Mechanism-Based Modeling of Rebound Tachycardia after Chronic \textit{l}-Propranolol Infusion in Spontaneous Hypertensive Rats\textsuperscript{1}

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ABSTRACT

The aims of the study were to characterize the rate and extent of the rebound effect after abrupt cessation of a chronic exposure of \textit{l}-propranolol in spontaneous hypertensive rats, using exercise-induced tachycardia as a pharmacodynamic endpoint. Thirty-two spontaneous hypertensive rats were randomized to receive either placebo or 4 or 8 mg/kg/day s.c. infusion of \textit{l}-propranolol for 11 days using osmotic minipumps. The heart rate was measured after standardized physical exercise before and during drug exposure and over 12 days after cessation, using a computerized tail-cuff method. Blood samples were collected after each effect measurement during the infusion. A similar reduction in exercise tachycardia was registered for the two doses. No apparent tolerance development was found, but both doses showed a clear rebound effect of similar extent and intensity. The maximal rebound effect was observed on the second day after cessation and was found to have a duration of about 6 days. A mechanism-based model was developed to describe the rate and extent of changes in \textit{\beta}-adrenoceptor up- and down-regulation with increased sensitivity of the transducer complex. The half-life of disappearance of up-regulated \textit{\beta}-adrenoceptors was estimated to be 2.0 days (1.0–3.9 days). The effect-versus-time data was analyzed by nonlinear mixed-effect modeling with the program NONMEM. A dose-dependent reduction in the growth of body weight was observed during drug treatment, which was reversible. A dose- and time-dependent increase in the \textit{\alpha}_1-acid glycoprotein concentration was also observed.

Rebound effects may occur on abrupt withdrawal of chronic \textit{\beta}-antagonist therapy and are a known phenomenon in patients with coronary heart disease. This phenomenon has been described for patients using propranolol, a nonselective \textit{\beta}-antagonist, but also for patients using other \textit{\beta}-antagonists (see Houston and Hodge, 1988, for a review). The symptoms seem to be related to the severity of the disease (Miller et al., 1975), where a worsening of angina, myocardial infarction, ventricular dysrhythmias, and sudden death have been observed after discontinuation of propranolol in patients with angina pectoris. Hypertensive patients have experienced transient symptoms of palpitations, tremors, headache, malaise, and sweating after drug cessation (Nattel et al., 1979).

Several different mechanisms have been proposed for these symptoms, but the hypothesis that has received the most interest is that a transient hyperadrenergic state follows drug cessation. This hypothesis has been confirmed in many but not all studies in humans (Boudoulas et al., 1977; Nattel et al., 1979) and in animals (Faulkner et al., 1973; Aarons et al., 1980; Cramb et al., 1984; Ebii et al., 1991). The hyper-sensitivity may relate in part to an increased \textit{\beta}-adrenoceptor density during chronic treatment (Aarons et al., 1980; Motulsky and Insel, 1982; Brodde et al., 1986). However, it could also be that there are changes in the efficiency of the coupling between the receptor and the second messenger system and/or an increased concentration of endogenous mediators such as catecholamines. Other explanations include the progression of underlying coronary artery disease, enhanced platelet aggregation or tri-iodothyronine levels, alteration in plasma renin activity, or continued high levels of physical activity despite withdrawal of propranolol (for reviews, see Prichard et al., 1983; Frishman, 1987).

The cardiovascular effects of \textit{\beta}-antagonists result in alterations in myocardial contractility and rate, which is the primary mechanism by which most \textit{\beta}-antagonists produce their therapeutic effects in conditions like angina and hypertension. Reduction in exercise-induced tachycardia is the most widely used method to measure the pharmacodynamic effect of \textit{\beta}-antagonists in humans. Exercise-induced tachycardia is

\textbf{ABBREVIATION:} AGP, \textit{\alpha}_1-acid glycoprotein.
also a reliable pharmacodynamic endpoint in spontaneous hypertensive rats when studying β-antagonists (Brynne et al., 1998). Changes in the response were detectable at low concentrations of the β-antagonists, and the effect was studied over a wide range of concentrations.

