Concentration-Effect Relationship of \( l\)-Propranolol and Metoprolol in Spontaneous Hypertensive Rats after Exercise-Induced Tachycardia\(^1\)

LENA BRYNNE, MATS O. KARLSSON and LENNART K. PAALZOW

Department of Pharmacy, Division of Biopharmaceutics and Pharmacokinetics, Uppsala University, Uppsala, Sweden

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ABSTRACT

The concentration-effect relationship of \( l\)-propranolol and \( dl\)-metoprolol were investigated in spontaneous hypertensive rats using reduction in exercise-induced tachycardia as a pharmacodynamic endpoint. The influence of protein binding on the effect relationship was also assessed. The rats were assigned to treatment or placebo groups, where each group received three randomly selected consecutively increasing steady-state infusions. Different pharmacodynamic effect models were fitted to the data, using nonlinear mixed effect modeling. The data were best described by a combined effect model, with a sum of an ordinary \( I_{\text{max}} \) and a linear model. At the lower concentration range, the ordinary \( I_{\text{max}} \) model dominated, although at higher concentrations, the effect was linearly related to the antagonist concentration. The \( I_{\text{max}} \) were 83 ± 6 and 103 ± 6 beats \( \cdot \) min\(^{-1} \) and the IC\(_{50} \) were 18.1 ± 4.3 and 50.6 ± 15.2 ng/ml for \( l\)-propranolol and \( dl\)-metoprolol, respectively. The slope in the linear model was steeper for \( l\)-propranolol than for \( dl\)-metoprolol, 28.9 ± 2.8 and 4.48 ± 0.39 beats \( \cdot \) ml \( \cdot \) (min \( \cdot \) \( \mu \)g\(^{-1} \), respectively. Plasma protein binding of \( l\)-propranolol was saturable. The unbound IC\(_{50} \) for \( l\)-propranolol was 1.14 ± 0.27 ng/ml. The concentration-effect relationship of \( l\)-propranolol was altered at higher plasma concentrations, due to saturable protein binding. The \( I_{\text{max}} \) and the linear concentration-effect relationship may be interpreted as a specific \( \beta\)-antagonist effect and a membrane-stabilizing effect, respectively. Using exercise-induced tachycardia as a pharmacodynamic endpoint, to study the effect of \( \beta\)-antagonists in spontaneous hypertensive rats, seems to give reliable results and can be a useful model to extrapolate to humans.

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**ABBREVIATIONS:** AGP, \( \alpha\)-1-acid glycoprotein; SHR, spontaneous hypertensive rat.
al., 1992), but corresponding animal studies have been few. SHR have been shown to be an appropriate animal model in studying essential hypertension in humans, and was used in this study to evaluate the concentration-effect relationship of two beta antagonists, using exercise-induced tachycardia as a pharmacodynamic endpoint.

One of the most widely prescribed beta antagonists, metoprolol, was selected, using propranolol as a reference. Because of the large difference in disposition between the two enantiomers of propranolol (Walle et al., 1988), the l-enantiomer was selected in this study. There is also a large difference in protein binding between the two beta antagonists. Propranolol is extensively bound (95%) to plasma proteins, a binding that decreases at higher concentrations (Smits and Struyker-Boudier, 1979), whereas the more hydrophilic metoprolol is negligibly bound (12%) (Brogden et al., 1977). Consequently, when an effect model is evaluated over a large concentration interval, the impact of protein binding on the concentration-effect relationship has to be considered.

Our objectives were to determine the concentration-effect relationships of l-propranolol and dl-metoprolol in conscious SHR, and to evaluate the use of exercise-induced tachycardia as a pharmacodynamic endpoint for future studies. The influence of plasma protein binding of l-propranolol was also determined.

Materials and Methods

Animals and drugs. Male SHR 300 ± 10 g (S.D.) were used (Møllegaard, Ejby, Denmark). The animals were housed under standardized conditions; room temperature 22 ± 1°C (S.D.), humidity 55 ± 5% (S.D.) and controlled light (7:00 A.M. to 7:00 P.M.) with free access to food and water. At least 1 wk before start of experiment, the rats were acclimatized to these conditions. During the experiment, the rats were kept in individual cages (CMA/120, CMA, Solna, Sweden). The study was approved by the Animal Ethics Committee of the University of Uppsala.

