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Pharmacokinetic/pharmacodynamic modelling of the anti-hyperalgesic and anti-nociceptive effect of adenosine A_1 receptor partial agonists in neuropathic pain

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Abstract

The objective of this investigation was to characterise the pharmacokinetic–pharmacodynamic correlation of adenosine A_1 receptor partial agonists in the chronic constriction injury model of neuropathic pain. Following intravenous administration of 8-methylamino-N^6-cyclopentyl-adenosine (MCPA; 10 mg/kg) and 2′deoxyribose-N^6-cyclopentyl-adenosine (2′dCPA; 20 mg/kg), the time course of the effect on the mechanical paw pressure threshold was determined in conjunction with plasma concentrations. Population pharmacokinetic/pharmacodynamic analysis was applied to derive individual concentration–effect relationships.

A composite model consisting of an E_{max} model for the anti-hyperalgesic effect in combination with a linear model for the anti-nociceptive effect accurately described the concentration–effect relationship. For both compounds, a full anti-hyperalgesic effect was observed. The values of the EC_{50} for the anti-hyperalgesic effect were (mean ± S.D.): 3170 ± 1460 and 2660 ± 1200 ng/ml for MCPA and 2′dCPA versus 178 ± 51 ng/ml for the reference full agonist 5′deoxyribose-N^6-cyclopentyl-adenosine (5′dCPA). The values of the slope for the anti-nociceptive effect were 1.9 ± 0.30 and 1.2 ± 0.20 g.μl/ng, respectively, versus 55 ± 8 g μl/ng for 5′dCPA.

Adenosine A_1 receptor partial agonists behave as full agonists with regard to the anti-hyperalgesic effect in neuropathic pain, but the anti-nociceptive effect is diminished.

Keywords: Chronic constriction injury model; 2′deoxyribose-N^6-cyclopentyl-adenosine; 8-methylamino-N^6-cyclopentyl-adenosine; Mechanical paw pressure threshold; Pharmacokinetics; Population pharmacokinetic/pharmacodynamic analysis

1. Introduction

In animal models of neuropathic pain, it has been demonstrated that A_1 receptor agonists display an anti-hyperalgesic effect in the sense that they reduce the hypersensitivity reaction to heat stimuli (Yamamoto and Yaksh, 1991), scratch behaviour (Sjölund et al., 1996), tactile stimulation (Cui et al., 1998, Lavand'homme and Eisenach, 1999), mechanical stimulation with von Frey hair monofilaments and cold and mechanical stimuli (Sjölund et al., 1998; Von Heijne et al., 1998). Several clinical studies have confirmed the anti-hyperalgesic effects of adenosine and related compounds in patients with neuropathic pain. Specifically, it has been demonstrated that intrathecally or systemically administered adenosine reduces both spontaneous pain and tactile allodynia, while touch-evoked pain thresholds are increased (Belfrage et al., 1995, 1999; Sollevi et al., 1995; Sjölund et al., 2001). Furthermore, spinal administration of the A_1 selective receptor agonist R-N^6-phenylisopropyladenosine (R-PIA) has been shown to abolish allodynia in a patient with chronic neuropathic pain (Karlsten and Gordh, 1995).

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The clinical application of adenosine A_1 receptor agonists in the treatment of neuropathic pain is hampered however by side effects, resulting from a lack of selectivity of action. Due to the ubiquitous distribution of adenosine receptors throughout the body, selective A_1 receptor agonists display a wide array of side effects including bradycardia and hypotension (Mullane and Williams, 1990; Olsson, 1996). Recent research efforts of improving the selectivity of action of A_1 receptor agonists have focused on the design of partial agonists (Van der Wenden et al., 1995; Roelen et al., 1996). It has been shown that due to differences in receptor density and in the efficiency of receptor–effector coupling between tissues, low-efficacy A_1 receptor agonists can have an improved selectivity of action in vivo. Specifically in pharmacokinetic/pharmacodynamic modelling studies, it has been demonstrated that various low-efficacy analogues of N^6-cyclopentyladenosine (CPA) still display a full anti-lipolytic effect, but are devoid of cardiovascular side-effects (Van Schaick et al., 1998; Van der Graaf et al., 1999). An important question is whether these partial agonists still display a full anti-hyperalgesic effect in neuropathic pain. In this respect, it is important that adenosine A_1 receptor expression in various parts of the brain and spinal cord is indeed quite high (Fredholm et al., 2001).

In this report, we describe the integrated pharmacokinetic/pharmacodynamic modelling of the anti-hyperalgesic and anti-nociceptive effects of 8-methylamino-N^6-cyclopentyl adenosine (MCPA) and 2′deoxyribose-N^6-cyclopentyl adenosine (2′dCPA), two structurally distinct adenosine A_1 receptor partial agonists (Van der Wenden et al., 1995; Roelen et al., 1996), in the chronic constriction injury model of neuropathic pain. A specific objective was to make a distinction between the anti-hyperalgesic effect (i.e. the elevation of the paw withdrawal threshold from the reduced value in chronic constriction injury rats to the baseline value in control animals) and the anti-nociceptive effect (i.e. the elevation of the paw withdrawal threshold above the baseline value in control animals).

An important factor with regard to investigations in animal models of neuropathic pain is that only a limited number of observations per animal can be obtained. Recently, a population pharmacokinetic/pharmacodynamic model for the effects of adenosine A_1 receptor agonists in neuropathic pain has been proposed which allows description of the full concentration–effect relationship in individual rats on the basis of sparse data (Schaddelee et al., 2004a). In this model, the concentration–effect relationship of the full agonist 5′-deoxy-N^6-cyclopentyl-adenosine (5′dCPA) was analysed on the basis of a composite pharmacodynamic model consisting of an E_{max} model for the anti-hyperalgesic effect in combination with a linear model for the anti-nociceptive effect. It has been shown that on the basis of this model the anti-hyperalgesic and the anti-nociceptive effects can indeed be separately quantified (Schaddelee et al., 2004a).

