, and 40 mg/min/kg, and $V_{SS}$ = 1232, 876, 1039, 974, 830, and 1075 ml/kg for 2dCPA, 3dCPA, 5dCPA, 8MCPA, SECPA, and 8BCPA, respectively), which were practically identical with values obtained previously by fitting individual pharmacokinetic profiles (Cl = 33, 58, 55, 44, 48, and 39 ml/min/kg, and $V_{SS}$ = 1050, 660, 740, 970, 840, and 1050 ml/kg for 2dCPA, 3dCPA, 5dCPA, 8MCPA, SECPA, and 8BCPA, respectively; Mathôt et al., 1995; Van Schaick et al., 1998). The pharmacokinetic parameter estimates were then used to simultaneously fit the individual $(n = 6–8)$ time-NEFA concentration profiles for each agonist to the physiological indirect response model (eq. 1) in combination with the Hill equation (eq. 2) to obtain estimates of the NEFA elimination rate constant ($k_e$) and upper asymptote, midpoint location, and midpoint slope parameters ($\alpha$, $pEC_{50}$, and $n_1$, respectively) of the concentration-effect relationship as described in Materials and Methods (Table 2; Fig. 3). The model converged in all cases, and the estimates of the rate constant for elimination of NEFA obtained for the different agonists were practically identical ($k_e$ = 0.05–0.08 min$^{-1}$, Table 2) and similar to our previously published estimates ($k_e$ = 0.07–0.08, Van Schaick et al., 1998), consistent with the results of previous studies.
with the assumption in the model that this parameter is ligand-independent. In the subsequent analysis with the operational model of agonism, \( k_i \) values were constrained to the Bayes’ estimates for each individual rat to eliminate a possible effect of the small between-animal variability.

**Mechanism-Based Pharmacokinetic-Pharmacodynamic Modeling.** Individual time-NEFA concentration profiles for all agonists were fitted simultaneously to the physiological indirect response model in combination with the operational model of agonism (eq. 3). The values of \( E_m \) and the associated variance describing the interindividual variability were constrained to the estimates of \( \sigma_i \) obtained for each agonist that displayed the highest intrinsic activity, \( k_i \), and error bars represent mean NEFA concentrations and S.E., respectively. The dashed lines represent the average population pharmacokinetic profile for each compound estimated by simultaneous fitting individual time-concentration data to a two-compartment model with NONMEM as described in the text. Estimates of interindividual variability are shown as CV in parentheses.

**Fig. 2.** Time profiles of concentrations of NEFAs in plasma (solid line, left ordinate) and drug in blood (dashed line, right ordinate) after i.v. administration of 5.2 mg/kg 2’dCPA (n = 7; A), 0.25 mg/kg 3’dCPA (n = 7; B), 0.16 mg/kg 5’dCPA (n = 8; C), 4.0 mg/kg 8MCPA (n = 7; D), 12.0 mg/kg 8ECPA (n = 8; E), and 20.0 mg/kg 8BCPA (n = 6; F) for 15 min in rats. ● and error bars represent mean NEFA concentrations and S.E., respectively. The values of \( k_{12} \) (rate constant for transfer from central to peripheral compartment), \( k_{21} \) (rate constant for transfer from peripheral to central compartment), and \( V_C \) (volume of central compartment) were obtained by fitting the time-concentration data to a two-compartment model with NONMEM as described in the text. Estimates of interindividual variability are shown as CV in parentheses.

**TABLE 1**

Pharmacokinetic parameter estimates for CPA analogs obtained after intravenous infusion for 15 min in conscious rats. Parameter estimates (mean ± S.E.; n = 6–9) of \( k_{10} \) (rate constant of elimination), \( k_{12} \) (rate constant for transfer from central to peripheral compartment), \( k_{21} \) (rate constant for transfer from peripheral to central compartment), and \( V_C \) (volume of central compartment) were obtained by fitting the time-concentration data to a two-compartment model with NONMEM as described in the text. Estimates of interindividual variability are shown as CV in parentheses.