Crystalline l-propranolol hydrochloride (99% purity) and dl-metoprolol tartrate (Sigma Chemical Co., St. Louis, MO) were completely dissolved in a physiological saline solution (Pharmacia & Upjohn AB, Stockholm, Sweden).

Surgical procedure and drug infusion. Two days before drug administration, two indwelling polyethylene catheters (PE50, Intramedic, KEBO, Spånga, Sweden) were implanted under light ether anesthesia. The left carotid artery was used to monitor heart rate and to sample blood while the study drugs were administered through the right jugular vein. The animals were trained during these two days to be accustomed to the handling and equipment.

The study was conducted in two separate parts. In each part, the rats were randomly assigned to receive drug or placebo treatment, and were given three consecutive intravenous infusions to steady-state (Harvard Apparatus Syringe Infusion Pump 22; B & K, Sollentuna, Sweden), where l-propranolol was given to 17 rats, dl-metoprolol to 13 and the corresponding placebo groups were five and six animals each. The steady-state levels were randomly picked among different aimed steady-state levels. Although, they were always attained from between the lowest up to the highest target concentration level. The desired steady-state plasma concentrations (C_s) were; 20, 70, 200, 400, 800, 1400, 2200, 3000 and 3500 ng/ml for l-propranolol and 50, 100, 200, 400, 800, 1600, 3200, 6400, 12800 and 25600 ng/ml for dl-metoprolol.

A technique using two consecutive intravenous infusions (Wagner, 1974) was implemented so that different pseudo steady-state plasma concentrations were rapidly attained. The following equations for calculating the infusion rates (Q_1 and Q_2) were used:

\[ Q_1 = \frac{Q_2}{1 - e^{-\frac{t}{\tau}}} \]  
\[ Q_2 = \frac{Dose \cdot C_{ss}}{A/\alpha + B/\beta} \]

where A, B, \( \alpha \) and \( \beta \) are pharmacokinetic macro-constants (Gibaldi and Perrier, 1982). The pharmacokinetic parameters used to calculate the rate of infusion required for a given steady-state concentration were derived from Terao and Shen (1983) and Vermeulen et al. (1993). A 30 min (T) loading infusion was followed by a maintenance infusion over 2 hr and 20 min, for each of the three desired levels. The drug solution was given in such a concentration that the rats received less than 3 ml during the total 8.5-hr infusion.

Effect measurements and blood sampling. Mean arterial blood pressure was measured by a pressure transducer (Statham P23 DC, Grass Instruments Co., Quincy, MA). Heart rate was captured from the pressure signal, which triggered a tachograph (7P4DE) and the signals were recorded by a polygraph (Grass model 7). The recorded data were instantly converted in a MacLab interface (ADInstruments, Castle Hill, Australia) and fed into a Macintosh LC IIvi computer. The data were stored on a hard disk for off-line analysis, using the MacLab software Chart, version 3.2.6 (ADInstruments, Castle Hill, Australia).

Exercise-induced tachycardia was obtained by having the animals run in a motorized wheel for 10 min (6 m · min \(^{-1}\)). Mean arterial blood pressure and heart rate were measured continuously for 15 min after exercise. In each rat, two effect measurements were recorded before start of infusion (baseline) and at each concentration level (effect values taken between 10 to 15 min postexercise). A mean value of each was used in the pharmacodynamic analysis.

Arterial blood samples, 200 \( \mu \)l, were collected in heparinized Eppendorf tubes. A total of 1200 \( \mu \)l was drawn from each animal. Two blood samples were drawn at each steady-state level after the effect measurement, and a mean value was used in the pharmacodynamic analysis. The samples were centrifuged at 7200 × g for 10 min whereby plasma was separated and frozen immediately (−70°C) until analysis.

Protein binding. The binding of l-propranolol to rat plasma was determined by equilibrium dialysis. Pooled plasma obtained from eight untreated rats that had undergone the same surgical procedure previously described, including time in the restraining cage, was frozen (−70°C) pending equilibrium dialysis. The plasma sample was adjusted to pH 7.40 using carbogen gas and spiked in triplicate with known amounts of l-propranolol to achieve the following concentrations: 20, 60, 100, 200, 300, 400, 600, 1000, 2000 and 3500 ng/ml. The Teflon chambers were filled with 0.8 ml plasma and 0.8 ml of 0.13 M phosphate buffer (pH 7.40). Before dialysis, the Spectropor 4 membranes (Spectrum Medical Industries Inc., Houston, TX; cut-off value of 12–14,000) were soaked in buffer for 2 to 3 hr. The cells were kept rotated at 37°C for 4.5 hr, by which time equilibrium has been reached. Volume shift was assessed by weighing the plasma/buffer solutions before and after equilibrium dialysis.