In the present investigation, the aforementioned population pharmacokinetic/pharmacodynamic model approach was applied to analyse the effects of the partial agonists 2′-deoxy-N^6-cyclopentyl-adenosine (2′dCPA) and 8-methylamino-N^6-cyclopentyl-adenosine (MCPA). Specifically, the objective of this study was to determine (1) whether partial agonists still behave as full agonists with regard to the anti-hyperalgesic effect in neuropathic pain, as reflected in the same value of the E_{max}, (2) whether partial agonists display a different slope of the concentration–anti-nociceptive effect relationship.

2. Material and methods

2.1. Chemicals

2-Deoxy-N^6-cyclopentyladenosine (2′dCPA), C8-methylamino-N^6-cyclopentyladenosine (MCPA) and GR79236 were kindly provided by GlaxoSmithKline (Stevenage, United Kingdom). Methanol (High Performance Liquid Chromatography (HPLC) application), acetonitrile (HPLC gradient application) and water (HPLC application) were obtained from Fisher Chemicals (Loughborough, United Kingdom). All other chemicals were of analytical grade (Fisher Chemicals).

2.2. Animals

The institutional ethics committee approved the protocol of this investigation. Male random hooded rats (Rodent Breeding unit- Bioscience Support GlaxoSmithKline, United Kingdom), weighing between 200 and 225 g, were used in this study. The animals were housed in groups in plastic cages with a normal 12 h light–dark cycle, fed on laboratory chow (Special Diet Services, Maldon, Essex, United Kingdom) and tap water ad libitum.

2.3. Pharmacokinetic study

The full pharmacokinetic profiles of MCPA (10 mg/kg) and 2′dCPA (20 mg/kg) were investigated in separate groups of rats ($n=5$). The compounds were administered as an intravenous bolus injection through the tail vein. Blood samples (50 to 400 μl) were drawn at predefined time-points after administration of MCPA in each rat: 5, 15, 30, 45, 75, 105, 165 min. For 2′dCPA, blood samples were obtained at the time points: 5, 15, 30, 60, 90, 120, 180 min. The samples were directly centrifuged to plasma (5 min, 13.000 rpm) and were stored at −20 °C until analysis.

2.4. Surgical procedures

The surgical procedure for the induction of neuropathic pain was according to the method described by Bennett and Xie (1988). Briefly, rats were anaesthetised with isoflurane. The common sciatic nerve was exposed at the level of the middle of the thigh by blunt dissection through biceps femoris. Proximal to the sciatic's trifurcation, the nerve was freed from adhering tissue and 4 ligatures (4.0 chromic gut, Braun Surgical, Melsungen, Germany) were tied loosely around it with 1 mm spacing. The surgical

procedure for the sham-operated control rats was identical except that the sciatic nerve was not ligated.

2.5. Experimental protocol

To determine the effect of MCPA and 2′dCPA, mechanical hypersensitivity was measured on basis of the mechanical paw pressure withdrawal threshold using an algesymeter (Ugo Basile, Comerio, Italy). One week after surgery, the behavioural measurements started. The actual experiments were performed 3 weeks after surgery, when the mechanical hypersensitivity and allodynia had reached a stable minimum. MCPA (10 mg/kg) and 2′dCPA (20 mg/kg) were administered by an intravenous bolus injection through the tail vein. Each treatment group consisted of 14 CCI and 10 sham rats. In each rat, 3 serial bleeds through the tail vein were obtained and 5 behavioural effect measurements were conducted. Behavioural anti-hyperalgesic measurements were performed at predefined time-points following administration: MCPA at 5, 15, 45, 90, 150, 210 min and 2′dCPA at 5, 15, 60, 120, 180, 240 min. At 24 h pre- and post-dosage additional behavioural anti-hyperalgesic measurements were conducted for determination of the baseline values. Blood samples (volume ranging between 50 to 400 μl, depending on the concentration) were drawn 5 min after the behavioural measurements. For MCPA, random blood samples were obtained at three of the following times after administration: 0, 10, 20, 50, 95, 155, 215 min. For 2′dCPA, these sampling times were 0, 10, 20, 65, 125, 185, 245 min after administration. The blood samples were directly centrifuged to plasma (5 min, 13.000 rpm) and were stored at −20 °C until the analysis took place.

2.6. Drug analysis

The concentrations of MCPA and 2′dCPA in the plasma samples were determined by High Pressure Liquid Chromatography with tandem mass spectrometry detection (LC-MSMS). Calibration standards were prepared by addition of aqueous solutions of the compounds to control plasma. GR79236 (N-[(1S,trans)-2-hydroxycyclopentyl]adenosine); (20 μl, 1000 ng/ml) was used as internal standard. The samples were extracted with acetonitrile (3 to 4 volumes). After centrifugation (5 min, 13000 rpm), the supernatant was transferred into clean tubes and dried under nitrogen at 40 °C. The residues were dissolved in 100 μl of a mixture of water and methanol (95:5 v/v) and a volume of 30 μl was injected into the LC system. HPLC was performed on a Hewlett Packard 1100 instrument (Hewlett Packard, Waldbronn, Germany). Chromatography was performed on a C18 column (50 mm × 2.1 mm I.D.; 5 μM particle size) (Capital HPLC, Broxburn, United Kingdom) with gradient elution at a flow rate of 1.0 ml/min. The mobile phase consisted of 2 solvents: (A) 100% water+0.1% formic acid and (B) 100% acetonitrile+0.1% formic acid. The profile was 0–2 min 95% A; 1–2 min linear gradient to 90% B; 2–3 min 90% B; 3–3.1 min linear gradient to 95% A; 3.1–5 min 95% A. Mass spectrometry was performed on a PE-Sciex API2000 instrument (Perkin Elmer Sciex Instruments, Foster City, CA, USA) equipped with a turbo ion spray source for electrospray ionisation used in the positive mode. Detection by tandem mass spectrometry was based on precursor ion transitions to the strongest intensity. Instrumental conditions were optimised to yield best sensitivity. The limit of quantification for a 50 μl sample was 5 ng/ml for both MCPA and 2′dCPA. The within-day and between-day variation ranged from 9 to 12% and from 14 to 19%, respectively.