In contrast to the abundance of reports on rebound effects after the abrupt withdrawal of β-antagonists, only limited attention has been given to the quantitative aspects of the rebound effect despite the extensive use of β-antagonist in the clinical setting. Because of the complexity in tolerance and rebound development, physiological models have been difficult to obtain, although attempts have been made using the effect of propranolol and increased β-adrenergic receptor density (Lima et al., 1989).

Because of the large difference in potency between the two enantiomers of propranolol (Walle et al., 1988), the l-enantiomer was selected for this study. The binding of l-propranolol to plasma proteins, primarily to α,-acid glycoprotein (AGP), is high in the rat and saturable at high concentrations (Brynne et al., 1998). Thus, changes in the AGP level may affect the pharmacokinetics of propranolol (Yasuhara et al., 1988). Thus, changes in the level may affect the pharmacokinetics of propranolol (Yasuhara et al., 1988). Thus, changes in the level may affect the pharmacokinetics of propranolol (Yasuhara et al., 1988).

Materials and Methods

Animals. Male spontaneous hypertensive rats of 285 ± 17 (S.D.) g and 3 to 3.5 months old were obtained from Møllegaard Ejby, (Denmark). They were kept in a regulated room with a 12-h light/dark cycle (lights on 7:00 AM to 7:00 PM), a temperature of 22 ± 1°C, and humidity of 55 ± 5%. Standard diet and water were provided ad libitum, except during the effect measurement. The rats were kept individually in Macrolon cages, where they received 25 ml of a D-glucose solution (50 mg/ml; KEBO Lab, Stockholm, Sweden) in separate drinking bottles twice a week. The body weights were monitored throughout the experiment. The protocol was approved by the Animal Ethics Committee of Uppsala University.

Drug Delivery. The rats were randomized into three groups to receive the drug or placebo for 11 days. Group I received one Alzet osmotic minipump 2 ML2 (Alza Co., Palo Alto, CA) (4 mg/kg/day, n = 11), group II received two minipumps (8 mg/kg/day, n = 11), and the group III (placebo, n = 10) received one minipump. The minipumps were used to obtain a constant β-receptor blockade and contained l-propranolol hydrochloride (99% purity) in physiological saline solution at a concentration yielding a dosage of 4.0 mg/kg/day (mean weight). The concentration of l-propranolol was given with respect to the free base and a release rate of 5.60 μl/h. The pumps were incubated before implantation in sterile physiological saline solution at 37°C overnight (>8 h). The pumps were then implanted s.c. via a short incision between the shoulder blades in a small pocket with the animals under brief ether anesthesia (Prolabo, Manchester, England). The stability of the drug solutions was tested by taking samples before and after the implantation.

Blood Sampling and Effect Measurement. Venous blood samples (250 μl) were drawn from the hind paw 3 days before the start of infusion and on days 1, 4, 5, 7, 8, and 11 during the infusion. All blood samples were drawn immediately after the effect measurement, except those taken after cessation of the infusion. The infusions were stopped by removing the pumps under light ether anesthesia, subsequently after effect measurements and blood sampling (day 11). Additional blood samples were drawn 40, 130, and 210 min postinfusion. The blood samples were collected in heparinized (Lovens, Ballerup, Denmark) Eppendorf tubes and centrifuged at 7200g for 10 min; the plasma was immediately separated and frozen (at −70°C) pending chemical analysis.

A computerized rat tail-cuff system configured on a Macintosh computer was used (Kent Scientific Corporation, Litchfield, CT) to measure the heart rate. The system consists of WorkBench Mac data acquisition software, an analogue/digital board, a stacking amplifier, an automatic pump, and a tail-cuff with sensor. The WorkBench acquisition system is used to derive the systolic blood pressure and heart rate values; the signals are displayed on the computer monitor, and the values captured are automatically saved to a disk. The WorkBench software also controls the automatic pump to initiate and terminate cuff inflation when the set cuff pressure has been attained by the pump.

