The 'free drug hypothesis' : fact or fiction?

Steeg, T.J. van

Chapter 4

Mechanism-based pharmacodynamic modelling of S(-)-Atenolol: Estimation of *in vivo* affinity for the beta1-adrenoceptor with an agonist-antagonist interaction model

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J. Pharmacol. Exp. Ther. (2008) 324:1234-1242

Summary

Background and purpose The aim of this study was the development of an agonist-antagonist interaction model to estimate the *in vivo* affinity of S(-)-atenolol for the β1-adrenoreceptor.
Methods Male Wistar Kyoto rats were used to characterise the interaction between the model drugs isoprenaline (to induce tachycardia) and S(-)-atenolol. Blood samples were taken to determine plasma pharmacokinetics. Reduction of isoprenaline-induced tachycardia was used as a pharmacodynamic endpoint.
Results The pharmacokinetic-pharmacodynamic relationship of isoprenaline was first characterised with the operational model of agonism model using the literature value for the affinity (K_A) of isoprenaline ($3.2x10^{-8}$ M; left atria WKY rats). Resulting estimates for baseline (E_0), maximum effect (E_{max}) and efficacy (t) were 374 (1.9%) bpm, 130 (5.9%) bpm and 247 (33%) respectively. Secondly, the interaction between isoprenaline and S(-)-atenolol was characterised using a pharmacodynamic interaction model based on the operational model of agonism that describes the heart rate response on the basis of affinity of the agonist (K_A), the affinity of the antagonist (K_B), the efficacy (τ), the maximum effect (E_{max}), the Hill coefficient (n), the concentrations of isoprenaline and atenolol, as well as the displacement of the endogenous agonist adrenaline. The estimated *in vivo* affinity (K_B) of S(-)-atenolol for the β1-receptor was $4.6x10^{-8}$ M. The obtained estimate for *in vivo* affinity of S(-)-atenolol ($4.6x10^{-8}$ M) is comparable to literature values for the *in vitro* affinity in functional assays.
Conclusions In conclusion, a meaningful estimate of *in vivo* affnity for S(-)-atenolol could be obtained using a mechanism-based pharmacodynamic modelling approach.

4.1 Introduction

The concentration-effect relationships of agonists depend on a combination of the binding to the target (affinity) and the efficiency in activating the target (intrinsic efficacy). On the other hand, the activity of a pure antagonist depends solely on binding as it results from the displacement of the (endogenous) agonist from the target (Hardman *et al*., 2001). Although in theory the mechanism of action of antagonists is less complex than that of agonists, the characterization and quantification of pharmacological effect of antagonists *in vivo* is challenging; it requires not only information on concentration of the antagonist but also on the concentration and the intrinsic efficacy of the agonist(s) to be replaced from the target (Kenakin, 1993).
In drug development, typically *in vitro* binding studies are performed to characterise the association between the new chemical entity (NCE) and receptor, by its ability to displace the radioligand at various concentrations (Sweetnam *et al*., 1993). Quantitative analysis of the displacement curve yields estimates of the affinity of the drug, which is usually represented as the equilibrium dissociation constant (K_D) (Copeland *et al*., 2006). In functional studies, the affinity of an antagonist can be obtained from a functional assay (i.e. cAMP accummulation) using the displacement of an agonist from the target (Jindal *et al*., 2003; Louis *et al*., 1999).
In general, the estimation of *in vivo* affinity on basis of pharmacodynamics (PD) endpoints (drug effects) is difficult and requires extensive knowledge of the system under investigation. Specifically, pertinent information is required on both the target affinity and the intrinsic efficacy, since both properties determine the shape and the location of the concentration-effect relationship (Ariens, 1954; Stephenson, 1956). In the current publication we show that a meaningful estimate of *in vivo* affnity can be obtained using a PD

interaction model for the agonist and the antagonist which is based on the operational model of agonism (Black and Leff, 1983).
The aim of the present study is the development of a mechanism-based PD interaction model for an agonist and an antagonist, which can be used for the estimation of *in vivo* affinity of S(-)-atenolol. To this end, the PD interaction between isoprenaline and S(-)-atenolol is characterised quantitively in conscious animals. The PD endpoint in this study is reduction in heart rate under isoprenaline-induced tachycardia and this is considered to be a biomarker for β_1-receptor binding.
Isoprenaline was selected as a model compound since it is very potent β-adrenoceptor agonist, which is also used in the clinical evaluation of the effects of β-blockers (Lipworth *et al*., 1991; Van Bortel *et al*., 1997). S(-)-atenolol, was selected because it is a β_1-selective hydrophilic β-blocker without intrinsic sympathomimetic activity, being eliminated predominantly via the kidneys (Kirch and Gorg, 1982; Reiter, 2004). Furthermore, the active enantiomer of this drug, S(-)-atenolol is not metabolised into (inter)-active metabolites *in vivo* and has negligible protein binding (Reeves *et al*., 1978a; Reeves *et al*., 1978b).
The first step in the development of the agonist-antagonist interaction model was the characterization of the concentration-effect relationship of isoprenaline on basis of the operational model of agonism. The second step, using the information from the first step, was the investigation of the PD interaction between isoprenaline and S(-)-atenolol. The PD interaction was characterised using the developed mechanism-based PD interaction model and yielded a sound estimate of the *in vivo* affinity of S(-)-atenolol for the β_1-receptor.