\( \alpha \)-Acid glycoprotein and drug analysis. The AGP concentrations were determined by using a modification of a previous published method (QR method) (Imamura et al., 1994). The analytical system consisted of a pump (ESA-580 Chelmsford, MA), a Triathlon autoinjector (Sparc Holland Emmen, the Netherlands), a fluorescence detector (Shimadzu RF-551, Kyoto, Japan), and an integrator (Shimadzu C-R5A Chromatopac). The detector was set at excitation and emission wavelengths of 496 nm and 590 nm, respectively. A 1/15 M phosphate buffer (pH 7.40) was used as mobile phase with a flow rate of 0.5 ml/min. All volumes used were one-fourth of that described in the original method, when preparing standard curves and samples. Standards and samples were placed in the autosampler, where 50 \( \mu \)l were injected. The difference in height of the
chromatograms, with and without QR solution, gave the fluorescence of AGP alone and was used as the response in the standard curves. Rat AGP showed a lower fluorescence response than human AGP. Human AGP standards were used to check the linearity of the system and rat AGP standards were used in the standard curve. The method was highly reproducible with an interday variability of <8%. The limit of quantification was 0.08 mg/ml with a coefficient of variation of 17% (n = 6).

The l-propranolol and dl-metoprolol concentrations in plasma and phosphate buffer were determined using high-performance liquid chromatography with fluorescence detection, as described by Rutledge and Garrick (1989), with some modifications. The analytes were extracted from 100 µl samples and standards in a 10-ml round-bottomed test tubes. Internal standard, 50 µl (12 µM metoprolol tartrate or 4 µM propranolol HCl in distilled water) was added to the 100-µl sample followed by 100 µl sodium hydroxide (4 M) and mixed briefly. Three mLs of diethyl ether was then added to the mixture. The contents of the test tube were vortexed for 2 min and centrifuged at 1400 × g for 10 min. The organic phase (upper layer) was transferred into a 10-ml conical glass tube and evaporated to dryness under a stream of oxygen free nitrogen, at 35°C. The residue was dissolved in 150 µl of 1 M H2SO4, vortexed and then centrifuged (10 min 2000 × g) before injection. In the protein binding study, the phosphate buffer samples were diluted with 5 mM H2SO4 and the plasma samples with blank plasma, when necessary, up to a total volume of 100 µl, to end up within the range of the standard curve. All solvents and reagents were of analytical grade.

The chromatographic equipment consisted of a pump (Shimadzu LC-9A, Kyoto, Japan), a µBondapak column (C18 column, 30 cm × 3.9 mm i.d., Waters Associates Milford, MA), a fluorescence detector (Shimadzu RF-535 or Jasco FP-920 Tokyo, Japan) and an integrator (Shimadzu C-R5A Chromatopac). The excitation and emission wavelengths were 235 nm and 335 nm using Shimadzu RF-535 for l-propranolol, and 280 and 305 nm using Jasco FP-920 for dl-metoprolol, respectively, in the animal study. For the protein binding analysis, the l-propranolol plasma and phosphate concentrations were analyzed using Jasco FP-920 with excitation and emission wavelengths of 222 and 350 nm, respectively. All sample injection was performed using a CMA/200 autoinjector (CMA, Solna, Sweden) fitted with a 100-µl sample loop. The composition of the mobile phase was a 65:35 mixture of solution A and B, respectively, when running plasma samples and changed to 70:30 when running samples of phosphate buffer (pH 7.40). Solution A contains 25 ml of l-heptanesulfonic acid (5 mM) in 1 liter acetic acid (1%) and solution B contains 25 ml of l-heptanesulfonic acid (5 mM) in 1 liter acetonitrile. The flow rate was 2.0 ml/min. Retention times for plasma samples were 2.7 and 4.7 min for metoprolol and propranolol.

