Consequence of Exercise on the Cardiovascular Effects of l-Propranolol in Spontaneously Hypertensive Rats¹

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ABSTRACT
The aim of this study was to elucidate time dependence in the development of rebound effect and to quantify the cardiovascular effects of chronic l-propranolol infusions in spontaneously hypertensive rats. Heart rate and systolic and diastolic blood pressures were monitored both during exercise performance and later by using telemetry. The pharmacodynamics were determined after different infusion lengths of l-propranolol (4 mg/kg/day) or placebo for 4, 8, or 12 days. A pronounced reduction in heart rate over time was found, which was interpreted as a positive influence of exercise on heart rate and was less marked in drug-treated animals. A mechanism-based model that accounts for competitive antagonism, spare receptors, the positive influence of exercise on heart rate, and circadian variations was used to describe the data. An empirical effect compartment model with an $E_{\text{max}}$ model was related to a circadian baseline and describes the relationship between plasma concentrations and reduction in blood pressures. The potencies for exercise and postexercise systolic blood pressure were similar with EC$_{50}$ values of 48 and 56 ng/ml, and the corresponding maximal effects were 17.8 and 21.9%, respectively. The EC$_{50}$ values and maximal effects for diastolic blood pressure were 26 and 5 ng/ml and 20.6 and 21.0%, respectively. The effect of l-propranolol could be quantified by a mechanism-based model in the presence of a positive influence of exercise on the heart rate. The effect of l-propranolol on the blood pressures is best described by an effect compartment model with circadian variations.

The β-adrenoceptor antagonists are among the most widely prescribed drugs for the treatment of diverse cardiovascular diseases. Soon after their introduction, case reports of abrupt withdrawal of β-antagonists resulting in hypertension, tachycardia, and occasional precipitation of myocardial ischemia in hypertensive patients were reported, which sometimes exceeded pretreatment levels (i.e., rebound effect) (see Houston and Hodge, 1988, for a review). The pathophysiology of rebound effects, its frequency and timing after withdrawal of β-antagonists, is contradictory, and there is disagreement regarding its very existence in the literature, as seen in studies in both humans (Boudoulas et al., 1977; Nattel et al., 1979) and animals (Aarons et al., 1980; Cramb et al., 1984; Ebii et al., 1991; Brynne et al., 1999).

The mechanisms behind the rebound phenomenon have been suggested to be a transient increase in the sympathetic nervous system after withdrawal of these drugs, due to up-regulation of the β-adrenoceptors during drug exposure (Aarons et al., 1980; Motulsky and Insel, 1982; Brodde et al., 1986). Besides an increase in receptor density and changes in the efficiency of the receptor stimulus transfer (i.e., coupling between receptor and second messenger system), mediators such as catecholamines may be depleted or physiological adaptation mechanisms may play a role. Other suggestions have been that the rebound effect could be a result of the recurrence of previously suppressed symptoms after the cessation of effective β-blockade or of progression of underlying disease, with symptoms being concealed during β-blockade due to a supersensitivity or rebound response to abrupt cessation of β-antagonist treatment (for reviews, see Prichard et al., 1983, and Frishman, 1987).

Rebound effect frequently, but not generally, occurs after withdrawal of a drug that has developed tolerance. Tolerance is often explained by a persistence of an opposing effect that will be revealed after disappearance of the drug and is tightly linked to the process of tolerance development. In a previous study, no apparent tolerance development was observed; however, a rebound effect occurred after the withdrawal of l-propranolol in spontaneously hypertensive rats (SHR) (Brynne et al., 1999). Because tolerance development is manifested as a time-dependent phenomenon (Shi et al., 1993), it is important to determine whether a time-dependent development of rebound effect occurs after the abrupt cessation of l-propranolol treatment. Pharmacodynamic modeling can offer a tool to quantify the rate and extent of rebound effect.

ABBREVIATIONS: AGP, α₁-acid glycoprotein; SHR, spontaneously hypertensive rats.
Because the mechanism of action of β-antagonists is known, a mechanism-based model was previously developed to account for the changes in the receptor density and the number of activated receptors over time (Bryne et al., 1999).

The β-antagonists reduce competitive catecholamine action, and the degree of cardiac β-blockade is assessed in the presence of increased adrenergic activity (McDevitt, 1989). In our studies, we have used exercise to induce tachycardia (stimulating the sympathetic nervous system), and it has been shown to be a reproducible pharmacodynamic end point in SHR when studying β-antagonists (Bryne et al., 1998).