<table>
<thead>
<tr>
<th>Compound</th>
<th>( k_{10} ) min(^{-1} )</th>
<th>( k_{12} ) min(^{-1} )</th>
<th>( k_{21} ) min(^{-1} )</th>
<th>( V_C ) mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>2’dCPA</td>
<td>0.18 ± 0.00 0.098 ± 0.009</td>
<td>0.018 ± 0.006</td>
<td>184 ± 19</td>
<td>(31%) (144%) (18%) (13%)</td>
</tr>
<tr>
<td>3’dCPA</td>
<td>0.39 ± 0.13 0.58 ± 0.26</td>
<td>0.14 ± 0.01</td>
<td>38 ± 12</td>
<td>(21%) (0%) (72%) (0%)</td>
</tr>
<tr>
<td>5’dCPA</td>
<td>0.39 ± 0.06 0.28 ± 0.05</td>
<td>0.071 ± 0.012</td>
<td>64 ± 9</td>
<td>(10%) (0%) (23%) (0%)</td>
</tr>
<tr>
<td>8MCPA</td>
<td>0.14 ± 0.01 0.12 ± 0.04</td>
<td>0.064 ± 0.009</td>
<td>108 ± 15</td>
<td>(33%) (12%) (22%) (0%)</td>
</tr>
<tr>
<td>8ECPA</td>
<td>0.12 ± 0.01 0.030 ± 0.008</td>
<td>0.039 ± 0.006</td>
<td>119 ± 15</td>
<td>(17%) (50%) (19%) (18%)</td>
</tr>
<tr>
<td>8BCPA</td>
<td>0.10 ± 0.07 0.045 ± 0.008</td>
<td>0.027 ± 0.004</td>
<td>129 ± 10</td>
<td>(7%) (13%) (0%) (1%)</td>
</tr>
</tbody>
</table>

**Fig. 3.** Average concentration-effect relationships for effects on NEFAs (solid lines) and heart rate (dotted lines) of 2’dCPA (A), 3’dCPA (B), 5’dCPA (C), 8MCPA (D), 8ECPA (E), and 8BCPA (F) in rats. The curves represent the NONMEM population fits obtained with the Hill equation (see Table 2 and Van der Graaf et al., 1997 for the parameter estimates) for the NEFA and heart rate effects, respectively. Effect is expressed as the percentage of the response to 5’dCPA.

**TABLE 2**

Parameter estimates for the antilipolytic effects of CPA analogs in rats obtained by pharmacokinetic-pharmacodynamic analysis with the indirect physiological response model in combination with the Hill equation. Parameter estimates (mean ± S.E.; n = 6–9) of \( k_i \) (first order rate constant for elimination of NEFAs), \( \alpha \) (intrinsic activity, expressed as fractional inhibition), \( n_{H} \) (Hill slope parameter), and pEC\(_{50}\) (potency) were obtained by fitting the time-concentration data for each agonist to eqs. 1 and 2 with NONMEM as described in the text. Estimates of interindividual variability are shown as CV in parentheses.