Peak area ratios of drug/internal standard were calculated and used in single-level calibration curves drawn through origin with replicates (n = 5) of the highest calibrator. The standard curves were linear within the range of 1.8 to 400 ng/ml and 3.9 to 800 ng/ml for l-propranolol and dl-metoprolol, respectively. The interday variation for l-propranolol in the concentration range 1.8 to 330 ng/ml was ≤5% and the accuracy varied between 92 to 100%. The interday variation for metoprolol in the concentration range 9.8 to 490 ng/ml was ≤11% and the accuracy varied between 90 and 100%. The absolute recovery was between 100% (1.8 ng/ml) to 96% (326 ng/ml) for l-propranolol, although it varied between 112% (3.9 ng/ml) and 93% (780 ng/ml) for dl-metoprolol. The limit of quantification was 1.8 ng/ml for l-propranolol and 3.9 ng/ml for dl-metoprolol, respectively.

**Data analysis.** Population models describing the concentration-effect relationships of the two β-antagonists were performed by relating the reduction of induced exercise-tachycardia directly to the measured steady-state plasma concentrations (time-independent) of the drugs. Both a linear (equation 3) and an ordinary Imax model (equation 4) were first fit to the data.

\[
E_{pred,ij} = E_{0,ij} - m_i \cdot C_{ss,ij} \tag{3}
\]

\[
E_{pred,ij} = E_{0,ij} - \frac{I_{max,i} \cdot C_{ss,ij}}{IC_{50,i} + C_{ss,ij}} \tag{4}
\]

\[
E_{pred,ij} = E_{0,ij} - \frac{I_{max,1} \cdot C_{ss,ij}}{IC_{50,1} + C_{ss,ij}} - m_1 \cdot C_{ss,ij} \tag{5}
\]

\[
E_{pred,ij} = E_{0,ij} - \frac{I_{max,2} \cdot C_{ss,ij}}{IC_{50,2} + C_{ss,ij}} \tag{6}
\]

The last model did not significantly improve the objective function value, and therefore the more simple dual effect model (equation 5) was chosen to describe the data.

The effect after placebo administration was evaluated separately using nonlinear mixed effect modeling, estimating the deviation from baseline values. No significant change in heart rate was found in the control groups of propranolol (n = 5) and metoprolol (n = 6). However, the data from the control rats were incorporated into the data analysis because they contain information about baseline and residual variability. All data were first analyzed separately for each drug. Simultaneous fits of data from both drugs were performed, as well as to total and unbound propranolol, to evaluate differences between the estimated population pharmacodynamic parameters.

The protein binding of l-propranolol was best described by the following equation using naive pooling in the nonlinear regression analysis:

\[
C = C_u + \frac{N \cdot C_p \cdot Pt}{1 + K_s \cdot C_u} \tag{7}
\]

where C and C_u represents total and unbound l-propranolol concentrations, respectively. N is the number of binding sites per mole protein, P_t is the total concentration of binding protein, i.e., AAGP, and K_s is the association constant between drug and binding site. The unbound fraction was calculated as the ratio of unbound and total drug concentrations. Individual unbound concentrations were calculated by using equation 7.

The nonlinear regression analysis was performed by using both the first-order (FO) and first-order conditional estimation (FOCE) methods in the software package NONMEM, version V (Beal and Sheiner, 1992). The NONMEM program is based on a statistical model that explicitly takes into account both the inter-animal variability in the parameters as well as intra-animal residual error. All data will be fitted simultaneously although preserving the individuality. This approach is particular suitable in cases where some of the concentration-effect curves are not completely defined, as all available information may provide reasonable estimates of the missing parts. The graphic analysis was performed using the Xpose package (Jonsson and Karlsson, 1998) running under Splus, version 3.3 (Statistical Sciences, 1993). An additive error model (equation 8) was used to characterize the residual error:

\[
E_{obs,ij} = E_{pred,ij} + e_{ij} \tag{8}
\]

where \(E_{obs,ij}\) and \(E_{pred,ij}\) are the jth observed and predicted effect for the ith individual, \(e_{ij}\) represents the residual error of variance for the ith individual, its distance between the jth observation and prediction. The values for \(e_{ij}\) are assumed to be symmetrically distributed,
with mean zero and variance \( \sigma^2 \). A proportional error model was used in the protein binding study:

\[
C_{obs} = C_{pred}(1 + \epsilon)
\]