The rats were familiarized with the environment and the equipment. Exercise-induced tachycardia was obtained by making the animals run in a motorized wheel for 10 min (6 m/min). Then they were transferred to a Macrolon cage with a thermostat-controlled warm pad (37°C) for 10 min. The mean of three measurements, performed within 10 min, was used in the data analysis. The heart rate was measured over 3 consecutive days before the start of infusion and then on days 1, 4, 5, 7, 8, and 11 after the start of infusion. Six hours after surgical removal of the minipumps, an effect measurement was performed and continued on days 12, 13, 14, 15, 16, 18, 19, 21, and 22. The measurements were performed every day at the same time.

Chemicals for Analysis. Crystalline l-propranolol hydrochloride (99%), dl-metoprolol tartrate, l-leptanesulfonic acid (5 mM), 5α-androstan-3,17-dione, quinidine red (rat and human), AGP, albumin, and γ-globulin were obtained from Sigma Chemical Co. (St. Louis, MO). Glacial acetic acid (1%), hydrogen chloride (3 M), sodium chloride, disodium hydrogen phosphate anhydrous, sodium dihydrogen phosphate monohydrate, sodium hydroxide (4 M), sulfuric acid (0.5 M), and acetonitrile were obtained from Merck (Darmstadt, Germany). Diethyl ether for drug analysis was purchased from Fluka (Buchs, Switzerland). All solvents and reagents were of analytical grade.

Protein and Drug Analysis. Individual AGP concentrations were determined by the quinidine red method (Imamura et al., 1994) using rat AGP (Sigma Chemical Co.). This method was automated by using an analytical system (Brynne et al., 1998). The interday variability was <5%, and the limit of quantification was 0.08 mg/ml with a coefficient of variation of 17% (n = 6).

The l-propranolol concentrations in plasma and pump solutions were determined using HPLC with fluorescence detection as described by Rutledge and Garrick (1989), with some modifications (Brynne et al., 1998). Extraction of the analyte was performed by liquid-liquid extraction from 100-μl samples and standards. The chromatographic equipment consisted of a pump (LC-9A; Shimadzu, Kyoto, Japan), a μBondapak column (C18, 30 cm × 3.9 mm i.d.; Waters Associates, Milford, MA), a fluorescence detector (RF-535; Shimadzu), and an integrator (C-R5A; Chromatopac Schimadzu). The excitation and emission wavelengths were 235 and 335 nm. All sample injections were performed using a CMA/200 autoinjector (CMA, Solna, Sweden) fitted with a 100-μl loop. The standard curve was linear within the range from 1.8 to 400 ng/ml. The interday variation for l-propranolol in the concentration interval from 1.8 to 330 ng/ml was ±5%, and the accuracy ranged from 92 to 100%. The absolute recovery was between 100 (1.8 ng/ml) and 96% (326 ng/ml). The limit of quantification was 1.8 ng/ml.

Data Analysis. The individual plasma concentration-time profiles of l-propranolol were nonparametrically described by linear interpolation between the consecutive observed plasma concentra-
tions in the pharmacodynamic analysis. In a pilot study, steady-state plasma concentrations were obtained 3 h after the start of infusion with osmotic minipumps. In the present study, the first blood samples were not drawn until 24 h after the start of infusion, and therefore, the individual 24-h plasma concentration values were used after 3 h as well as after 24 h to obtain a more accurate plasma concentration-time profile. Individual terminal half-lives were calculated using standard procedures (Gibaldi and Perrier, 1982).