2.7. Data analysis

The pharmacokinetics and pharmacodynamics of MCPA and 2′dCPA were quantified on basis of a population pharmacokinetic/pharmacodynamic model as described previously (Schaddelee et al., 2004a). In this approach, the data from all individual rats were fitted simultaneously while explicitly taking into account both the inter-individual variability in the model parameters as well as intra-individual residual error (Schoemaker and Cohen, 1996). All fitting procedures were performed in the non-linear-mixed-effect-modelling software NONMEM (GloboMax, Hanover, MD, US). The model was fitted using subroutine ADVAN 7 in NONMEM, a general linear model that makes use of numerical approximations to the matrix exponential.

Briefly, the structure of the pharmacokinetic/pharmacodynamic model was as follows. The final pharmacokinetic model was set up as a one compartment model with linear elimination. Thereby, the plasma concentration versus time data were modelled according to the following mono-exponential equation, which was selected on basis of the Akaike information criterion (Akaike, 1974):

$$[A]_i = [A_0]_i \cdot e^{-\frac{CL_i}{V_i}t} \quad (1)$$

where $[A]_i$ is the plasma concentration of agonist i at time t, $[A_0]_i$ the initial plasma concentration of agonist i, CL_i the total plasma clearance and V_i the volume of distribution at steady state of agonist i. Inter-individual variability on the parameters was modelled according to an exponential equation:

$$\theta_i = \theta \cdot \exp(\eta_i) \quad (2)$$

where θ is the population mean parameter value, θ_i is the individual parameter value and $\exp(\eta_i)$ is a random term from a normal distribution with mean zero and variance ω^2. Only the inter-individual variation in clearance (CL) could be identified. The residual error was characterised by a proportional error model:

$$[A_m]_{ijk} = [A]_{ijk} \cdot (1 + \varepsilon_{ij}) \quad (3)$$

where $[A]_{ijk}$ is the kth plasma concentration of agonist i for the jth individual predicted by the model, A_m is the measured plasma concentration, ε_{ij} is a random noise term from a normal distribution with mean zero and variance σ^2. The size of σ^2 is a measure of the intra-individual residual error in the model. From the individual post hoc estimates values for clearance (CL) and volume of distribution (V), the elimination half-life ($t_{½}$) was calculated following standard procedures (Gibaldi and Perrier, 1989). In the subsequent pharmacokinetic/pharmacodynamic analysis, the plasma concentrations at the time-points of the pharmacodynamic measurements were estimated on basis of the population pharmacokinetic model for each individual rat.

2.7.1. Pharmacodynamics: mechanical hypersensitivity

No hysteresis was observed between plasma concentration and the effect on mechanical hypersensitivity. This justifies an approach in which the two are directly related to each other. A previously proposed population pharmacodynamic model (Schaddelee et al., 2004a) was applied to the analysis of the complex

concentration–effect relationships. Briefly, in this "two component" model, the effect on mechanical hypersensitivity is divided into two effects, the anti-hyperalgesic effect and the anti-nociceptive effect. The anti-hyperalgesic effect was defined as the increase in paw pressure threshold from the baseline value of a chronic constriction injury rat to a maximum of the level of the baseline value in the sham-operated rats. Therefore, per definition, the anti-hyperalgesic effect is observed only in the chronic constriction injury rats. The anti-hyperalgesic effect was described with an E_{max} model according to:

$$E_{au}(t) = E_{0,CCI} + \left(\frac{E_{max} \cdot [A]_i}{EC_{50i} + [A]_i}\right) \quad (4)$$

where E_{au} (t)=the anti-hyperalgesic effect at time t, $[A]$;=the plasma concentration of agonist i at time t, E_{max} the maximum anti-hyperalgesic effect, EC_{50} the concentration where 50% of the anti-hyperalgesic effect is reached. An important feature of this model is that allows differences in the intrinsic activity (E_{max}) of the anti-hyperalgesic effect to be detected. The concentration effect profile for the sham rats, where per definition only an anti-nociceptive effect could be observed, was described with a linear model:

$$E_{sham}(t) = E_{0,sham} + a_i \cdot [A]_i \quad (5)$$

where $E_{sham}(t)$ is the effect at time t in the sham rats, $[A]_i$ the plasma concentration of agonist i at time t, $E_{0,sham}$ the baseline value of the sham rats and a_i is the slope of the linear concentration–effect relationship for agonist i. The concentration effect profiles for the chronic constriction injury rats were

Table 1
Pharmacokinetic parameter estimates obtained intravenous administration of MCPA (10 mg/kg), 2′dCPA (20 mg/kg) and 5′dCPA (0.3 and 0.75 mg/kg)[a]

	n	$t_{½}$ (min)	Cl (ml·min^{-1})	V_{ss} (ml)
MCPA				
Mean of individual post hoc estimates	29	24.5±2.4	3.6±0.35	128±4.1
Population mean value	29	23.9±1.1	3.7±0.13	128±4.1
2′dCPA				
Mean of individual post hoc estimates	29	27.4±2.3	6.6±0.55	261±7.5
Population mean value	29	27.0±1.0	6.7±0.16	261±7.5
5′dCPA[a]				
Mean of individual post hoc estimates	58	21.3±2.9	8.5±1.2	260±15
Population mean value	58	22.7±1.8	7.9±0.4	260±15
Inter-subject variability	116	NA	15%	≪0.01%
Residual error (%)	116		27±9.6	

The values are presented as (1) the mean±S.E. of the post hoc Bayesian estimates and (2) the population mean value±S.E.
NA: not applicable.
[a] Based on data from Schaddelee et al. (2004a).