4.2 Methods

Animals
All animal procedures were performed in accordance with Dutch laws on animal experimentation. The study protocol was approved by the Animal Ethics Committee of Leiden University (UDEC no. 02112). Male Wistar Kyoto rats (291 g ± 37, n=42) obtained from Janvier (Le Genest Saint Isle, France) were housed individually at a constant temperature of 21ºC and a 12-hour light/dark cycle. Prior to the surgery the rats were acclimatised for at least 5 days. The rats had *ad libitum* access to acidified water and food (laboratory chow, Hope Farms, Woerden, The Netherlands), except during the experimental procedures.

Drugs and Chemicals
S(-)-atenolol ((-)-4-[2-Hydroxy-3-[(1-methylethyl)amino]propoxy]benzeneacetamide), (±)-sotalol hydrochloride (N-[4-[1-Hydroxy-2-(isopropylamino)ethyl]phenyl]methanesulfonamide hydrochloride) and (-)-isoprenaline hydrochoride (isoprenaline) were purchased from Sigma-Aldrich BV (Zwijndrecht, the Netherlands). Ketanest-S® ((S)-ketaminebase) was purchased from Parke-Davis (Hoofddorp, The Netherlands). Domitor® (medetomidine hydrochloride) was obtained from Pfizer (Capelle a/d IJssel, The Netherlands). Polyvinylpyrrolidone (PVP) was obtained from Brocacef (Maarsen, The Netherlands). Heparin (20 IU/ml) was obtained from the LUMC (Leiden University Medical Center) Pharmacy (Leiden, The Netherlands) and 0.9% (g/v) saline from B. Braun Melsungen AG. (Melsungen, Germany).

Surgery
The rats were anesthetised with a subcutaneous injection of 0.1 ml/100 g Ketanest-S® and an intramuscular injection of 0.01 ml/100 g Domitor®. During surgery the rats were placed on a heating pad to maintain body temperature at 37 °C. Seven days prior to the experiment, the rats were instrumented

with four indwelling blood cannulas (Portex Limited, Hythe, Kent, England); two cannulas in the right jugular vein (Polythene 14 cm, ID 0.58 mm, OD 0.96 mm) for drug administration and one in the left and the right femoral artery (Polythene, 4 cm ID 0.28 mm, OD 0.61 mm + 20 cm ID 0.58 mm, OD 0.96 mm) for blood sampling and heart rate measurements respectively. The blood cannulas were subcutaneously tunnelled and externalised at the dorsal base of the neck. To prevent blood clotting, the arterial cannulas were filled with a 25% (w/v) PVP solution in a 0.9 % saline solution containing 20 IU/ml heparin. The venous cannula was filled with a saline solution containing 20 IU/ml heparin.

Experimental Design

Experiments were performed to characterise the pharmacokinetics (PK) and pharmacodynamics (PD) of isoprenaline, S(-)-atenolol and the PD interaction between both compounds.

Isoprenaline The PK and PD of isoprenaline were determined as described previously (van Steeg *et al.*, 2007). Briefly, experiments were performed to characterise the PK of Isoprenaline. Male WKY rats received an intravenous infusion of 25 or 50 $\mu g\ kg^{-1}$ and blood samples were taken at predefined time points. Thereafter the concentrations in plasma were determined using HPLC-ECD. To identify the PKPD relationship for isoprenaline, PD experiments were performed. Rats received multiple intravenous infusions of isoprenaline, the following doses were used: 0.001, 0.01, 0.05, 0.1, 0.2, 0.5, 1, 2.5, 5 and 10 $\mu g\ kg^{-1}\ h^{-1}$. Heart rate at the steady state isoprenaline concentration was used as a pharmacodynamic endpoint.

Interaction S(-)-atenolol and isoprenaline The rats were randomly divided into five groups. The rats in the three treatment groups received 0.5 (n=7), 1 (n=9) or 5 mg kg^{-1} (n=9) of S(-)-atenolol under isoprenaline-induced tachycardia. In two control groups the treatment consisted of 5 mg kg^{-1} S(-)-atenolol without isoprenaline-induced tachycardia (non-isoprenaline, n=8) and isoprenaline-induced tachycardia only (non-atenolol, n=9). S(-)-atenolol was dissolved in saline and administered as an intravenous infusion in 15 minutes (20 $\mu l\ min^{-1}$). Isoprenaline-induced tachycardia consisted of a continuous intravenous infusion of 5 $\mu g\ kg^{-1}\ h^{-1}$ isoprenaline in 0.1% SMBS saline solution. In the control groups, the infusions were replaced by vehicle solutions.