where \( C_{obs} \) and \( C_{pred} \) are the observed and predicted total plasma concentration. The inter-individual variability in the pharmacodynamics is described using an exponential variance model, assuming log-normal distribution of the pharmacodynamic parameters:

\[
P_i = \bar{P} \cdot \exp(\eta_i)
\]

in which \( P_i \) is the value of the \( i \)th individual of an arbitrary pharmacodynamic parameter, \( \bar{P} \) is the population mean parameter value, and \( \exp(\eta_i) \) expresses the (random) difference between \( P_i \) and \( \bar{P} \). The values for \( \eta_i \) are assumed to be independently multivariate distributed, with mean zero and diagonal variance-covariance matrix \( \Omega \) with diagonal elements \( \omega_1^2, \ldots, \omega_m^2 \). The values of the population parameters \( \theta_1, \sigma^2 \), and \( \Omega \) are estimated from the data, where \( \theta_1 \) is the population parameter estimate.

Discrimination between different models was calculated via comparison of the objective function values (\(-2\log likelihood\)) by NONMEM and by visual inspection of the goodness of fit plots. The difference between the objective function values for two hierarchical models is approximately chi-square distributed and may consequently be used for model selection purposes. In this study, \( P < .05 \) was used as the significance level. All data are given as mean ± S.E. unless otherwise stated.

## Results

**Protein binding.** The relationship between unbound fraction and total plasma concentration of \( l \)-propranolol was described by a binding model for different independent sets of equivalent binding sites (fig. 1). The number of binding site was 1.08 ± 0.07 and the association constant was 0.736 ± 0.059 \( \mu M^{-1} \) at an AGP concentration of 22.6 \( \mu M \) (MW 43,500). The fraction unbound of \( l \)-propranolol was 5.3 ± 0.5% (mean ± S.D.) and linear in the 60 to 1400 ng/ml concentration range (fig. 1). At more than 1400 ng/ml, the free fraction increased, and at 3500 ng/ml, it was 10 ± 0.7% (mean ± S.D.). No results were obtained for the lowest studied concentration (20 ng/ml) because of assay limitations. No apparent volume shift was seen during the 4.5-hr equilibration time.

**Concentration-effect relationship.** The baseline variation between the two treatment groups was not significantly different. The maximal observed decrease in heart rate for \( l \)-propranolol and \( dl \)-metoprolol was 168 and 216 beats \( \cdot \) min\(^{-1} \), respectively. At concentrations higher than 3000 ng/ml of \( l \)-propranolol, acute bradycardia occurred, and consequently the highest steady-state level has been excluded in this evaluation.

The same results were obtained when data were analyzed separately or simultaneously, and therefore the latter is presented here. The concentration-effect relationships of the two drugs was best described by a combined effect model, represented as the sum of an ordinary \( I_{\text{max}} \) model and a linear model. The individual baseline normalized effect values, transformed after the modeling, are shown in figure 2. The pharmacodynamic parameter estimates are presented in table 1. No significant differences between \( l \)-propranolol and \( dl \)-metoprolol were found in either the \( I_{\text{max}} \) or the \( IC_{50} \) values. The \( I_{\text{max}} \) values were 21.3% for \( l \)-propranolol and 26.0% for \( dl \)-metoprolol, and their corresponding \( IC_{50} \) values were 18.1 ng/ml (69.8 nM) and 50.6 ng/ml (94.6 nM). A significant difference in the linear slopes (\( P < .001 \)) was found between the two drugs. The concentration interval at \( I_{\text{max}} \), where a plateau occurred, was longer for \( dl \)-metoprolol than for \( l \)-propranolol.

Figure 3 illustrates the simulations of the partial and the combined concentration-effect relationships for \( l \)-propranolol. The inter-animal variability was low (<12%) for all parameters except for the \( IC_{50} \) values. The estimated \( IC_{50} \) values with 95% confidence intervals were predicted to be 18.1 (4–74) and 1.14 (0.28–4.67) ng/ml for total and unbound \( l \)-propranolol and 50.6 (15–173) ng/ml for \( dl \)-metoprolol. The intra-animal variability was 13 beats \( \cdot \) min\(^{-1} \). The first-order (FO) and first-order conditional estimation (FOCE) methods performed equally, and therefore only the results of the former are presented.