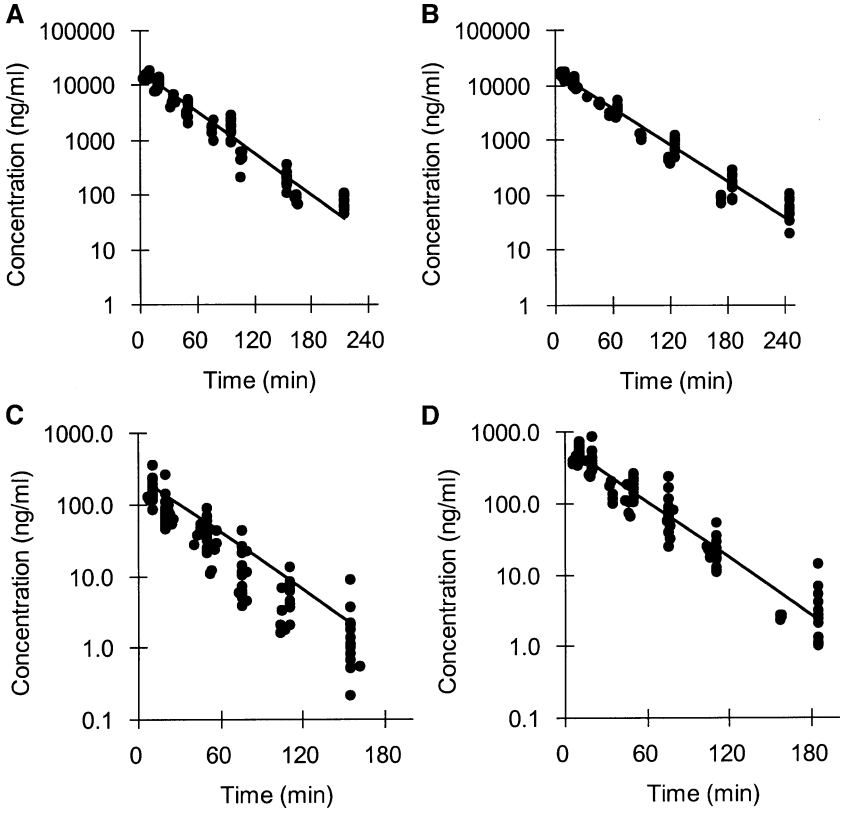

Fig. 1. Plasma concentration time-profiles after intravenous bolus injection of 10 mg/kg MCPA (A), 20 mg/kg 2′dCPA (B), 0.3 mg/kg 5′dCPA (C) or 0.75 mg/kg 5′dCPA (D). The solid line in the graph represents the population predicted mean plasma concentration observed on fitting of a one-compartment pharmacokinetic model to the data.

described with a combination model of the E_{max} model and the linear model:

$$E_{CCI}(t) = E_{0,CCI} + \left(\frac{E_{max} \cdot [A]_i}{EC_{50i} + [A]_i}\right) + a_i \cdot [A]_i \quad (6)$$

where $E_{CCI}(t)$ is the effect at time t in the chronic constriction injury rats, $E_{0,CCI}$ the baseline value of the chronic constriction injury rats, E_{max} the maximum anti-hyperalgesic effect, EC_{50i} the concentration where 50% of the anti-hyperalgesic effect is reached for agonist i. In the modelling initially the value of E_{max} was estimated as an independent value. In the final analysis, on the basis of a statistical evaluation of the goodness of fit, the E_{max} was set to a value identical to the population estimate of the baseline in the sham-operated rats. The statistical model used for the concentration effect curves had the following general from:

$$E_{ijk} = f\left([A]_{ijk}, \theta_i\right) + \varepsilon_{jk} \quad (7)$$

where E_{ijk} and $[A]_{ikj}$ correspond to effect and concentration of agonist i for the kth data point in the jth concentration–effect curve, f is the function, θ_i is the individual parameter value (e.g. E_{max} and EC_{50}) of the concentration–effect curve j and ε_{jk} is a random noise term from a normal distribution with mean zero and variance σ^2. The inter-individual variability in the pharmacodynamic parameters $E_{0,CCI}$, a and EC_{50} were modelled according to the exponential equation used in the pharmacokinetic model (Eq. (2)).

In the pharmacokinetic/pharmacodynamic analysis, the data from the present study on the low-efficacy agonists MCPA and 2′dCPA were simultaneously analysed with the data of the a previous investigation on high-efficacy A_1 receptor agonist 5′dCPA (Schaddelee et al., 2004a). To this end, the integrated pharmacokinetic/pharmacodynamic model was fitted to the pharmacokinetic and pharmacodynamic data of MCPA, 2′dCPA and 5′dCPA in both the chronic constriction injury and the sham-operated rats. The first-order Bayesian estimation method implemented in the NONMEM software was used to calculate population and individual parameter estimates. All fitting procedures were performed on an IBM-compatible personal computer (Pentium®, 133 MHz) running under Windows NT using the Microsoft FORTRAN Powerstation 4.0 compiler with NONMEM version IV, level 2 (double precision) and Visual NONMEM version 2.2.2 (RDPP, Montpellier, France).