Serial arterial blood samples were collected in heparin tubes at pre-defined time intervals for determination of S(-)-atenolol concentrations. Plasma samples were obtained by centrifugation (5 min; 5000 rpm) and stored at – 20°C until analysis. Heart rate was recorded continuously throughout the experiment.

Pharmacodynamic measurements

All experiments started between 8.00 and 9.00 AM to avoid influences of circadian rhythms on the outcomes. The baseline heart rate was recorded for 30 min, thereafter isoprenaline-induced tachycardia was recorded for 30 min before commencing with the S(-)-atenolol infusion. At the end of each experiment, approximately 480 min after the start of the S(-)-atenolol infusion, the continuous infusion of isoprenaline was stopped and heart rate was recorded for another 20 min. Arterial blood pressure and heart rate were measured from the cannulas in the femoral artery using a P10EZ-1 pressure transducer (Viggo-Spectramed BV, Bilthoven, The Netherlands), equipped with a plastic diaphragm dome (TA1017, Disposable Critiflo Dome, BD, Alphen a/d Rijn, The Netherlands). During the experiment the diaphragm dome was flushed with saline at a rate of 500 $\mu l\ h^{-1}$ (Harvard 22-syringe pump, Harvard Apparatus Inc., South Natick, MA, USA). The pressure transducer was placed at the level of the heart of the rats, when in normal position, and connected to a blood pressure amplifier (AP-641G, Nihon Kodhen Corporation.,

Tokyo, Japan). Heart rate was captured from the pressure signal. The signals were passed through a CED 1401*plus* interface (Cambridge, Electronic Design LTD, Cambridge, England) into a Pentium 4 computer using the data acquisition program Spike 2 (Spike 2 Software, version 3.11, Cambridge, England) and stored on a hard disk for off-line analysis.

Drug analysis
S(-)-atenolol were quantified using reversed phase HPLC following liquid-liquid extraction as briefly described below.
The HPLC-system consisted a LC-10AD HPLC pump (Shimadzu, 's Hertogenbosch, The Netherlands), a Waters 717 plus autosampler (Waters, Etten-Leur, The Netherlands), and a FP 920 fluorescence detector (Jasco Co, Tokyo, Japan) with an excitation wavelength of 235 nm and an emission wavelength of 300 nm. Chromatography was performed on Spherisorb ODS-2 3 μm column (4.6 mm I.D. x 100 mm) (Waters, Millford, MA, USA) equipped with a refill guard column (2 mm I.D. x 20 mm) (Upchurch Scientific, Oak Harbor, WA, USA) packed with pellicular C18 (particle size 20-40 μm) (Alltech, Breda, The Netherlands). The mobile phase consisted of 77.5% (v/v) 0.1 M sodium acetate buffer (pH 3.4) containing 5 mM octane-sulfonic acid (OSA) and 22.5% (v/v) acetonitrile. Sample (50 μl plasma), internal standard (50 μl sotalol 5 μg/ml in water), sodium hydroxide solution (3 M, 100 μl), water (200 μl) and ethyl acetate (5 ml) were mixed, shaken (5 min) and centrifuged (4000 rpm, 10 min). The organic layer was taken and evaporated to dryness. Subsequently the residue was reconstituted in 100 μl mobile phase and 50 μl was injected into the HPLC-system.

Data analysis
The PK and the concentration-effect relationship of isoprenaline served as an input for the data-analysis in this study (van Steeg *et al.*, 2007). The PK of S(-)-atenolol and the PD interaction between S(-)-atenolol and isoprenaline were quantified using non-linear mixed-effects modelling as implemented in NONMEM software version V, level 1.1 (Beal and Sheiner, 1999). The approach takes into account structural effects and both intra- and interanimal variability. Parameters were estimated using the first-order conditional estimation method with η-ε interaction (FOCE interaction). Modelling was performed on an IBM-compatible computer (Pentium IV, 1500 MHz) running under Windows XP with the Fortran compiler Compaq Visual Fortran version 6.1. An in-house available S-PLUS 6.0 (Insightful Corp., Seattle, WA, USA) interface to NONMEM version V was used for data processing and management and graphical data display. Model selection was based on the Akaike Information Criterion (AIC) (Akaike, 1974). Goodness-of-fit was determined using the objective function and by visual inspection of various diagnostic plots.