Besides heart rate, both systolic and diastolic blood pressures are simultaneously recorded when using telemetry. The cardiovascular system is very complex and involves many control mechanisms with different time domains and gains (short- or long-term activity; Struyker Boudier, 1992). Except for a reduction in myocardial contractility and cardiac output, β-antagonists affect the circulatory system through a number of mechanisms. The major control mechanisms involved in cardiovascular regulation could be divided into four main mechanisms: neuronal reflexes, endocrine mechanisms, renin mechanisms, and structural adaptations. Because of this complexity, pharmacodynamic modeling of blood pressures is often empirically modeled.

The study objectives were to elucidate time dependence in the cardiovascular effects of l-propranolol in SHR during and after exercise, with a special emphasis on rebound effects. Circadian rhythms in both heart rate and blood pressures were assessed.

Materials and Methods

Animals. The study was performed on 21 male SHR (Møllegaard, Ejby, Denmark) with a mean weight of 295 ± 10 g and an age of 3 to 3.5 months. They were housed individually under controlled conditions in a temperature- (22 ± 1°C) and humidity- (55 ± 5%) regulated room, with a 12-h light/dark cycle (7:00 AM to 7:00 PM light). Standard diet and water were freely available, and their body weights were monitored throughout the experiment. The protocol was approved by the Swedish Animal Experimental Committee.

Implantation of Telemetry Transmitters. At least 1 week before the start of an experiment, the telemetry transmitters (C40 implants; Data Sciences International, St. Paul, MN) were placed in the descending aorta under inhalation anesthesia (2.5% enflurane [Abbott Laboratories, Chicago, IL] and 1.5% nitrous oxide balanced with 1.5% oxygen) using a Tec5 Vaporizer (Ohmeda Inc., Madison, WI) for animals. During surgery, the body temperature was monitored and maintained at 37°C by using a CMA-150 animal warmer (CMA, Solna, Sweden). Hair was removed from the abdominal area, and the rat was put in a sterile drape. The peritoneal cavity was opened by a 3- to 4-cm-long incision in the midline, and a small hole was made in the aorta using a 22-gauge needle. The catheter of the telemetry transmitter was inserted into the abdominal aorta and secured with small medical-grade tissue adhesive from a cellulose fiber patch. A small amount of lidocaine solution (Astra AB, Södertälje, Sweden) was used to relax the aorta after cannulation to prevent thrombi. The body of the telemetry transmitter was fixed with resorbable 3-0 silk sutures in the midline of the muscle layer before closing up the skin with wound clips. The rats were transferred to a 37°C warming pad with towels during recovery from anesthesia, and gentamicin (5 mg/kg i.p.; Schering-Plough, Madison, NJ) was administered twice during 1 day after surgery.

Telemetry System (Data Sciences International). The system consists of blood pressure sensors (TA11PA-C40), receivers (RLA1020), and a consolidation matrix (BCM100) that relay information from the telemetry receivers. One ambient pressure monitor (APR-1) was coupled to the consolidation matrix to calibrate the analog output signals with the ambient atmospheric pressure during data collection to convert telemetered waveforms to pressure in millimeters of mercury. The implantable transmitter is a 4.5-ml cylinder with an attached fluid-filled catheter. The tip of the catheter is filled with a patented gel and coated with an antithrombogenic film to inhibit thrombus formation. The system was configured to monitor each rat for 10 s every 2 min (sampling rate of 500 Hz) at each effect measurement period. The diastolic, mean, and systolic blood pressures, as well as heart rate and activity, were recorded and analyzed by the Dataquest IV system (Data Sciences International). The sampling procedure was regulated by the program Dataquest IV Data Acquisition LabPro version 3.0, which uses a Win/OS-2 operating system. All online data were saved to disk (AST Bravo MS P133 16/2GB). The battery could be switched on and off by use of a magnet. The gross activity was registered as counts from the receivers. All transmitters work on the same radiofrequency, and the animals are therefore housed singly.