2.7.2. Statistical analysis

The pharmacokinetic and pharmacodynamic post hoc Bayesian estimates and the population estimates were compared using parametric analysis of variance (ANOVA) (Graphpad Instat® version 3.00). A value of $p < 0.05$ was considered as a statistically

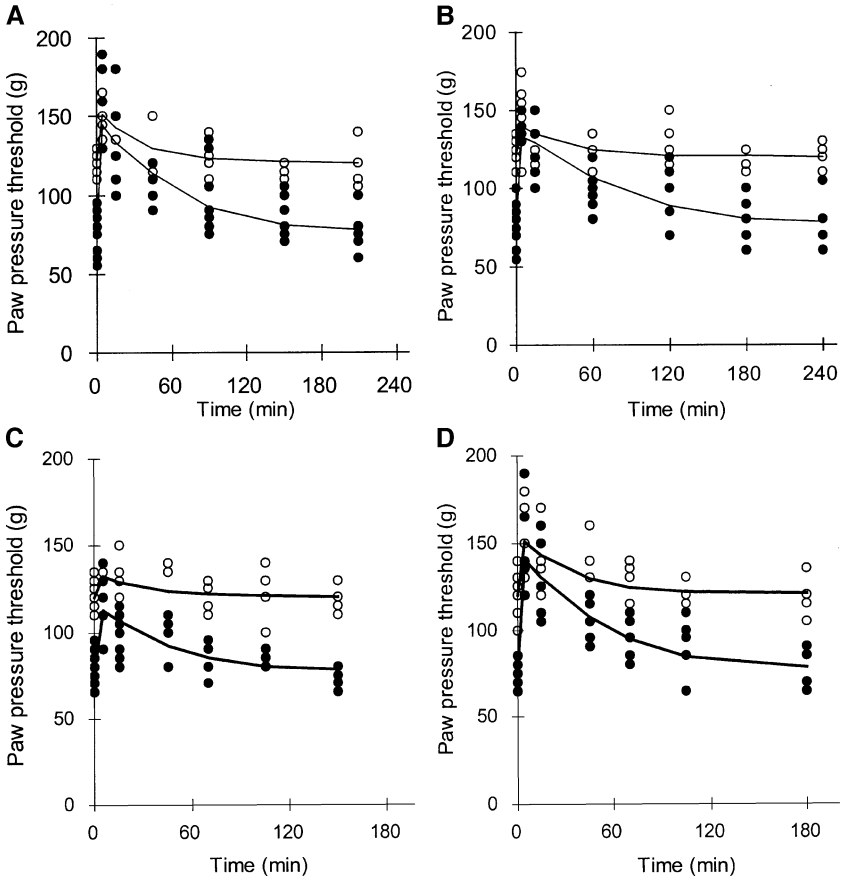

Fig. 2. The time course of the paw pressure withdrawal threshold (mechanical hypersensitivity) after intravenous bolus injection of 10 mg/kg of MCPA (A), 20 mg/kg of 2′dCPA (B), 0.3 mg/kg 5′dCPA (C) or 0.75 mg/kg 5′dCPA (D). The solid line in the graph represents the population predicted mean effect on fitting of the combined E_{max} and linear model to the data. (●) Chronic constrictive injury; (○) Sham.

significant difference. All data are reported as mean±standard error.

3. Results

3.1. Pharmacokinetics

The plasma concentration time profiles after intravenous administration of MCPA (10 mg/kg), 2′dCPA (20 mg/kg) and 5′dCPA (0.3 and 0.75 mg/kg) are shown in Fig. 1. The solid lines represent the population fit. A one-compartment model with linear elimination was found to best describe the concentration–time profiles. In the population analysis, chronic constriction injury could not be identified as a covariate. Thus, there is no difference in the pharmacokinetic parameters between chronic constriction injury rats and the sham-operated controls. From the population analysis, the individual values of the pharmacokinetic parameters were estimated by Bayesian post hoc analysis. The values of the (population) pharmacokinetic parameter estimates of MCPA, 2′dCPA and 5′dCPA are summarised in Table 1.

3.2. Mechanical hypersensitivity

The time course of the mechanical hypersensitivity (paw pressure withdrawal threshold) after intravenous administration of MCPA and 2′dCPA is shown in Fig. 2. At the start of the experiment, there was a wide and stable difference in baseline values between the chronic constriction injury operated rats versus the sham-operated controls of 77.1±1.2 and 120±2.7 g, respectively. Administration of MCPA and 2′dCPA produced a rapid and reversible increase in the paw pressure withdrawal threshold, in both the chronic constriction injury and sham-operated rats. A dual effect was observed, an anti-hyperalgesic effect (the reversal from chronic constriction injury baseline to the sham baseline level) and an anti-nociceptive effect (increase in paw withdrawal threshold above sham baseline level). No hysteresis was observed between the plasma concentration and effect and therefore no effect-compartment was necessary to link the pharmacokinetics to the pharmacodynamics. The composite pharmacodynamic model consisting of an E_{max} model for the anti-hyperalgesic effect in combination with a linear model for the anti-nociceptive effect was found to best describe the relationship between plasma concentration and mechanical hypersensitivity. The resulting plasma concentration effect relationships for the paw pressure withdrawal threshold are shown in Fig. 3. The solid lines represent the population mean value of the paw pressure withdrawal threshold. The individual values of the pharmacodynamic parameters describing the concentration–effect relationship were estimated from the population on the basis of Bayesian post hoc analysis. The values of the (population) pharmacodynamic parameter estimates of MCPA, 2′dCPA and 5′dCPA are summarised in Table 2. No differences in mean baseline values of the chronic constriction injury rats were found between the different groups of rats and therefore one population value was estimated for this parameter in the final pharmacokinetic/pharmacodynamic model. This was also the case for the sham-operated control rats (Fig. 4). The low-efficacy agonists MCPA and 2′dCPA behaved as full agonists with respect to the effect on mechanical hypersensitivity in the neuropathic pain model as the effect of both agonists reached the E_{max} of the anti-hyperalgesic effect, which was indistinguishable from the