<table>
<thead>
<tr>
<th>Compound</th>
<th>( k_i )</th>
<th>( \alpha )</th>
<th>pEC(_{50})</th>
<th>( n_{H} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>2’dCPA</td>
<td>0.07 ± 0.01 0.62 ± 0.02</td>
<td>6.81 ± 0.08</td>
<td>1.49 ± 0.18</td>
<td>(7%) (0%) (100%) (19%)</td>
</tr>
<tr>
<td>3’dCPA</td>
<td>0.06 ± 0.01 0.61 ± 0.09</td>
<td>7.67 ± 0.18</td>
<td>1.21 ± 0.17</td>
<td>(69%) (20%) (9%) (89%)</td>
</tr>
<tr>
<td>5’dCPA</td>
<td>0.08 ± 0.01 0.80 ± 0.06</td>
<td>9.18 ± 0.25</td>
<td>0.64 ± 0.15</td>
<td>(48%) (9%) (10%) (82%)</td>
</tr>
<tr>
<td>8MCPA</td>
<td>0.05 ± 0.01 0.68 ± 0.12</td>
<td>7.13 ± 0.47</td>
<td>1.01 ± 0.23</td>
<td>(32%) (0%) (4%) (100%)</td>
</tr>
<tr>
<td>8ECPA</td>
<td>0.07 ± 0.02 0.71 ± 0.05</td>
<td>6.80 ± 0.21</td>
<td>0.73 ± 0.29</td>
<td>(41%) (0%) (7%) (39%)</td>
</tr>
<tr>
<td>8BCPA</td>
<td>0.06 ± 0.01 0.66 ± 0.05</td>
<td>6.00 ± 0.09</td>
<td>1.85 ± 0.21</td>
<td>(48%) (0%) (5%) (120%)</td>
</tr>
</tbody>
</table>
agonist (Table 2). The model converged and estimates of in vivo affinity (pK_A) and efficacy (log τ) for each agonist were obtained (Table 3). These estimates were highly correlated with those obtained in the previous study (Van der Graaf et al., 1997) for the effect on heart rate (r = 0.68 and 0.96 for pK_A and log τ, respectively), and a formal comparison was made using the least-squares procedure explained in Materials and Methods (Fig. 4). This analysis showed that the relationship between the pK_A estimates did not deviate significantly from a straight line (F_{3,50} = 1.00, P > .1) with unit slope (F_{1,50} = 0.11, P > .5). However, the intercept was significantly less than zero (F_{1,50} = 8.35, P < .01), indicating a constant difference in the pK_A estimates for the NEFA and heart rate effects (Fig. 4A). The comparison of log τ values also indicated a constant difference between the two systems [i.e., there were no significant deviations from the straight line (F_{1,50} = 0.56, P > .5) and unit slope models (F_{1,50} = 1.96, P > .1) but the intercept was significantly greater than zero (F_{1,50} = 203.4, P < .0001, Fig. 4B)]. Note that the log τ value for 5’dCPA was estimated by constraining the pK_A value to the pK_E estimate obtained in binding studies because the “comparative method” cannot yield independent estimates of affinity and efficacy for the reference agonist.

As explained in Materials and Methods, in the case of a high-efficacy system, the relationship between τ and EC_{50}/K_A approximates to a simple linear relationship such that a double-logarithmic plot of EC_{50}/K_A against τ yields a straight line with a slope of −1 (Fig. 1). Figure 1 shows that the outcomes of the present analysis of the NEFA effect were highly consistent with this predicted linear relationship (r = −0.99, slope = −0.95).

**Discussion**

Recently, we have shown that the operational model of agonism can be used in pharmacokinetic-pharmacodynamic analysis of in vivo drug effects, and we have demonstrated that it is possible to estimate agonist affinity and efficacy at cardiac adenosine A_1 receptors that are highly consistent with results from in vitro radioligand-binding studies (Van der Graaf et al., 1997). In the present study, we extended this mechanism-based approach by combining the operational model of agonism with an indirect response model to analyze antilipolytic effects to predict tissue-dependent expression of efficacy.

**TABLE 3**

Parameter estimates for the antilipolytic effects of CPA analogs in rats obtained by pharmacokinetic-pharmacodynamic analysis with the indirect physiological response model in combination with the operational model of agonism

<table>
<thead>
<tr>
<th>Parameter estimate (mean ± S.E.; n = 6–8)</th>
<th>pK_A</th>
<th>log τ</th>
</tr>
</thead>
<tbody>
<tr>
<td>2’dCPA</td>
<td>5.73 ± 0.11</td>
<td>1.09 ± 0.09</td>
</tr>
<tr>
<td>3’dCPA</td>
<td>6.07 ± 0.18</td>
<td>1.30 ± 0.01</td>
</tr>
<tr>
<td>5’dCPA</td>
<td>5.68 ± 0.22</td>
<td>1.35 ± 0.20</td>
</tr>
<tr>
<td>8MCPPA</td>
<td>5.27 ± 0.13</td>
<td>1.40 ± 0.25</td>
</tr>
<tr>
<td>8CEPPA</td>
<td>5.18 ± 0.10</td>
<td>0.94 ± 0.07</td>
</tr>
</tbody>
</table>

* Interindividual variability (CV): 39.4%.  
* Estimated with pK_A constrained to pK_E value (6.35) for adenosine A_1 receptor obtained in binding studies (Van der Wenden et al., 1995).