Drug Administration. The rats received either l-propranolol (4 mg/kg/day for 4, 8, and 12 days, n = 5, 5, and 5, respectively) or placebo (physiological saline solution; Pharmacia & Upjohn AB, Stockholm, Sweden) for 4 (n = 1), 8 (n = 1), or 12 (n = 4) days. To obtain a constant β-blockade, osmotic minipumps (Alza Co., Palo Alto, CA) were used and filled with l-propranolol hydrochloride (99% purity, Sigma Chemical Co., St. Louis, MO) in physiological solution (Pharmacia and Upjohn AB, Stockholm, Sweden) 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 osmotic minipumps were incubated overnight (>8 h) in sterile physiological saline solution at 37°C before implantation. The pumps were then implanted s.c. via a short incision between the shoulder blades in a small pocket under brief ether anesthesia (Prolabo, Manchester, England).

Blood Sampling and Effect Measurement. Blood samples of 100, 200, and 320 µl were drawn venously from the hind paw both 5 days after start of the infusion in all groups and thereafter at days 1, 2, 3, and 4 in the 4-day infusion group; Pharmacia & Upjohn AB, Stockholm, Sweden) for 4 (n = 1), 8 (n = 1), or 12 (n = 4) days. To obtain a constant β-blockade, osmotic minipumps (Alza Co., Palo Alto, CA) were used and filled with l-propranolol hydrochloride (99% purity, Sigma Chemical Co., St. Louis, MO) in physiological solution (Pharmacia and Upjohn AB, Stockholm, Sweden) 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 osmotic minipumps were incubated overnight (>8 h) in sterile physiological saline solution at 37°C before implantation. The pumps were then implanted s.c. via a short incision between the shoulder blades in a small pocket under brief ether anesthesia (Prolabo, Manchester, England).

Blood Sampling and Effect Measurement. Blood samples of 100, 200, and 320 µl were drawn venously from the hind paw both 5 days after start of the infusion in all groups and thereafter at days 1, 2, 3, and 4 in the 4-day infusion group; at days 1, 2, 4, 6, 7, and 8 in the 8-day infusion group; and at days 1, 2, 5, 6, 7, 10, 11, and 12 in 12-day infusion groups. On day 1, blood samples were drawn at 1.5, 3, and 6 h after start of the infusion in all groups. When the infusions were stopped, the first blood sample was taken within 20 to 110 min and the second at 2.5 h after the first one (sampling window). The samples were collected in heparinized (Loven, Ballerup, Sweden) Eppendorf tubes and centrifuged at 7200g for 10 min, and plasma was immediately separated and frozen (−70°C) pending chemical analysis.

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 their individual Macrolon cages. Effect measurements were performed four times a day (between 8:00 and 10:00 AM, 11:00 AM and 1:00 PM, 2:00 and 4:00 PM, and 5:00 and 7:00 PM) during the 2 baseline days and on the days of infusion start and stop. All other effect measurements were performed twice daily (between 8:00 and 10:00 AM and 5:00 and 7:00 PM). The rats were always measured at the same time of day. The effects were captured at the end of the 10-min exercise and after a 12-min rest.

Protein and Drug Assay. Individual α1- and α2-adrenergic receptor 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 (Bryne et al., 1998). The interday variability was <8%, 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 were determined us-
ing 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 column, 30 cm × 3.9 mm i.d.; Waters Associates, Milford, MA), a fluorescence detector (RF-535; Shimadzu), and an integrator (C-R5A Chromatopac; Shimadzu). The excitation and emission wavelengths were 235 and 335 nm. All sample injections were performed using a CMA/A200 autoinjector (CMA, Solna, Sweden) fitted with a 100-μl loop. The standard curve was linear within the range 1.8 to 400 ng/ml. The interday variation for l-propranolol in the concentration interval 1.8 to 330 ng/ml was ≤5%, and the accuracy varied between 92% and 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. Model fitting was carried out within the NONMEM V software (Beal and Sheiner, 1992) using the first-order approximation method. Mean population parameters were assessed as well as interanimal and residual variability. Individual parameter values were obtained from the bayesian estimation. An exponential variance model was used to describe the interanimal variability for all parameters except for the blood pressure baselines, where an additive variance model was used. The residual errors in the pharmacodynamic models were characterized by an additive error model. Statistical discrimination between different models was made both by comparing the objective function values (two times the log likelihood value), as calculated by NONMEM, and by visual inspection of the goodness-of-fit plots in the program Xpose (version 2) (Jonsson and Karlsson, 1998). The difference between the objective function values for two hierarchical models is approximately χ²-distributed and may consequently be used for model selection purposes. In this study, P < .05 was used as the statistical significance level.