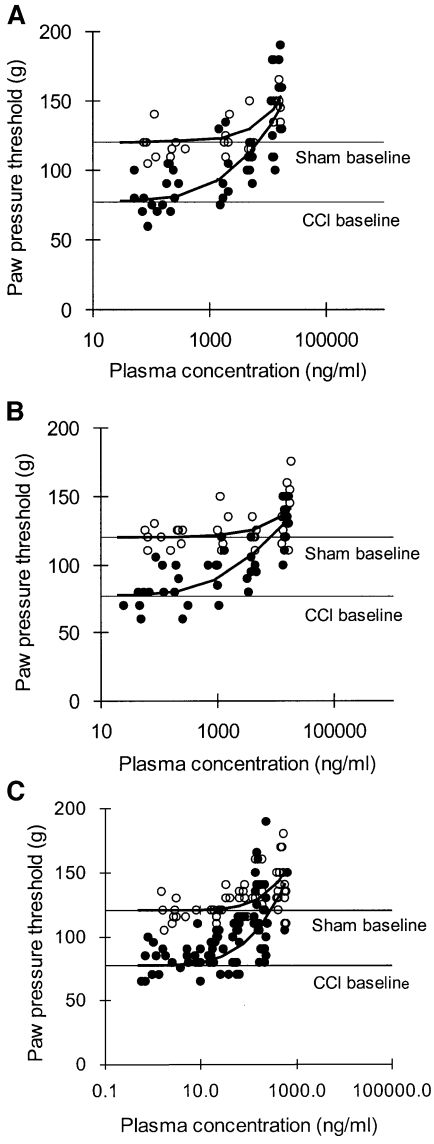

Fig. 3. The plasma concentration–effect relationship for the paw pressure withdrawal threshold after intravenous bolus injection of 10 mg/kg MCPA (A), 20 mg/kg 2′dCPA (B) or 0.3 and 0.75 mg/kg 5′dCPA (C). The solid lines in the graph represent the population predicted concentration effect relationships. (●) Chronic constrictive injury; (○) Sham.

baseline value in the sham-operated controls. Specifically, analysis of the data on the basis of a model allowing estimation of an E_{max} which differs from the baseline paw withdrawal threshold in sham-operated rats, did not result in a statistically significant improvement of the goodness of fit (data not shown). Thus, for the three compounds tested (2′dCPA, 5′dCPA and MCPA), the value of the E_{max} of the anti-hyperalgesic effect is the same indicating that they behave as full agonists with respect to this effect. The EC_{50} of the high-efficacy agonist 5′dCPA was significantly lower than for the low-efficacy agonists, i.e. 178±51 ng/ml for 5′dCPA versus 2660±1200 and 3170±1460 ng/ml for 2′dCPA and MCPA, respectively. A significant anti-nociceptive effect could be identified for both MCPA and 2′dCPA, on the basis of the linear component of the composite pharmacodynamic model. Interestingly, the slope of MCPA and 2′dCPA was significantly different from the value obtained with high intrinsic efficacy agonist

Table 2
Pharmacodynamic parameter estimates obtained intravenous administration of MCPA (10 mg/kg), 2′dCPA (20 mg/kg) and 5′dCPA (0.3 and 0.75 mg/kg)[a]

	Treatment	*n*	Baseline paw pressure withdrawal threshold (g)	EC_{50} (ng/ml)	Slope (g·µl/ng)
MCPA					
Individual post hoc	Chronic constriction injury	24	77.2±5.9	4596±2067	2.5±1.0
	Sham		120±2.7		1.7±0.50
Population mean	Chronic constriction injury	24	77.1±1.2[b]	3170±1460	1.9±0.30
	Sham		120±2.7[c]		1.9±0.30
2′dCPA					
Individual post hoc	Chronic constriction injury	24	77.7±5.2	3298±1111	1.4±0.45
	Sham		120±2.7		1.4±0.71
Population mean	Chronic constriction injury	24	77.1±1.2[b]	2660±1200	1.2±0.20
	Sham		120±2.7[c]		1.2±0.20
5′dCPA[a]					
Individual post hoc	Chronic constriction injury	46	76.3±3.5	200±93	61±23
	Sham		120±2.7		60±24
Population mean	Chronic constriction injury	46	77.1±1.2[b]	178±51	55±8.0
	Sham		120±2.7[c]		55±8.0
Inter-subject variability		94	8%	76%	21%
Residual error		94		10.6±2.9	

The values are presented as (1) the mean±S.E. of the post hoc Bayesian estimates and (2) the population mean value±S.E.
[a] Based on data from Schaddelee et al. (2004a).
[b] The data from the three different treatment groups were simultaneously analysed yielding a single population mean value in chronic constriction injury rats.
[c] The data from the three different treatment groups were simultaneously analysed yielding a single population mean value in Sham rats.

5′dCPA. The values of this parameter were 55±8.0 g·µl·ng^{-1} for 5′dCPA versus 1.9±0.30 versus 1.2±0.20 g·µl·ng^{-1} for MCPA and 2′dCPA, respectively.

4. Discussion

The results of this investigation show that two adenosine A_1 receptor partial agonists MCPA and 2′dCPA behave as full agonists with respect to the anti-hyperalgesic effect in the chronic constriction injury model of neuropathic pain. Furthermore, the slope of the concentration–anti-nociceptive effect relationship was significantly lower than for the reference full adenosine A_1 receptor agonist 5′dCPA.

MCPA and 2′dCPA were chosen as model drugs since it is well established that they act as partial agonists both in vitro (Van der Wenden et al., 1995; Roelen et al., 1996) and in vivo (Mathôt et al., 1995; Van Schaick et al., 1997). Specifically, the values of the GTP-shift in a rat brain membrane preparation were 4.1±1.2 and 3.8±1.5 for 2′dCPA and MCPA, respectively, versus 6.4±1.9 for the reference full agonist 5′dCPA (Van der Wenden et al., 1995; Roelen et al., 1996). A particularly important property is that both compounds have much reduced haemodynamic (side) effects (Van der Graaf et al., 1997). By pharmacokinetic/pharmacodynamic modelling, it has been confirmed that these compounds have an improved selectivity of action in favour of the anti-lipolytic effect (Van der Graaf et al., 1999).