A mechanism-based model was developed (Fig. 1) in which l-propranolol competitively inhibits norepinephrine to activate the β-adrenoceptors and the transducer complex. This competitive antagonism results in an increased β-adrenoceptor density and a reduced heart rate. The classic equation for competitive antagonism (Gaddum, 1937)

$$\frac{R^*_0}{R_T} = \frac{C_{NE}/K_{D,NE}}{1 + C_{Prop}/K_{D,Prop} + C_{NE}/K_{D,NE}} \quad (1)$$

gives the fractional receptor occupancy by the agonist (R*/R_T) for any given concentrations of agonist and antagonist. The total amount of receptors (R_T) cannot be measured in vivo and therefore was set to start at one unity. This equation predicts that in the presence of a competitive antagonist, the fractional receptor occupancy by the agonist will be lower than that in absence of antagonist. In the equation, C_{NE} corresponds to the plasma concentration of norepinephrine, C_{Prop} corresponds to the l-propranolol concentration, and K_{D,NE} and K_{D,Prop} are the equilibrium dissociation constants for norepinephrine and l-propranolol, respectively. For simplicity, the ratio between the norepinephrine concentration and its K_{D} value is estimated. When no drug is present, the fractional number of activated receptors (R*_0/R_T) is described by the following equation:

$$\frac{R^*_0}{R_T} = \frac{C_{NE}/K_{D,NE}}{1 + C_{NE}/K_{D,NE}} \quad (2)$$

The change in total β-adrenoceptor density (R_T) over time is described by the following function:

$$f(R_T) = \frac{dR_T}{dt} = k_{rec} \cdot \left( 1 + \frac{R^*_0 - R^*}{R^*_0} \right) - k_{rec} \cdot R_T \quad (3)$$

where k_{rec} is the first order rate constant for degradation of β-adrenoceptor density. A linear slope (SL) is used to relate the fractional change in the number of activated receptors to the change in total β-adrenoceptor density. Two models were developed according to the hypothesis of mechanisms of the rebound phenomenon.

Model A—A competitive antagonist model with a change in transducer efficiency (T_s) (Black and Leff, 1983) due to the increased receptor density was described as follows:

Fig. 1. Schematic illustration of the mechanism-based model. Norepinephrine (NE) and propranolol (P) concentrations at the receptor (R) site forms an agonist-receptor (NE-R) and antagonist-receptor (P-R) complex. These complexes are regulated by a dissociation constant (K_D) and the agonist- or antagonist-receptor complex leads to activation (+) or inhibition (−) of the receptor, respectively. The strength of the signal from the activated (agonist-occupied) receptor (R*) is dependent on the fractional receptor occupancy (R*/R_T) where R_T represents the total amount of receptors. The feedback of the up- and down-regulation of the receptor density, which is controlled by a first order rate constant (k_{rec}), is related to the number of activated receptors. The sensitization of the transduction system (T_s) during drug exposure is controlled by a first order rate constant, k_{trans}, which is similar the change in receptor density and is assumed to be directly related to the heart rate (E).

$$T_s = \frac{E_{max} \cdot R^*}{K_T + R^*} - \frac{E_{max} \cdot R_0^*}{K_T + R_0^*} \quad (4)$$

where E_{max} is the maximal effect in response, and K_T is the fraction of activated receptors for producing 50% of maximal response.

Model B—A competitive antagonist model with both a change in receptor density (model A) and an increased sensitization of the transducer efficiency over time. The sensitization time profile is assumed to resemble the change in receptor density over time [f(R_T)], which is therefore denoted k_{trans} in Fig. 1.

$$T_s = f(R_T) \cdot \frac{E_{max} \cdot R^*}{K_T + R^*} - \frac{E_{max} \cdot R_0^*}{K_T + R_0^*} \quad (5)$$

In this model, the estimated rate constant for degradation of the β-adrenoceptor density (k_{rec}) from model A was used to estimate the increase in the extent of rebound tachycardia because there was a large correlation between k_{rec} and the slope (SL).

In both models, the change in efficiency of the transducer complex was directly related to the exercise-induced tachycardia (E), according to the following relationship:

$$E = E_0 \cdot (1 + T_s) \quad (6)$$

where E_0 is the heart rate baseline. The K_T value in the transducer equations was set to 3% but subject to sensitivity analysis because it is known that the β-adrenoceptor systems have a large amount of spare receptors in rats. Only 1.5 to 3% of β-adrenoceptors have to be occupied by isoproterenol to cause 50% of maximal response (Brown et al., 1992). The effect after placebo administration and the increase in body weight were not accounted for in the pharmacokinetic/pharmacodynamic data analysis.