Pharmacokinetics PK analysis for S(-)-atenolol was performed by fitting a standard three compartment model to the concentration-time profile. The PK compartmental model for isoprenaline consisted of a standard two compartment model. Interanimal variability of the PK parameters was described according to an exponential distribution model:

$$P_i = \theta \cdot \exp(\eta_i) \qquad (1)$$

in which P_i is the individual value of model parameter P, θ is the typical value (population value) of parameter P and η_i is the random deviation of P_i from P. The values of η_i are assumed to be independently normally distributed with mean zero and variance ω^2. Selection of an appropriate residual

error model was based on inspection of goodness-of-fit plots. On this basis, a proportional error model was selected to describe residual error in the plasma drug concentration:

$$C_{obs,ij} = C_{pred,ij} \cdot (1 + \varepsilon_{ij}) \quad (2)$$

in which $C_{obs,ij}$ is the *j*th observed concentration in the *i*th individual, $C_{pred,ij}$ is the predicted concentration, and ε_{ij} accounts for the residual deviation of the model predicted value from the observed value. The values for ε_{ij} are assumed to be independently normally distributed with mean zero and variance σ^2. For both S(-)-atenolol and isoprenaline, the pharmacokinetics served as an input for the pharmacological model.

Pharmacodynamics The developed PK models for isoprenaline and S(-)-atenolol served as an input for the PD modelling. A mechanism-based model was applied to the concentration-effect relationship of isoprenaline under the assumption that the maximal obtainable effect in the system equals the maximal effect of isoprenaline (Kenakin, 1993). An estimate for the *in vitro* affinity of isoprenaline was obtained from literature (Doggrell *et al*., 1998). The efficacy (τ) of isoprenaline was estimated using the operational model of agonism (equation 3) and the concentration-effect data reported previously (van Steeg *et al*., 2007).

$$E = E_0 + \frac{E_{\max} \cdot \tau . [A]^n}{(K_A + [A])^n + \tau . [A]^n} \quad (3)$$

where *E* is the effect of the drug at concentration [*A*], E_0 is the baseline heart rate, E_{max} is the maximum effect (i.e. the system maximum), K_A and τ are the affinity and efficacy of isoprenaline and *n* is the slope factor, which determines the steepness of the transducer function (Black and Leff, 1983).
Subsequently a mechanism-based PD interaction model for isoprenaline and S(-)-atenolol was used to describe the heart rate response in rats. The overall effect on heart rate was described using equation 4.

$$Effect = E_{BSL}(t) - E_{ADR}(B) + E(A,B) \quad (4)$$

In which E_{BSL} is the baseline heart rate as a function of time, E_{ADR} is the influence of adrenaline (epinephrine) on the baseline heart rate as a function of the antagonist concentration and *E(A,B)* is the drug interaction effect as a function of the concentration antagonist and agonist.
A baseline model (E_{BSL}) was used in order to describe the data more accurately. The baseline model consisted of a linear decrease over time and is defined as follows:

$$E_{BSL} = E_0 - DEC \cdot time \quad (5)$$

In which E_0 is the unique baseline heart rate and DEC is the parameter describing the linear decrease over time. In addition to the linear decrease over time an influence of adrenaline was observed in the baseline data. Therefore the model was extended using an E_{max} model (equation 6) for the interaction of atenolol with adrenaline and the subsequent reduction in baseline heart rate.

$$E_{ADR} = \frac{E^B_{\max} \cdot [B]^n}{(EC^B_{50})^n + [B]^n} \quad (6)$$

In which *[B]* is the concentration of the antagonist, $E_{max}{}^{B}$ is the maximal decrease due to displacement of adrenaline in the system, $EC_{50}{}^{B}$ is the concentration which causes half-maximal effect and n is the slope factor.
The interaction of isoprenaline and S(-)-atenolol was described using a mechanism-based PD interaction model which is based on the operational model of agonism (equation 7).

$$E = \frac{E_{\max}.(\tau.[A])^n}{(K_A + [A] + ([B].\frac{K_A}{K_B}))^n + (\tau.[A])^n} \quad (7)$$

In which *E* is the overall effect of the agonist at concentration [*A*] and the antagonist at the concentration [*B*], E_{max} is the maximum effect (i.e. the system maximum), K_A and τ are the affinity and efficacy of the agonist respectively, K_B is the receptor affinity of the antagonist and *n* is the slope factor. The slope factor in this equation is assumed to be equal to the slope factor in equation 6, since both effects are the result of interaction with the same receptor. All groups, treatment (0.5, 1 and 5 mg kg^{-1} S(-)-atenolol) and control (non-atenolol and non-isoprenaline) were analysed simultaneously. For the treatment groups the heart rate effect is described using equation 8.

$$Effect = E_0 - DEC \cdot time - \frac{E^B_{\max} * [B]^n}{(EC^B_{50})^n + [B]^n} + \frac{E_{\max}.(\tau.[A])^n}{(K_A + [A] + ([B].\frac{K_A}{K_B}))^n + (\tau.[A])^n} \quad (8)$$

For the non-atenolol group equation 8 reduces to equation 9. Drug effect for isoprenaline in this equation is equal to equation 3.

$$Effect = E_0 - DEC \cdot time + \frac{E_{\max}.(\tau.[A])^n}{(K_A + [A])^n + (\tau.[A])^n} \quad (9)$$

Finally, for the non-isoprenaline group the equation reduces to equation 10.

$$Effect = E_0 - DEC \cdot time - \frac{E^B_{\max} \cdot [B]^n}{(EC^B_{50})^n + [B]^n} \quad (10)$$