The l-propranolol plasma concentration-versus-time profile for each rat was modeled nonparametrically with linear interpolation between consecutive plasma concentration observations in the pharmacodynamic evaluation. Individual terminal half-lives were calculated using standard procedures (Gibaldi and Perrier, 1982).

Two different physiological conditions were assessed in the pharmacodynamic evaluations: at the end of the 10-min exercise (exercise data) or after a 12-min rest (postexercise data). The pharmacodynamic responses were observed to change over time in the placebo group. A steep descending slope described the exercise heart rate. For both systolic and diastolic blood pressure, slowly ascending slopes were used for both exercise and postexercise data. The heart rate and blood pressure baselines displayed circadian rhythm in both the placebo and the drug models. Because only a limited part of the rhythm was recorded, two baseline values, one for the morning and evening, were estimated in the models, and a slope between these baseline values was calculated from intermediate data.

Different pharmacodynamic models for the drug effect were tested on both heart rate and blood pressure data. In a previous study, we presented a mechanism-based model for exercise-induced tachycardia, where l-propranolol competitively inhibits norepinephrine to activate the β-adrenoceptors, which via the transducer complex produce cAMP, which is assumed to be directly related to the heart rate (see Fig. 1) (Brynne et al., 1999). The pharmacological effect is assumed to be a function of the concentration of agonist-occupied receptors (R*),

\[ R^* = \frac{R_T \cdot (C_{NE}/K_{D,NE})}{1 + C_{PROP}/K_{D,PROP} + C_{NE}/K_{D,NE}} \]  

where \( R_T \) is the total amount of receptors (100%). In the equation, \( C_{NE} \) and \( C_{PROP} \) correspond to the plasma concentration of norepinephrine and l-propranolol, respectively. \( K_{D,NE} \) 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 \( K_0 \) value is estimated. In absence of drug, the ratio between \( C_{PROP} \) and its \( K_0 \) value approaches zero in eq. 1.

The change in total β-adrenoceptor density (\( R_T \), normalized so that at baseline \( R_T \) is 1) over time is described by the following function:

\[ \frac{dR_T}{dt} = k_\infty \cdot \left(1 + \frac{S \cdot (R^* - R^\infty)}{R^\infty}ight) - k_{rec} \cdot R_T \]  

where \( k_{rec} \) is the first order rate constant for degradation of β-adrenoceptor density and \( R^\infty \) is the number of activated receptors when no drug is present. At baseline, the production rate of receptors is equal to the degradation rate and because \( R_T \) at baseline is set to 1, the zero order production rate constant \( k_\infty \) will be equal in value to the first order degradation rate constant \( k_{rec} \). 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. Due to lack of information about \( k_{rec} \) and SL in these present data, these parameters were fixed to values obtained in a previous study (Brynne et al., 1999).

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

\[ E = E_0 \cdot \left(1 - \frac{E_{max} \cdot R^\infty}{K_T + R^\infty} - \frac{E_{max} \cdot R^\infty}{K_T + R^\infty}ight) \]  

where \( E_0 \) is the heart rate baseline, \( E_{max} \) is the maximal effect in response, and \( K_T \) is the fraction of activated receptors for producing 50% of maximal response. The \( K_T \) value in the transducer equations was set to 3%, as it is known that the β-adrenoceptor system has a large amount of spare receptors in rats (Brown et al., 1992). The positive influence of exercise was described by a descending slope, which was affected by the drug during the infusion. Simulations of a ±50% change in \( k_{rec} \), SL, \( K_{D,PROP} \), and \( K_0 \) were performed, because fixed values of these parameters were used in the data analysis (Brynne et al., 1999).

Because the reduction of blood pressure is a multifactorial process, empirical models are often used. Two empirical effect models with different time delays were tested: the transit compartment model (Sun and Jusko, 1998) and the effect compartment (link) model (Sheiner et al., 1979). In the transit compartment model, the time delay was between the direct receptor-elicited response and the observed change in systolic or diastolic blood pressure according to the following equations:

\[ R = \frac{E_{max} \cdot C}{EC_{50} + C} \]
where $R$ is the response, $EC_{50}$ is the concentration that corresponds to 50% of the maximal response ($E_{\text{max}}$), $E$ is the change from baseline in the observed cardiovascular effect, and $\tau$ is the transit time constant. To allow for different shapes in the delay, different numbers of transit compartments, from one to five, were tested.