Both concentration- and effect-versus-time data were analyzed by nonlinear mixed-effect modeling within the program NONMEM Version V (Beal and Sheiner, 1992), using the first order approximation method and ADVAN 8 as subroutines. Mean population parameters were assessed, as well as interanimal and residual variabilities. An exponential variance model was used to describe the interanimal variability, and an additive error model was used to characterize the residual errors in the pharmacodynamic models. Individual parameter values are obtained from the Bayesian estimation. Statistical discrimination between different models was made by comparison of the objective function values (−2log likelihood) calculated by NONMEM and by visual inspection of the goodness-of-fit plots in the program Xpose (Jonsson and Karlsson, 1998) running under Splus, Version 3.3 (Statistical Sciences, 1993). The difference between the objective function values for two hierarchical models is approximately χ² distributed and may consequently be used for model selection purposes.

All data are given as mean ± S.D. or with a 95% confidence interval unless otherwise stated. For statistical comparison of stability of the drug over time, the Student’s t test for paired data was used. The differences in AGP and weight between groups were compared by using one-factor ANOVA, followed by the Fisher PLSD test (StatView; Abacus Concepts, Inc., Berkeley, CA). The significance level was set to 95%.

Results

The stability test of the drug solution showed no significant difference after 11 days of s.c. infusion. The body weight of the animals increased during the study, but a reduced rate of change was observed during l-propranolol exposure at the higher dose level (Fig. 2), an effect that was reversible when l-propranolol was withdrawn. The difference in the body weight was only significant (p < .05) for the rats that received 8 mg/kg/day l-propranolol on day 11.
Pharmacokinetics. A statistical significant increase in AGP levels was observed on day 11 at the lower L-propranolol dose and on day 5 during the higher L-propranolol dose (Fig. 3), but no changes were observed in the control group.

The mean plasma concentration time profiles of L-propranolol for the two dose groups are presented in Fig. 4. A relatively large concentration range was obtained (221–448 and 89–213 ng/ml for the higher- and lower-dose group, respectively, on day 1). The rate of infusion from the minipumps declined over time. The terminal half-lives of L-propranolol, 1.31 ± 0.24 h and 1.13 ± 0.26 h, did not deviate between the two dose groups.

Postexercise Heart Rate over Time. The mean basal exercise-induced heart rate was 386 ± 15 beats/min (n = 11) and 383 ± 17 beats/min (n = 11) for the lower- and higher-dose groups, respectively, and 388 ± 19 beats/min in the control group, before the start of infusion (Fig. 4).

Similar reductions in exercise-induced heart rate during the L-propranolol infusion were observed in both groups (Fig. 4; the individual observed and predicted heart rate values were normalized to the corresponding baseline values). The mean reductions for the lower- and higher-dose groups were similar and varied between 12 and 14%. No apparent tolerance development was observed, but a clear rebound effect was obtained after the cessation of the drug. The rebound effect was similar for the two dose groups, where the rebound effect reached its maximum 2 days after cessation and had a duration of about 6 days for both doses. The intensity of the rebound effect was also similar in the two dose groups (about 6% increase). The placebo effect showed a transient increase in exercise-induced heart rate with a maximal effect on day 5.

Pharmacodynamic Modeling. The population pharmacodynamic estimates for model A (competitive model with increased receptor density) and model B (model A with increased transducer sensitivity) are listed in Table 1. The estimated half-life of the production and disappearance of the β-adrenoceptor was 2.0 days (1.0–3.9 days). The interanimal variability for each pharmacodynamic estimate is shown in Table 1, and the S.D. of the residual variability was 15 beats/min. Only a small rebound effect was predicted by model A, whereas model B gave a more accurate fit to data when adding a function describing increased sensitization of the transducer complex.

Fig. 2. The fractional increase in body weight before, during, and after drug treatment, showing a dose-dependent reduction in the growth rate for rats that have received 8 mg/kg/day (solid line; n = 11) compared with 4 mg/kg/day L-propranolol (dashed line; n = 11) or physiological saline solution (dotted line; n = 10; mean ± S.D.). *Significantly different from the control group and the lower-dose group.