Interanimal variability of the PD parameters was described according to an additive (eq. 11) or an exponential (eq. 12) distribution model:

$$P_i = \theta + \eta_i \quad (11)$$

$$P_i = \theta \cdot \exp(\eta_i) \quad (12)$$

in which P_i is the individual value of model parameter *P*, θ is the typical value (population value) of parameter *P* and η_i is the random deviation of P_i from *P*. The values of η_i are assumed to be independently normally distributed with mean zero and variance ω^2.
On this basis of visual inspection, an additive error model was proposed to describe residual error in the drug effect:

$$C_{obs,ij} = C_{pred,ij} + \varepsilon_{ij} \quad (13)$$

in which $C_{obs,ij}$ is the *j*th observed concentration in the *i*th individual, $C_{pred,ij}$ is the predicted concentration, and ε_{ij} accounts for the residual deviation of the model predicted value from the observed value. The values for ε_{ij} are assumed to be independently normally distributed with mean zero and variance σ^2.

4.3 Results

S(-)-atenolol

Pharmacokinetics A three-compartment PK model best described the S(-)-atenolol data obtained at doses of 0.5, 1 and 5 mg kg^{-1}. The PK of S(-)-atenolol was linear across all doses used. All PK parameters were estimated precisely with coefficients of variation ranging between 3.8 and 37.8% (Table 1). Interanimal variability was identified for clearance (*CL*) and the volumes of distribution for both peripheral compartments (*V2* and *V3*). Correlations between the values of interanimal variability were evaluated by using a full omega matrix. A significant correlation was obtained for *CL* and *V3* and this correlation was taken into account in the final model. Validation of the PK model for S(-)-atenolol was performed by a visual predictive check. The visual predictive check showed that the population PK model could well predict the time course of S(-)-atenolol in rats for all dose groups (Figure 1). In addition no difference was observed between the 5 mg kg^{-1} dose with and without isoprenaline-induced tachycardia. The PK parameter estimates for S(-)-atenolol are displayed in Table 1.

Table 1, Population PK model for S(-)-atenolol: Estimates and coefficient of variation (CV%) for PK parameters, interanimal variability (ω) and residual error (σ)

Parameter	Value	CV (%)
Structural parameters[a]		
Cl (ml min^{-1})	11.9	3.8
V1 (ml)	112	8.3
Q2 (ml min^{-1})	15.3	8.0
V2 (ml)	171	8.7
Q3 (ml min^{-1})	8.73	4.8
V3 (ml)	857	4.7
Interanimal variability[a]		
ω_{CL}^2	0.0359	21
ω_{V2}^2	0.158	30
ω_{V3}^2	0.0230	37
$\omega_{CL,V3}^2$ *(covariance)*	0.0251	38
Residual error		
σ_{PK}^2	0.0265	9.9

[a] *Abbreviations: CL (Clearance), V1 (Volume of distribution of the central compartment), Q2 (intercompartmental clearance to the 2nd compartment), V2 (Volume of distribution of the 2nd compartment), Q3 (intercompartmental clearance to the 3rd compartment), V3 (Volume of distribution of the 3rd compartment), ω (inter-individual variability), σ (residual variability)*

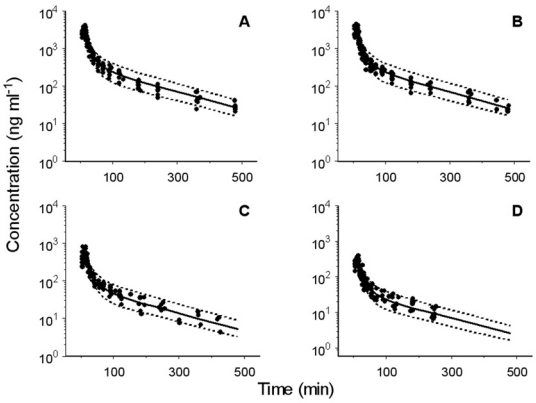

Figure 1, Visual predictive check (VPC) population PK model for S(-)-atenolol. Panel A, B, C and D show the VPC for 5 mg kg^{-1} S(-)-atenolol (non-isoprenaline), 5 mg kg^{-1} S(-)-atenolol, 1 mg kg^{-1} S(-)-atenolol and 0.5 mg kg^{-1} S(-)-atenolol respectively. The range between the dashed lines depicts the 90% inter-quantile range. The solid line presents the population prediction. The solid dots are the observed concentrations.

Isoprenaline

Pharmacokinetics A population PK model has been developed previously and this model served as an input also in this study (Van Steeg *et al.*, 2007). In short, a two compartment model adequately described the PK of isoprenaline. Since the maximal effect of isoprenaline is already observed at concentrations below the limit of quantification in rats (1 ng ml^{-1}), the two compartment model was used for the prediction of the concentrations of isoprenaline in the PD experiments.