The studies were conducted in the chronic constriction injury model of neuropathic pain as described by Bennett and Xie (1988). This model offers the advantage that a stable level of neuropathy develops within two to three weeks. A limitation is however that in each animal only a limited number of observations are possible. For this reason, non-linear mixed effects analysis was applied to derive individual concentration–effect relationships on the basis of sparse data using an existing and validated population pharmacokinetic/pharmacodynamic model (Schaddelee et al., 2004a). Under this paradigm, the data of all animals (chronic constriction injury and sham-operated controls) and drugs (MCPA, 2′dCPA and 5′dCPA) are simultaneously analysed. Non-linear mixed effects analysis offers the advantage that in addition to precise estimation of the values of the pharmacokinetic parameters also estimates of the inter-individual variability and the individual residual error are obtained (Schoemaker and Cohen, 1996).

The pharmacokinetics of MCPA, 2′dCPA and 5′dCPA were successfully described by a 1-compartment pharmacokinetic model with linear elimination. In the analysis, chronic constriction injury was considered as a covariate but this did not result in a statistically significant improvement of the goodness of fit. This justifies the conclusion that the pharmacokinetics is identical in chronic constriction injury rats and sham-operated controls. The values of the pharmacokinetic parameters for the various compounds obtained in the present investigation are identical to those obtained previously using more traditional sampling designs and data analysis techniques (Mathôt et al., 1995; Van Schaick et al., 1997).

The pharmacodynamics was determined using the mechanical paw pressure withdrawal threshold as an endpoint. In this manner, a continuous and reproducible measure of the pharmacodynamic response is obtained

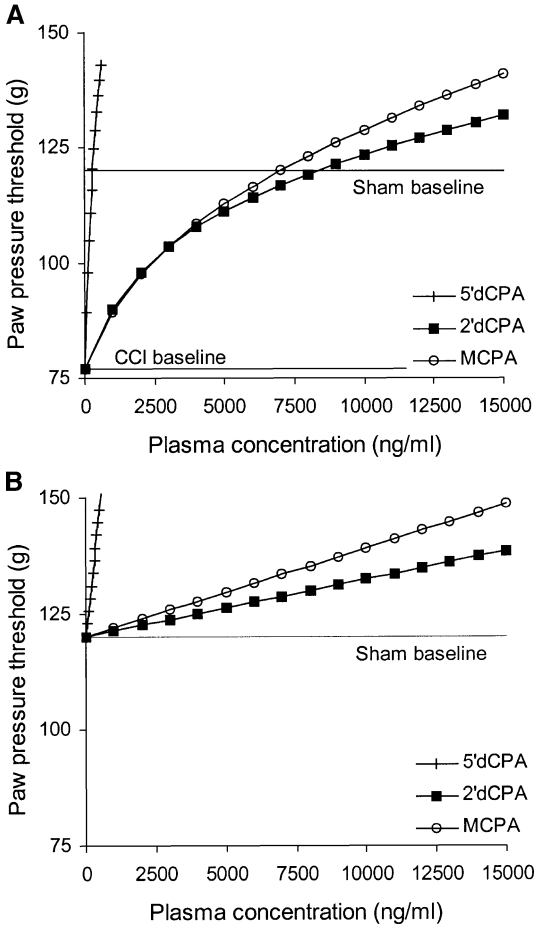

Fig. 4. The population mean plasma concentration–effect relationship for the paw pressure withdrawal threshold after intravenous bolus injection of 5′dCPA (0.3 and 0.75 mg/kg), MCPA (10 mg/kg) or 2′dCPA (20 mg/kg) in the chronic constrictive injury rat (A) and in the sham-operated control rat (B).

(Dingemanse et al., 1988). Various other endpoints, including the von Frey Hair withdrawal threshold, yield categorical rather than continuous data, which complicates the pharmacokinetic/pharmacodynamic data analysis. Furthermore, for synthetic A_1 receptor agonists, the reproducibility of these measures has been shown to be rather poor, with a significant percentage of non-responders in the population (Schaddelee et al., 2004a). A limitation of the mechanical paw withdrawal threshold is lack of selectivity of action in the sense that both the anti-hyperalgesic and the anti-nociceptive effect are detected. Therefore, a novel composite effect model was used to describe the concentration effect relationships (Schaddelee et al., 2004a). This model bears similarity to an earlier model for the anti-convulsant effect of midazolam in rats (Hoogerkamp et al., 1996), and consists of an E_{max} model to describe the anti-hyperalgesic effect in combination with a linear model for the anti-nociceptive effect. An important feature of the pharmacokinetic/pharmacodynamic model is the strict distinction between the anti-hyperalgesic and the anti-nociceptive effect. In this manner, estimates of the potency (EC_{50}) and intrinsic activity (E_{max}) can be obtained, which are not confounded by the anti-nociceptive effect (Schaddelee et al., 2004a).

In the pharmacodynamic analysis, the effect on the paw withdrawal threshold was directly related to the drug concentration in plasma. This is justified by the observation that there is no hysteresis between the drug concentration in plasma and the effect, indicating that the drug concentrations in brain decline in parallel with the concentrations in plasma. This is corroborated by the results of another study showing that the drug concentration in brain (as determined by intra-cerebral microdialysis) parallels indeed the concentration in plasma (Schaddelee et al., 2004a,b).

An important factor in this respect is also that the mechanism of the distribution of adenosine A_1 receptor agonists between plasma and brain is passive diffusion and that the distribution is linear with concentration (Schaddelee et al., 2003). Specifically, the *es* nucleoside transporter is not involved in the transport of 2′dCPA, 5′dCPA and MCPA across the blood–brain barrier (Schaddelee et al., 2003, 2005). The assessment of the plasma concentration–effect relationship is thus not confounded by non-linearities in brain distribution.