Fig. 3. The AGP concentration time profiles for the three groups, showing a dose- and time-dependent increase in concentration in rats that have received 8 mg/kg/day (●; n = 11) compared with 4 mg/kg/day of L-propranolol (○; n = 11) or physiological saline solution (△; n = 10; mean ± S.D.). **Significantly different from control group. ***Significantly different from the lower dose group.

Fig. 4. The plasma concentration time profiles (mean ± S.D.) of L-propranolol for the two dose groups (dashed line, 4 mg/kg/day; solid line, 8 mg/kg/day; top). The observed changes (mean ± S.E.M.) in exercise-induced tachycardia for the placebo (●; n = 10), 4 mg/kg/day (○; n = 11), and 8 mg/kg/day (●; n = 11) dose versus time, and the mean of baseline-normalized individual predictions in each dose group (dashed line, 4 mg/kg/day; solid line, 8 mg/kg/day) for model A (A) and model B (B), respectively.
The increases in the receptor density are shown in the re-
played in Fig. 5, using parameter estimates from model B. 
concentration levels on the exercise-induced tachycardia, re-
garding the magnitude of the transducer sensitivity time profile (\(k_{\text{trans}}\) in Fig. 1), although with no success. The \(\beta\)-adrenoceptor rate constant \(k_{\text{rec}}\) was fixed in model B, and an increase in this rate constant resulted in a decrease in the linear slope (SL) and only a small increase in the equilibrium dissociation constant for propranolol \(K_{\text{D,Prop}}\). The initial \(\beta\)-receptor occupancy of norepinephrine was 12%, which corresponded to a maximal response of 80%, when \(K_T\) was set to 3%. An increase in the fraction of activated receptors for producing 50% of maximal response \(K_T\) resulted in a small decrease in the \(K_{\text{D,Prop}}\) value, a large increase in the ratio between the norepinephrine concentration and its \(K_N\) value, but no changes in the \(E_{\text{max}}\) value.

The \(\beta\)-receptor density was estimated to increase by 32% during drug exposure.

Predictions of the effect of different steady-state plasma concentration levels on the exercise-induced tachycardia, receptor density, and fractional activated receptors are displayed in Fig. 5, using parameter estimates from model B. The increases in the receptor density are shown in the response at steady-state concentrations around \(E_{\text{C50}}\) or below (in an ordinary \(E_{\text{max}}\) model). The different steady-state concentrations showed no larger difference in duration and intensity of the rebound effect. A larger concentration level will result in a larger increase in the receptor density, a larger reduction in activated receptors during drug infusion, and a larger amount of available receptors to be activated after drug withdrawal. The influence of different terminal half-lives of the drug on the response was also predicted by using parameter estimates from model B (Fig. 6). The longer half-life of the drug or the more slowly the drug is eliminated from the body, the less rebound effect will be displayed due to the smaller amount of receptors that will be available to activate.

### Discussion

The present study demonstrates that rebound tachycardia occurred after abrupt cessation of a chronic s.c. infusion of \(\beta\)-propranolol in spontaneous hypertensive rats, using exercise-induced tachycardia as the pharmacodynamic endpoint. The rebound effect was seen without any apparent tolerance development, which might be due to the limited information that could be obtained after the initial surgery. The rebound maximum, 2 days after drug withdrawal, and the 6-day duration correspond well with studies in hypertensive patients, where the transient hypersensitivity to isoproterenol or exercise-induced tachycardia commenced 2 to 6 days after cessation and lasted for 3 to 13 days (Nattel et al., 1979). The absence of a difference in effect between the two doses could be because the concentration range was near or at the maximal effect of the drug (about 15% reduction), which is close to an earlier observed maximal effect \((I_{\text{max}} = 21\%); Brynne et al., 1998). The transient increase in exercise-induced heart