Pharmacodynamics In this study, the operational model of agonism (Black and Leff, 1983) was used to describe the concentration-effect relationship of isoprenaline and to obtain an estimate for the efficacy (τ) of this agonist. The *in vivo* affinity (K_A) was assumed to be equal tot the *in vitro* affinity (K_i) and the estimates for E_0, E_{max} and τ were 374 (1.9%), 130 (5.9%) and 247 (33%) respectively. No inter-individual variability was used in the final model, since the goodness of fit decreased as judged on basis of the diagnostic plots. The model fit for the operational model of agonism and the E_{max} model is shown in Figure 2.

S(-)-atenolol and isoprenaline

Pharmacodynamic interaction The basal resting heart rate in rats was 363 ± 45 bpm. The basal mean arterial pressure values were within the normal range (Mathôt *et al.*, 1994). All groups, both control (non-isoprenaline and non-atenolol) and treatment (isoprenaline-atenolol), were analysed simultaneously with a PD interaction model and the individual plots for the worse, median and best individual fit are shown in figure 3. As a consequence of the isoprenaline infusion a steep increase in heart rate was observed directly after the start of the intravenous infusion. Thereafter upon S(-)-atenolol administration the heart rate decreased again and slowly recovered in a concentration dependent fashion while S(-)-atenolol was cleared from the system. Finally, after the stop of the steady state infusion of isoprenaline the heart rate returned to baseline. Some individuals showed a slight linear

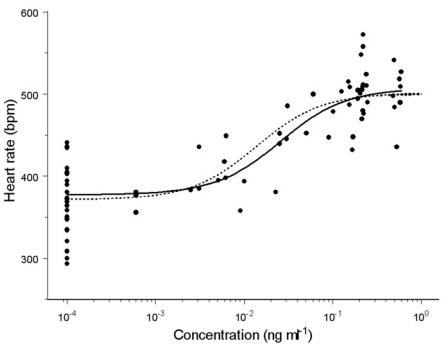

Figure 2, The operational model of agonism in comparison to the Emax model for isoprenaline. The solid line and the dashed line represent the model population prediction for the operational model of agonism and for the sigmoid Emax model respectively. The solid dots represent the observations for heart rate.

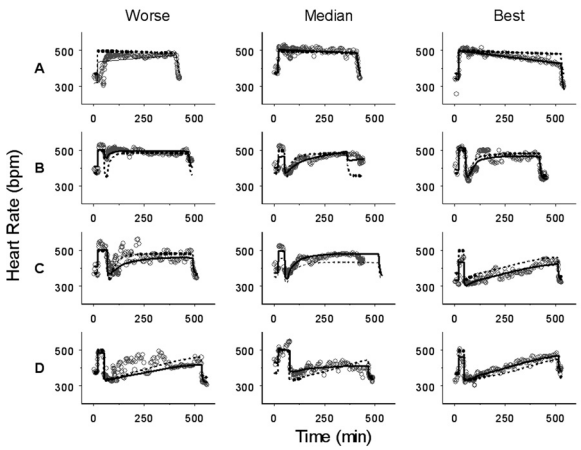

Figure 3, Individual fits for the mechanism-based interaction model for the effect of isoprenaline and S(-)-atenolol on heart rate. Row A, B, C and D represent the worse, median and best individual fit for non-atenolol, 0.5, 1 and 5 mg kg^{-1} S(-)-atenolol respectively. The solid line and the dashed line represent the individual and population prediction respectively. The open dots represent the heart rate observations.

decrease in heart rate, this decline over time being most clearly visible in control animals (i.e. panel A – Best, Figure 3). On top of that, in several rats, the drop in heart rate following S(-)-atenolol administration resulted in heart rate values below the initial baseline (i.e. panel D – Median, Figure 3). To account for these two observations, a baseline model was incorporated into the interaction model. The two control groups, non-isoprenaline and non-atenolol, were essential for the fit of the baseline model. Administration of S(-)-atenolol without the isoprenaline infusion resulted in a small decrease in heart rate and this effect was incorporated into the baseline model by means of an sigmoid E_{max} equation (van Steeg *et al*., 2007). All populations parameters were estimated with good precision (CV < 50%). The values for the population parameter estimates and the estimates for variability are displayed in Table 2. Inter-individual variability was identified for baseline, *DEC*, E_{max} and K_B.

4.4 Discussion and conclusion

The estimation of *in vivo* affinity for antagonists is usually complicated, since under normal physiological conditions the pharmacological activity of antagonists is small. The aim of this study was to develop an agonist-antagonist interaction model to estimate the *in vivo* receptor affinity of the β-receptor antagonist S(-)-atenolol using changes in heart rate under isoprenaline-induced tachycardia as biomarker for β_1-adrenoceptor binding in conscious rat. The developed interaction model adequately described the heart rate profiles of S(-)-atenolol (Figure 3). The obtained estimate for affinity of S(-)-atenolol, 4.62×10^{-8} M, was comparable to values reported in literature for the *in vitro* affinity in functional assays. The current analysis shows that meaningful estimates for affinity can be obtained *in vivo* when using a mechanism-based modelling approach.