With the “comparative method” (Barlow et al., 1967; Leff et al., 1990), a full agonist is required to provide an estimate of the maximal system response. 5’dCPA produced the highest response and was assumed to act as a full agonist. The validity of this assumption is supported by the almost 1000-fold difference between EC_{50} and apparent K_A for 5’dCPA (Fig. 1), indicative of the presence of a large receptor reserve. Furthermore, in the previous study of heart rate effects, 5’dCPA was also found to produce the highest response, and of all the adenosine ligands tested, it displays the highest in vitro “GTP shift” (see below).

The parameters E_m and n are ligand-independent and should in principle be constant for a particular system. Recently, however, we have shown that interindividual variability in E_m can produce significant bias on affinity and efficacy estimates (Van der Graaf and Danhof, 1997b), and therefore we used a population approach described recently (Van der Graaf et al., 1997) to allow for differences between animals. Initially, we attempted to use the same approach to account for interindividual variability in n, but the steepness of individual concentration-effect curves varied considerably between experiments (Table 2), and it was not possible to fit all data simultaneously with a single transducer slope. Therefore, because in the case of high-efficacy agonists the Hill slope (n_H) is indistinguishable from n (Black and Leff, 1983), the estimates of n_H obtained for each agonist were assumed to represent different transducer slopes. At present, we have no explanation for the slope variability. Interestingly, however, the average value of n_H for the different agonists (1.16) was almost identical with the value of the transducer slope (1.18) estimated for the effect on heart rate (Van der Graaf et al., 1997).

In contrast to the parameters E_m and n, the affinity con-
stant (pK_A) is assumed to be independent of the response system. The comparison of the pK_A values associated with the antilipolytic and bradycardic effects indicated a strong correlation but also a significant deviation from the line of identity of ~0.4 log unit (Fig. 4A). Although the reason for this observation requires further investigation, there are at least two possible explanations. First, the difference between pK_A estimates could indeed reflect different affinities, which would imply the involvement of more than one receptor type. However, the ligands used in this study have been characterized as selective adenosine A_1 receptor agonists (Van der Wenden et al., 1995; Roelen et al., 1996), and it is generally believed that a homogeneous population of adenosine A_1 receptors mediates inhibition of cardiac function and lipolysis (see Ralevic and Burnstock, 1998). A second, in our opinion more likely, explanation would be that the pK_A differences are due to a different relationship between measured and active drug concentrations for the two effects. For example, du Souich et al. (1993) pointed out that the relationship between drug binding to plasma proteins and pharmacological response is complex. In our analysis of the effect on heart rate, it was found that pK_A estimates based on whole blood concentrations were virtually identical with pK_V values for the adenosine A_1 receptor in rat brain homogenates, whereas pK_A estimates based on free plasma concentrations were ~0.5 log unit higher than the pK_V values. Therefore, we investigated whether the discrepancy between the pK_A values could be accounted for, at least in a quantitative manner, by expressing the estimates for the NEFA effect on the basis of free drug concentration in plasma rather than on total blood concentration. Figure 5 shows that after this correction, the pK_A estimates became indistinguishable; that is, the best-line fit did not deviate significantly from the line of identity (F_1,50 = 0.43, P > .5). However, we have no explanation for the possibility that plasma protein binding would affect only the interaction with the adenosine A_1 receptors on adipocytes and not with those on the heart, and the involvement of other processes (e.g., cellular uptake and enzymatic degradation) that reduce the concentration of drug available to act at the receptors on adipocytes cannot be excluded. The discrepancy in the pK_A estimates was similar for all six ligands and therefore appears not to be related to binding to blood cells because the plasma/blood ratio (P/B) of the 8-alkylamino analogs is significantly greater than unity (P/B = 2.0, 1.7, and 1.2 for 8MCPA, 8ECPA, and 8BCPA, respectively; Van Schaick et al., 1997a), whereas the deoxyribose analogs display ratios below unity (P/B = 0.64, 0.54, and 0.55 for 2′dCPA, 3′dCPA, and 5′dCPA, respectively; Mathôt et al., 1995).