Simulation of the total and unbound \( l \)-propranolol and \( dl \)-metoprolol concentration-effect relationships are illustrated in figure 4A. The corresponding two parts of the concentration-effect relationships are given in figures 4B and C. The \( IC_{50u} \) value of \( l \)-propranolol was 1.14 ± 0.27 ng/ml (4.40 nM). The \( IC_{50u} \) value of \( dl \)-metoprolol was calculated to be 44.5 ng/ml (83.3 nM), assuming linear protein binding and that the unbound fraction of \( dl \)-metoprolol is 88% (Brogden et al., 1977). About a 20-fold difference in the \( IC_{50u} \) values was found when the two drugs were compared. There were large differences between the slopes of the linear model; 343 ± 51 and 28.9 ± 2.8 beats \( \cdot \) ml \( \cdot \) (min \( \cdot \) \( \mu g \))\(^{-1} \) for unbound and total \( l \)-propranolol and 4.48 ± 0.39 beats \( \cdot \) ml \( \cdot \) (min \( \cdot \) \( \mu g \))\(^{-1} \) for \( dl \)-metoprolol.

## Discussion

In earlier pharmacodynamic studies of \( \beta \)-antagonists, simplified concentration-effect relationships such as linear or log-linear models have frequently been used, mainly due to the small dose/concentration range studied in humans. In this study, we clearly demonstrate that the concentration-effect relationship of \( l \)-propranolol and metoprolol is more...
complex and better described by a combined relationship of at least two different components. This was obtained by using a well-defined stimulus (exercise-induced tachycardia) in combination with a large drug concentration interval.

When using a large dose/concentration interval, saturation processes might be revealed, such as nonlinear protein binding. Differences in the free fraction are of minor importance in experiments with only one compound, but when a highly bound agent is compared to a virtually unbound drug, this needs to be accounted for. The protein binding in this study was linear at low plasma \( l \)-propranolol concentrations, which is in agreement with previous findings in Wistar rats (Chindavijak et al., 1988). An increase in free fraction has earlier been found for \( dl \)-propranolol in SHR (Smits and Struyker-Boudier, 1979), where the nonlinearity started at around 100 ng/ml in rats with a normal AGP level. From the literature it is known that the AGP level can increase 6-fold 2 days after surgery (Yasuhara et al., 1983; Lin et al., 1987), suggesting that the nonlinearity in this study would occur at higher drug concentrations.

In addition to surgery, infections, inflammations and stress, are important conditions that will increase the AGP concentration. It has been shown that the AGP level has a large influence on both the pharmacokinetics and the apparent pharmacodynamics of propranolol when total plasma \( l \)-propranolol concentrations are used in the evaluation (Yasuhara et al., 1983), and consequently the levels of AGP is essential to quantify and explain interindividual variability. The saturation of the protein binding in our study affects the response of \( l \)-propranolol at high plasma concentrations. It increases the steepness of the concentration-effect relationship at high plasma levels, suggesting a nonlinear increase of the effect at

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**TABLE 1**
Pharmacodynamic estimates obtained by population analysis of concentration-effect data after \( l \)-propranolol and \( dl \)-metoprolol administration to SHR (mean ± S.E.)

<table>
<thead>
<tr>
<th></th>
<th>( l )-Propranolol</th>
<th>( dl )-Metoprolol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( n ) = 22</td>
<td>( n ) = 19</td>
</tr>
<tr>
<td>Total</td>
<td>388 ± 4</td>
<td>388 ± 4</td>
</tr>
<tr>
<td>Unbound</td>
<td>388 ± 4</td>
<td>388 ± 4</td>
</tr>
<tr>
<td>Total</td>
<td>396 ± 6</td>
<td>396 ± 8</td>
</tr>
</tbody>
</table>

\( E_0 \) (beats \cdot min^{-1}) | \( l_{\text{max}} \) (beats \cdot min^{-1}) | \( IC_{50} \) (ng \cdot ml^{-1}) | \( m \) (beats \cdot ml \cdot (min \cdot \mu g)^{-1}) | \( f_u \) (%)
---|---|---|---|---
Total | 388 ± 4 | -83 ± 6 | 18.1 ± 4.3 | 28.9 ± 2.8 | 5.3 ± 0.5^b
Unbound | 388 ± 4 | -89 ± 7 | 1.14 ± 0.27 | 343 ± 51
Total | 396 ± 6 | -103 ± 6 | 50.6 ± 15.2 | 4.48 ± 0.39 | 88^c

\(^a\) All animals.
\(^b\) Free fraction at the linear relationship.
\(^c\) Data taken from Brogden et al., 1977.