Over the years a limited number of studies on the PKPD correlations of competitive antagonists have been reported, often on the basis of the interaction with a full agonists (e.g. Mandema *et al*., 1992; Appel *et al*., 1995; Zuideveld *et al*., 2002). The characterisation of the PKPD correlation of competitive antagonists is not without complications. To our knowledge the current investigation is the first to propose a mechanism-based PKPD model for the effect of atenolol on heart rate in rats which is based on concepts from receptor theory (Danhof *et al*., 2007). The added value of this model is that instead of estimation of the potency on the basis of a descriptive PD parameter (EC_{50}), the actual affinity (K_B) of S(-)-atenolol is estimated *in vivo* using the agonist-antagonist interaction model. The main limitation of the current model is that the system under investigation should be well characterised in order to asses the validity of the assumptions made

Table 2, Population PD interaction model for isoprenaline and S(-)-atenolol: Estimates and coefficient of variation (CV%) for PD parameters, interanimal variability (ω) and residual error (σ).

Parameter	Value	CV (%)
Structural parameters[a]		
E_0 (bpm)	373	1.8
DEC (h^{-1})	2.1	39
E_{max} (bpm)[b]	130	(5.9)
τ[b]	247	(34)
$K_A = K_i$ (ng ml^{-1})[c]	6.33	FIX
K_B (ng ml^{-1})	12.9	24
n	1.74	18
EC_{50B} (ng ml^{-1})	36.6	17
E_{maxB} (bpm)	77.7	37
Interanimal variability[a]		
ω_{DEC}^2	1210	24
ω_{E0}^2	25.4	29
ω_{Emax}^2	1320	29
ω_{KB}^2	1.14	36
Residual error		
σ_{PD}^2 (bpm^2)	829	28

[a] *Abbreviations: E_0 (Baseline heart rate), DEC (linear decrease over time), E_{max} (Maximal heart rate), (efficacy of isoprenaline), K_A (affinity of isoprenaline), K_B (affinity of S(-)-atenolol), n (Hill coefficient), EC_{50B} (concentration which causes half-maximal effect for baseline model), E_{maxB} (maximal decrease due to displacement of endogenous agonist), ω (inter-individual variability), σ (residual variability)* [b] *Parameter estimated using the Emax model and fixed in the agonist-antagonist interaction model*
[c] *Parameter obtained from literature and fixed in the agonist-antagonist interaction model*

for the curve fitting. In addition, the curve fitting requires a sufficient amount of experimental data for both the agonist and the interaction between agonist and antagonist. Despite the limitations, this model constitutes a solid basis for further investigations on the role of e.g. plasma protein binding as a determinant of the effects of β-adrenoceptor agonists.

The estimation of *in vivo* affinity for 'silent' agonists is usually complicated, since the pharmacological activity of antagonists is small under normal physiological conditions. If considerable drug effects are observed, these are as a rule the result of competition between the antagonist and the 'endogenous' agonist (Hardman *et al*., 2001). Exact quantification of antagonist effects requires knowledge on the concentration of the agonist and this information is typically not obtained or is difficult to acquire in *in vivo* studies (Kenakin, 1993). The use of a non-endogenous agonist in interaction studies can, therefore, be advantageous. A drawback of introducing another agonist into the system is that the overall observed effect is the result of three compounds. Isoprenaline, however, is an very potent agonist for the β-

adrenoceptor and the influence of the endogenous agonist, adrenaline, on the overall drug effect on heart rate is small (Rang *et al*., 1999).

The tachycardia produced by isoprenaline is primarily due to β-adrenoceptor activity in the heart (Chiu *et al*., 2000). Although β_1, β_2 and β_3 adrenoceptors are present in mammalian heart, the positive chronotropic effect of isoprenaline *in vivo* is brought about by β_1-adrenoceptors (Nandakumar *et al*., 2005; Piercy, 1988; Wellstein *et al*., 1987). It is occasionally suggested that β_2-adrenoceptors are involved in the effect on heart rate. However, only a small population of functional β_2-adrenoceptors is present in the heart of WKY rats (Doggrell and Surman, 1994). Heart rate can therefore be used as a biomarker for β_1-adrenoceptor binding *in vivo* in rat.

The effect of agonists in a given biological system is determined by a combination of the affinity (binding) and the intrinsic efficacy (activation of the receptor) (Kenakin, 1993; Ariens, 1954; Stephenson, 1956). For the accurate estimation of *in vivo* affinity of the antagonist it is important to take into account the efficacy of the agonist. In this study, therefore, we first evaluated the PKPD relationship of isoprenaline using the operational model of agonism (Black and Leff, 1983; Van Der Graaf and Danhof, 1997a).