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**TABLE 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Model A</th>
<th>Model B</th>
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<tbody>
<tr>
<td>(E_b) (beats/min)</td>
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<td>383</td>
</tr>
<tr>
<td>(E_{\text{max}}) (%)</td>
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<td>17.3</td>
</tr>
<tr>
<td>(K_{\text{D,Prop}}) (ng/ml)</td>
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<td>1.71</td>
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<tr>
<td>(C_{\text{NE}}/K_{\text{D,NE}})</td>
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<td>0.141</td>
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<tr>
<td>SL</td>
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<td>1.0</td>
</tr>
<tr>
<td>(k_{\text{rec}}) (day(^{-1}))</td>
<td>0.35</td>
<td>0.35</td>
</tr>
</tbody>
</table>

\(^a\) \(E_b\), heart rate baseline; \(E_{\text{max}}\), effect of maximal response; \(K_{\text{D,Prop}}\), the equilibrium dissociation constant for \(\beta\)-propranolol; \(C_{\text{NE}}/K_{\text{D,NE}}\), ratio between the norepinephrine concentration and its equilibrium dissociation constant; SL, linear slope that relates the fractional change in the number of activated receptors to the change in total \(\beta\)-adrenoceptor density; \(k_{\text{rec}}\), first-order rate constant for degradation of \(\beta\)-adrenoceptor density.

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rate could be viewed as a change in the endogenous norepinephrine concentration over time; however, at maximal drug effect, the relative contribution of an increased norepinephrine concentration is negligible.

Rebound effects generally occur after withdrawal of a drug to which tolerance has been developed. This is explained by the persistence of the effect being counteracted after drug cessation, which is often linked to the process of tolerance development (Bauer and Fung, 1994). Several pharmacodynamic models that describe both tolerance development and a rebound effect have been proposed. These models differ in the driving force of the effect, which could be either the drug concentration or the drug effect (see Gårdmark et al., 1999, for a review), and they require more or less tolerance development to be able to account for the rebound effect. They also are more or less empirical with respect to the absence of knowledge of the mechanism of drug action.

The β-antagonists act by preventing catecholamines from binding to the β-adrenoceptors and thereby inhibit the activation of adenylyl cyclase, which catalyzes the intracellular production of cyclic AMP and its stimulation of protein kinases. This cascade will result in a decreased level of intracellular calcium, which activates the cardiac troponin complex, thereby lowering the heart rate. The β-adrenoceptor density and the sensitivity of the adenylyl cyclase will increase during the exposure of antagonist and slowly return to normal conditions after cessation of the drug (Aarons et al., 1980; Motulsky and Insel, 1982). We propose a mechanism-based model that includes a time-dependent increase in the β-adrenoceptor density during drug exposure and spare receptors (model A). An additional function describing a sensitization of the transducer complex was needed to better describe the extent of the rebound effect (model B). Both models described the fast reduction in exercise-induced tachycardia during drug exposure. A drawback was that none of the models were successful regarding the peak time of the rebound effect. Although efforts were made to include both magnitude and time delay in the sensitization of the transducer complex, increasing model size resulted in either unacceptably low parameter precision or numerical difficulties of the integration routine used. The rate-limiting step could be the distribution of the drug to the receptor or at the postreceptor level. Recently, Sheiner and Verotta (1995) presented a general model in which they proposed that the pharmacodynamic response consists of a cascade of dynamic and static functions. Depending on whether distributional or postreceptor events form the rate-limiting step, the general model would collapse into a direct (Sheiner et al., 1979) or an indirect (Dayneka et al., 1993) response model, respectively, and these models should be considered as submodels of the general model. The present mechanism-based model includes both prereceptor and postreceptor events and thus is related to the general model. The time course of reduction in exercise tachycardia after a single dose of propranolol is reflected by the antagonist concentration present in plasma samples at each time point, and the exercise tachycardia parallels the β1-adrenoceptor occupancy in humans (Wellstein et al., 1985; De Mey, 1997). This means that the plasma concentrations of antagonist are representative of the concentrations in the effect compartment. Therefore, it is most likely that the sensitization of the transducer complex or other postreceptor events should be the rate-limiting step. Another rate-limiting step could be down-regulation of β-receptors, where the synthesis of the receptor could be reduced or the degradation be enhanced (Lohse, 1993).