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**Fig. 2.** The concentration-effect relationships of \( l \)-propranolol and \( dl \)-metoprolol, observed (●) and predicted (——).

**Fig. 3.** Simulated concentration-effect relationship of \( l \)-propranolol, showing both the combined and partial effect models.
In a study performed on dogs, both propranolol and metoprolol equipotently reduced exercise-induced heart rate in a similar fashion (Åblad et al., 1980). In our study, we did not find large differences in the pharmacodynamics between the two drugs either. However, a 20-fold difference appears when the IC$_{50}$ values are corrected for protein binding, making propranolol the more potent drug. The difference in IC$_{50}$ values of different studies could be due to the exercise test used, i.e., duration, power and type. As the drugs are competitive antagonists, a higher degree of exercise (agonist) will shift the effect relationship toward higher concentrations (Wellstein et al., 1985).

Except for the large difference in protein binding between propranolol and metoprolol, they also differ in such factors as their respective affinities to the beta-1 and beta-2 receptors, membrane stabilizing activity and lipophilicity. Selectivity is a relative rather than an absolute property, because increasing doses of beta-selective antagonists results in a dose-related beta-2 adrenoceptor blockade (Lipworth et al., 1991) and because higher doses of a beta-2 adrenoceptor selective antagonist will reduce exercise-induced tachycardia (Harry et al., 1988). The different roles of beta-1 and beta-2 receptors in the genesis of cardiovascular responses have proved to be more complex, because of their coexistence in the heart (Brodd et al., 1983). The rat’s left ventricle contains about 74% beta-1 and 26% beta-2 adrenergic receptors (Vago et al., 1984). Due to the high density of beta-1 receptors in the heart, one would expect that they would contribute more to the positive inotropic and chronotropic responses than would beta-2 (Molenaar and Summers, 1987), and that the latter would play only a minor role in the heart rate alteration.

A major effect of beta antagonist therapy is increased AV nodal conduction time resulting in a prolonged AV nodal refractoriness (increased PR interval). Hence, beta antagonists are useful in terminating reentrant arrhythmias that involve the AV node and in controlling ventricular response in atrial fibrillation or flutter (Rodin, 1996). As it is difficult to standardize and use arrhythmias as pharmacodynamic endpoints, many studies have been performed in vitro. Pruett and coworkers (1977, 1980) found that d- and dl-propranolol shortened action potential duration with similar potency at a concentration as low as 100 ng/ml. The antiarrhythmic activity correlated better with the tissue propranolol concentration, and when this was taken into account, the minimum plasma concentration expected to produce an electrophysiologic effect in patients was 150 ng/ml. This was confirmed when 40% of the patients with ventricular arrhythmias responded to the drug at plasma concentrations in excess of 150 ng/ml, a level associated with a high degree of beta receptor blockade (Woosely et al., 1979). When d-propranolol was given separately, it suppressed ventricular arrhythmias both

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**Fig. 4.** The predicted effect models showing total and unbound plasma concentration; (——) dl-metoprolol, (— — ) l-propranolol and (— — — ) unbound l-propranolol. The combined effect model (A) for both drugs shows similar $I_{\text{max}}$ values (B) but large differences in the linear slopes (C) between the drugs.

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The maximal response after exercise-induced heart rate in humans occurred at plasma dl-propranolol concentrations from 100 ng/ml and above (Hager et al., 1981; Lalonde et al., 1987; Sowinski et al., 1995), which is in agreement with our data. The $I_{\text{max}}$ value was 21.3% for l-propranolol in this study, which is lower in comparison values reported from human studies, 33.3 to 38.3%, where dl-propranolol was given orally (Lalonde et al., 1987; Sowinski et al., 1995). The estimated IC$_{50}$ value in our study, 18.1 ng/ml (l-propranolol), was within the range reported in these human studies (10.2–24.4 ng/ml, dl-propranolol). The unbound IC$_{50}$ values in these human studies were 1.29 to 2.77 ng/ml, which is similar to our observation (1.14 ng/ml). The corresponding values reported for dl-metoprolol are in agreement with the present findings (Abrahamsson et al., 1990).

high doses. During saturated protein binding the half-life is likely to increase, because the relative change in the volume of distribution is expected to be larger than in clearance (Evans et al., 1973). In addition, the beta-antagonists at these high concentration levels will decrease the blood flow and thereby the clearance, which suggest that an even longer half-life should be observed. The consequence on duration of action is difficult to interpret, but changes in kinetics is suggested to be larger than the difference in the effect slope.