Isoprenaline is the most potent agonist known for the effect on heart rate and it can be assumed that the maximum effect of the system (i.e. the system maximum) equals the maximal effect of isoprenaline (Leff *et al*., 1990). The obtained estimate for efficacy (τ) of isoprenaline is 247 ± 84. According to receptor theory, the transducer ratio (τ) can be calculated by dividing the total concentration of receptors [R_0] by the concentration of occupied receptors that elicits half-maximal effect (K_E) (Black and Leff, 1983). In the case of a linear relationship between the concentration of occupied receptors and the effect, the efficacy can be calculated from the total number of receptors in rat left atrium ([R_0]= 0.85×10^{-6} M) and the K_{AR} (the equilibrium dissociation constant for the agonist-receptor-transducer complex) for isoprenaline (K_{AR} = 2.7×10^{-9}). This calculated value, 314, is very close to the value obtained for the efficacy in the present study. Dogrell reported that half-maximal response for isoprenaline in WKY left atrium is already reached at 3% receptor occupation. Isoprenaline is, therefore, an efficient agonist in a system with a large receptor reserve and this observation is in agreement with the high value obtained for τ in the system.

The PK parameters obtained from the description of the profiles by a three compartment model are comparable with literature (Belpaire *et al*., 1993; De Lange *et al*., 1994; Mehvar *et al*., 1990). In the literature however, the plasma concentrations of atenolol following intravenous administration are described by a two-compartment model. This difference might be explained by the duration of the experiments, which is usually 2-3 h in literature compared to 8 h in our experiment.

In the interaction experiments, the heart rate quickly increased from baseline values of around 365 bpm to approximately 500 bpm upon isoprenaline administration. An important question is to what extent reflexive heart rate effects originating from *in vivo* homeostatic feedback mechanisms may have interfered with the characterisation of the PKPD relationship of atenolol. In designing the present PKPD investigations for atenolol, we have considered elucidation of the contribution of homeostatic feedback mechanisms. To evaluate this, we have determined the PKPD correlation of atenolol following the administration of a wide range of different doses (0.5, 1.0 and 5.0 mg kg^{-1}). In the PD profiles following the administration these widely different dosages no indications for profound homeostatic feedback control mechanisms were obtained. Moreover, unique estimates of the K_B (affinity) have been obtained which are independent of the administered dose of atenolol. Finally, the obtained estimates of the *in vivo* K_B of atenolol are close to the values obtained *in vitro* bio-assays (Nandakumur *et al*., 2005). These findings confirm that *in vivo* homeostatic feedback mechanisms play a minor role in the estimation of these PD parameters. This observation is consistent with earlier investigations on the PKPD correlations for the

effects on heart rate of A_1 adenosine receptor agonists (Mathôt *et al.*,1994; Van der Graaf *et al.*, 1997b).

Upon S(-)-atenolol administration, isoprenaline is displaced from the receptor and a decrease in heart rate was observed. In some animals, the heart rate dropped below the original baseline and this observation is most likely the result of the presence of endogenous agonist (adrenaline) in the system under normal physiological conditions. To allow the model to describe the decrease below baseline accurately the effect of adrenaline (E_{ADR}) was added to the model. This addition improved model stability and precision and did not alter the estimation of K_B significantly. Since a more mechanistic description would require information on the concentration of adrenaline and the concentration-effect relationship of adrenaline, the endogenous agonist effect was added in a rather descriptive manner.

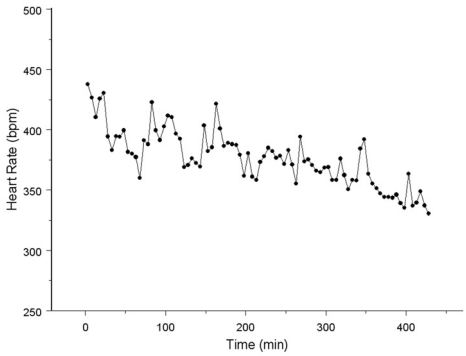

Figure 4, Heart rate for a typical control rat, which did not receive any drug treatment

The implementation of baseline model was feasible by incorporation of pertinent information from the control groups (non-atenolol and non-isoprenaline groups). The linear decrease over time might have been the result of a circadian cycle in heart rate and/or the down regulation of β-adrenoceptors (van den Buuse, 1999; Henry *et al.*, 1990). It is known that β-adrenoceptors are susceptible to down regulation in the presence of agonists (Matthews *et al.*, 1996; Lu and Barnett, 1990). The linear decrease over time, however, was also observed in some of the control rats, which received no drug at all (Figure 4). Therefore, we conclude that the most important cause for the linear decrease in heart rate during the experiment is a circadian cycle.

In conclusion, a PD interaction model has been developed which allows the estimation of the *in vivo* affinity of S(-)-atenolol for the β_1-receptor using heart rate as a biomarker for receptor binding. In future studies the developed interaction model can be evaluated further using other β-blockers.

4.5 Acknowledgements

The authors gratefully acknowledge the technical assistance of M.C.M. Blom-Roosemalen, S.M. Bos-van Maastricht and E. Suidgeest and the discussions with Dr. F. MacIntyre (Pfizer, research and development).

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