Both models predicted rebound tachycardia, although model B did so to a larger extent, thus suggesting that a sensitization of the transducer complex occurs during the drug exposure. The steady-state concentration used in the present study resulted in similar reductions and rebound tachycardia. It would have been interesting to use a lower dose to obtain concentrations below the IC50 value of I-propranolol (18.1 ng/ml; Brynne et al., 1998) to be able to register the increase in receptor density by less reduction in heart rate during steady state, according to the simulations in Fig. 5. However, registrations of small changes in heart rate (at low plasma concentrations) are difficult due to the variability in the tail-cuff technique. The influence on the rebound tachycardia due to different half-lives of the drug was also simulated, which was shown to be large. This indicates that the pharmacokinetics of the drug (longer half-life) or a slow withdrawal of the drug will reduce the extent of rebound tachycardia (see Fig. 6).

The β-adrenoceptor density was estimated to increase by 32% (model B), which is similar to values reported from human studies on β2-adrenoceptors lymphocytes (25–51%; Fitzgerald et al., 1981; Brodde et al., 1986; van den Meiracker et al., 1989). The half-life of β-receptor degradation was 2.0 days (1.0–3.9 days) in the present study. It is little less than the mean recovery rates reported in healthy
volunteers, where the half-life was 2.7 days (Reeves et al., 1989). The large variability in the half-life of the β-receptor degradation and the linear receptor relationship in our model were probably due to a large variability in the rate and extent of rebound tachycardia between rats. A similar mechanism-based model was presented previously by Lima et al. (1989), using literature values for computer simulations. This model uses a relationship between the parasympathetic and sympathetic nervous systems to simulate the heart rate response over time, but it does not account for spare receptors or sensitization of the transducer complex. Furthermore, this model showed a very small extent of rebound tachycardia in comparison with the reduction in isoprotein-induced tachycardia during drug exposure (maximal effect of 100%). This model also uses a half-life of 1.5 days for the β-receptor on lymphocytes (Aaron and Molinoff, 1982), which is low in comparison with the 4.0 days reported in healthy volunteers (Reeves et al., 1989).

The dose- and time-dependent increase in the AG concentration could be due to activation of the immune system at high propranolol concentrations. It is known that elevated sympathetic activity can modulate the immune system, and that withdrawal of this activity by the administration of drugs such as propranolol can induce the activity of the immune system (Maiel et al., 1991); although this has not been observed previously for AG, it has been observed for other parameters of immunity. Another possible explanation for the increase in AG level could be infection, inflammation, or stress because the AGP level increases in these situations. However, because no changes were observed in the control group, a non-drug-induced increase is unlikely. The increase in AG was unexpected, and further investigations are needed for correct interpretation of this phenomenon. However, during both infusions, the $E_{\text{max}}$ value was obtained, and a change in AG will have only a marginal influence on the effect. Consequently, fluctuations in AG concentration are likely to be important around and below the EC$_{50}$ value, that is, when the infusions have been stopped.

The dose-dependent reduction in the growth rate observed in this study was reversible after l-propranolol withdrawal. Similar findings have been shown in Wistar rats at high propranolol doses (Paraskevopoulos et al., 1991) without any alterations in the plasma growth hormone or hypothalamic somatostatin concentrations. We observed that the rats receiving the higher dose became sedated during drug exposure. A possible explanation is that the rats that received the higher-dose level were too sedated to eat and had a more pronounced lack of appetite. The reason behind the growth retardation is unknown at present and requires further investigation.

In conclusion, similar intensities and durations of the rebound effect have been reported in human studies as in the present study, suggesting that this could be a suitable animal model in which to study rebound phenomenon, although further studies must be performed. This is an attempt to mechanistically characterize the rate and extent of the rebound effect in spontaneous hypertensive rats after l-propranolol infusion. The result suggests that additional studies on cAMP turnover and/or mRNA regulation of β-receptors are necessary for a better characterization of the rebound phenomenon.

References

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