The efficacy parameter τ is given by the ratio of the total receptor concentration and the midpoint location of the transducer function, which relates agonist-occupied receptor concentration to pharmacological effect. Therefore, changes in the cross-tissue expression of efficacy can be due to differences in receptor concentration and/or the efficiency of coupling, which includes both tissue- and compound-dependent components. However, the ratio of τ values for one agonist in two tissues is expected to be constant and independent of the compound used, and a plot of τ values estimated for two responses (τ_1 and τ_2) is expected to yield the straight line τ_2 = τ_1 * r, where r is the τ ratio. Accordingly, a plot of log τ against log τ_1 yields a straight line with unit slope and intercept of log r. The analysis of the log τ data shown in Fig. 4B is fully consistent with these expectations for the involvement of a single receptor and indicates that the in vivo coupling and density of adenosine A_1 receptors mediating antilipolytic effects is ~38 times higher compared with the receptors mediating bradycardia. Although the contributions of receptor density and coupling to the increased expression of efficacy cannot be distinguished on the basis of the present data, it is of interest to note that radioligand-binding studies of rat tissues have demonstrated a ~25-fold higher density of adenosine A_1 receptors in adipocytes compared with the heart (Linden, 1984; Lohse et al., 1987; Martens et al., 1987). Similar, although slightly higher, differences in adenosine A_1 receptor density have also been found in human tissues (Böhm et al., 1989; Green et al., 1989).

One of the advantages of the approach used in this study is that it allows for integration of in vitro and in vivo data. Previously, we have shown that in vivo log τ estimates for the heart rate effect correlate significantly with GTP shifts (the ratio between apparent affinity in the presence and absence of GTP) obtained in radioligand-binding studies and that a direct prediction of intrinsic activity in vivo can be made on the basis of in vitro data (Van der Graaf et al., 1997). Figure 6 shows that the log τ estimates obtained in the present study were also significantly correlated with GTP shift values (r = 0.88, P < .05). Without prejudice to mechanism, by combining the linear relationship between log τ and GTP shift (log τ = 0.44 × GTP shift – 0.21) with eq. 4, a direct relation can be made between intrinsic activity in vivo and the in vitro data as described previously (Van der Graaf et al., 1997). Figure 7 shows the predicted differences between the bradycardic and antilipolytic effects for the adenosine A_1 receptor agonists tested. This analysis indicates that even ligands with GTP shift values close to unity (i.e., ligands that appear to behave as antagonists in vitro) may still produce significant inhibition of lipolysis in vivo, whereas they are expected to be devoid of cardiodepressant side effects.

In conclusion, we extended our mechanism-based pharmacokinetic-pharmacodynamic analysis of adenosine A_1 receptor-mediated effects with a component that can predict tissue selectivity in vivo on the basis of in vitro data. Our prediction that some ligands that appear to behave as “silent” antagonists in vitro may act as agonists in vivo shows that residual
efficacy that is not easily detected in in vitro systems may be amplified to significant physiological effects in vivo. This underscores the danger of missing key pharmacological properties by relying too much on simplified in vitro screening assays and illustrates the potential of preclinical, mechanism-based pharmacokinetic-pharmacodynamic modeling. In the light of the lack of success of programs that have aimed to develop adenosine A1 receptor antagonists into drugs without cardiodepressant side effects, it might be of interest to re-evaluate the in vivo pharmacological properties of ligands that have been classified as antagonists only on the basis of in vitro binding assays.

References


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