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In a study performed on dogs, both propranolol and metoprolol equipotently reduced exercise-induced heart rate in a similar fashion (Åblad et al., 1980). In our study, we did not find large differences in the pharmacodynamics between the two drugs either. However, a 20-fold difference appears when the IC$_{50}$ values are corrected for protein binding, making propranolol the more potent drug. The difference in IC$_{50}$ values of different studies could be due to the exercise test used, i.e., duration, power and type. As the drugs are competitive antagonists, a higher degree of exercise (agonist) will shift the effect relationship toward higher concentrations (Wellstein et al., 1985).

Except for the large difference in protein binding between propranolol and metoprolol, they also differ in such factors as their respective affinities to the beta-1 and beta-2 receptors, membrane stabilizing activity and lipophilicity. Selectivity is a relative rather than an absolute property, because increasing doses of beta-selective antagonists results in a dose-related beta-2 adrenoceptor blockade (Lipworth et al., 1991) and because higher doses of a beta-2 adrenoceptor selective antagonist will reduce exercise-induced tachycardia (Harry et al., 1988). The different roles of beta-1 and beta-2 receptors in the genesis of cardiovascular responses have proved to be more complex, because of their coexistence in the heart (Brodd et al., 1983). The rat’s left ventricle contains about 74% beta-1 and 26% beta-2 adrenergic receptors (Vago et al., 1984). Due to the high density of beta-1 receptors in the heart, one would expect that they would contribute more to the positive inotropic and chronotropic responses than would beta-2 (Molenaar and Summers, 1987), and that the latter would play only a minor role in the heart rate alteration.

A major effect of beta antagonist therapy is increased AV nodal conduction time resulting in a prolonged AV nodal refractoriness (increased PR interval). Hence, beta antagonists are useful in terminating reentrant arrhythmias that involve the AV node and in controlling ventricular response in atrial fibrillation or flutter (Rodin, 1996). As it is difficult to standardize and use arrhythmias as pharmacodynamic endpoints, many studies have been performed in vitro. Pruett and coworkers (1977, 1980) found that d- and dl-propranolol shortened action potential duration with similar potency at a concentration as low as 100 ng/ml. The antiarrhythmic activity correlated better with the tissue propranolol concentration, and when this was taken into account, the minimum plasma concentration expected to produce an electrophysiologic effect in patients was 150 ng/ml. This was confirmed when 40% of the patients with ventricular arrhythmias responded to the drug at plasma concentrations in excess of 150 ng/ml, a level associated with a high degree of beta receptor blockade (Woosely et al., 1979). When d-propranolol was given separately, it suppressed ventricular arrhythmias both
through non-beta-mediated effect (includes membrane-stabilizing activity) and beta-mediated effect (Murray et al., 1990). Propranolol and metoprolol display a large difference in partition coefficient, where propranolol is much more lipophilic (logD 20.2) than metoprolol (logD 0.98). The large difference in lipophilicity could explain the difference in membrane-stabilizing activity, due to membrane dissolving capacity. The steeper slope of propranolol should then indicate that it would display more membrane-stabilizing activity as compared to metoprolol, which is in agreement with the literature (Hoffman and Leikowitz, 1996). Concluding that the linear component in the dual effect model could be membrane-stabilizing activity.

In conclusion, using spontaneous hypertensive rats to study the concentration-effect relationship of beta-blockers, with exercise-induced tachycardia as the pharmacodynamic endpoint, gives reliable results and may be a useful model to extrapolate to humans. The combined I\textsubscript{max} and linear concentration-effect relationship can be interpreted as a specific beta antagonist effect and a membrane-stabilizing effect, respectively.

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Send reprint requests to: Dr. Lena Brynne, Department of Pharmacy, Division of Biopharmaceutics and Pharmacokinetics, Box 580, S-751 23 Uppsala, Sweden.