The results of the present investigation show that the low intrinsic efficacy adenosine A_1 agonists display a clear anti-hyperalgesic effect in the chronic constriction injury model. To make a distinction between the anti-hyperalgesic effect and the anti-nociceptive effect, the concentration–effect relationships were analysed on the basis of the two-component analysis described above. In the final analysis, the maximum (E_{max}) of the anti-hyperalgesic effect was identical to the baseline value of the paw withdrawal threshold in the sham-operated control. This was justified since analysis of the data on the basis of a more complex model, allowing estimation of a separate value of the maximum effect, which is different from the baseline in the sham-operated controls, did not result in a statistically significant improvement of the goodness of fit. Thus, it appears that the low intrinsic efficacy adenosine A_1 receptor agonists MCPA and 2′dCPA both behave as full agonists with respect to the anti-hyperalgesic effect. Similar observations have recently been made with regard to the anti-lipolytic effects of these compounds (Van Schaick et al., 1997). By mechanism-based pharmacodynamic modelling, it has been demonstrated that the improved selectivity of action of the low intrinsic efficacy adenosine A_1 receptor agonists can be explained by a 40-fold difference in receptor expression between adipose tissue and cardiac tissue (Van der Graaf et al., 1999).

An important observation is that the anti-hyperalgesic effect of MCPA and 2′dCPA occurs at high concentrations as reflected in the values of the concentration at half-maximal effect (EC_{50}) of 3170±1460 and 2660±1200 ng/ml, respectively. The reasons for this low sensitivity relative to the haemodynamic and the anti-lipolytic effects (Van der Graaf et al., 1997, 1999) are presently not fully understood.

In theory, however, the combination of low receptor affinity and a low intrinsic efficacy of MCPA and 2′dCPA can explain the observed high EC_{50} values (Van der Graaf and Danhof, 1997). However, the poor distribution to the site of action is likely to be the most important factor. In this respect, it is important that the ratio of the EC_{50} values in the present investigation and the K_I values at the adenosine A_1 receptor reported in the literature (Van der Wenden et al., 1995; Roelen et al., 1996) is indeed very high, with values of 127, 151 and 867 for 5′dCPA, 2′dCPA and MCPA, respectively. Recent investigations have shown that the transport of MCPA and 2′dCPA across the blood–brain barrier is indeed highly restricted (Schaddelee et al., 2003, 2004b). Interestingly, in an in vitro model of the blood–brain barrier, the clearance for passive diffusion for both 2′dCPA and 5′dCPA was approximately 6–8 times higher than for MCPA (Schaddelee et al., 2003). Thus, there appears to be an inverse correlation between the EC_{50}/K_I ratio in vivo and the clearance for passive diffusion in the in vitro blood–brain barrier model, supporting the view that restricted blood–brain barrier transport is an important determinant of the anti-hyperalgesic effect in the neuropathic pain model. Presumably the same restriction in the transport applies to the transport to the site of action in the spinal cord: it has been demonstrated that that the blood–spinal cord barrier is similar in anatomy and function to the blood–brain barrier (Noble et al., 1996). The observation that the high EC_{50} values are at least partly caused by poor distribution to the site of action is of considerable interest. Specifically, by improving their brain penetration, it is in theory possible to obtain adenosine A_1 receptor partial agonists with an improved selectivity of action, which acts at reasonable dose levels.

In addition to the anti-hyperalgesic effect, also a modest anti-nociceptive effect (i.e. an elevation of the paw withdrawal threshold above the baseline value in sham-operated controls) was observed. It seems unlikely that this observation of an anti-nociceptive effect is an artifact resulting from motor impairment caused by bradycardia and hypotension. In this respect, it is important that the partial agonists tested in this investigation cause only moderate cardiovascular effect (Van der Graaf et al., 1997). Furthermore, we did not observe overt signs of motor impairment in the investigations. Thus, the elevation of the paw withdrawal threshold above the values in sham-operated controls appears to reflect an anti-nociceptive effect indeed.

A remarkable observation of the present investigation is the difference in the slope of the concentration–effect relationship for the anti-nociceptive effect. Specifically, this slope was much lower for the MCPA and 2′dCPA with values of 1.9 ± 0.3 and 1.2 ± 0.2 $g \cdot \mu l \cdot ng^{-1}$, respectively, relative to the value of 55 ± 8 $g \cdot \mu l \cdot ng^{-1}$ for 5′dCPA, which is highly significant. The differences in these slopes, should be considered with some caution, since they might be confounded by a small distribution equilibrium between plasma and brain, shortly following drug administration. However, if present at all, such a disequilibrium is small (Schaddelee et al., 2004a,b). The much lower slope of the concentration anti-nociceptive effect relationship and thus the low sensitivity for the anti-nociceptive effect is a favourable property of the partial agonists since this effect is in principle undesired in the treatment of neuropathic pain.

In conclusion, a composite effect model was successfully applied to determine the concentration–effect relationships of low-efficacy A_1 receptor agonists in the chronic constriction injury model of neuropathic pain, allowing a separation between the anti-hyperalgesic and the anti-nociceptive effect. The results of this analysis showed that the adenosine A_1 receptor partial agonists MCPA and 2′dCPA behave as full agonists for the effect on the mechanical paw pressure threshold, which is consistent with the presumed high receptor density and the high efficacy of transduction of A_1 receptors in the CNS. The potency of both compounds as reflected in the in vivo EC_{50} values, was quite low, which can be explained by the poor distribution to the site of action in the central nervous system. Interestingly, the anti-nociceptive effect was much decreased relative to the high-efficacy agonist 5′dCPA, as is reflected in the low slope of the concentration anti-nociceptive effect relationship.

These findings indicate that it might be of interest to develop novel adenosine A_1 receptor partial agonists, which much improved blood–brain barrier transport characteristics for the treatment of neuropathic pain.

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