In the effect compartment model, the first order time delay was between plasma and effect compartment concentration, as governed by the rate constant, $k_e$. Different functional forms (i.e., linear, $E_{\text{max}}$, and sigmoid $E_{\text{max}}$) were tested, where an $E_{\text{max}}$ model related to baseline best described the relationship between the effect-site concentration of $\beta$-propranolol and blood pressure, according to the following equation (Holford and Sheiner, 1982):

$$E = E_0 \cdot \left(1 - \frac{E_{\text{max}} \cdot C_e}{EC_{50} + C_e}\right)$$  

(6)

where $C_e$ is the effect site concentration, $E$ is the cardiovascular response, and $E_0$ is the no-drug response. The placebo effect was assumed to be additive to the drug effect, affecting the baseline in all pharmacodynamic models.

Unless otherwise indicated, all data are reported as the mean ± S.D. The differences in half-lives, steady-state concentrations, and weight between the groups were compared by using a one-factor ANOVA (StatView; Abacus Concepts, Inc., Berkeley, CA). The significance level was set at 95%.

Results

Pharmacokinetic Data. All rats gained weight by 4.5 ± 1.1% per week, with no difference among the four groups. The steady-state plasma concentration of $\beta$-propranolol declined by one-third to one-half during the infusion (Fig. 2). Similar steady-state concentrations were observed in the three dose groups, and the half-lives were 2.00 h regardless of infusion length. The AGP levels were constant over time in all groups, except for an increase at the end of the 12-day infusion.

Descriptive Pharmacodynamics. The time course of the exercise heart rate is shown in Fig. 3. A pronounced reduction of about 100 beats/min was observed in the placebo group over time, which is interpreted as a positive influence of exercise on the heart rate. Initially, $\beta$-propranolol produced a rapid decrease in heart rate, which was more pronounced in the 4-day treatment group than in the 8- and 12-day treatment groups. The heart rate did not return to pre-drug-administered values but rather to a level that was very near the placebo baseline level for the 4-day infusion group. However, the 8- and 12-day infusion groups’ heart rate did not return to a level above their placebo baselines but rather to a level that was less than the pre-drug-administered value. None of the infusion lengths displayed rebound effect.

Similar time profiles were observed for both systolic and diastolic blood pressure and exercise or postexercise data. Figure 4 depicts the time course of systolic blood pressure during exercise for the three infusion lengths and placebo. A small increase in the systolic and diastolic blood pressure

![Fig. 2. Individual plasma concentration-time profiles of $\beta$-propranolol for the three s.c. infusion periods (4, 8, and 12 days).](image)

![Fig. 3. Mean ± S.D. exercise-induced heart rate. Observations over time for placebo (top) and the 4- (top middle), 8- (bottom middle), and 12- (bottom) day infusion groups. The fit of the mechanism-based model, including circadian rhythm (solid line) and the linear placebo model (dotted line), is also shown in all graphs.](image)
baselines over time was observed, in both exercise as well as in postexercise data. The effects of \( l \)-propranolol infusions on blood pressures were less rapid during exercise compared with postexercise. A larger reduction in systolic blood pressure was observed during exercise in comparison with postexercise. However, similar reductions were observed in the diastolic blood pressure for the three infusion lengths, both during exercise and postexercise. Both systolic and diastolic blood pressure returned to values slightly above the predrug-administration values, which were similar to the placebo baseline. No time-dependent rebound effect was observed in exercise or postexercise or systolic or diastolic blood pressure data, respectively.

Pharmacodynamic Models. Our final population pharmacodynamic estimates and interanimal variability for exercise heart rate are shown in Table 1. The positive influence of exercise on heart rate was estimated to be similar in the placebo and 4-day treatment groups, which was less marked in the 8- and 12-day treatment groups (Fig. 3). The total number of receptors was found to increase by approximately 17% during drug treatment. The initial \( \beta \)-receptor occupancy of norepinephrine was 14%, which corresponded to a maximal response of 82% when the fraction of activated receptors for producing 50% of maximal responses (\( K_T \)) was set to 3%. The maximal reduction in exercise-induced tachycardia was predicted to be 18.2% (\( E_{\text{max}} \)).

Because some of the parameters of the model were fixed, we investigated the influence of the present model. A \( \pm 50\% \) change in the rate of \( \beta \)-receptor degradation (\( k_{\text{rec}} \)) had no effect on the pharmacodynamic profile, and the slope (\( SL \)) had only a minor effect on the pharmacodynamic profile, displaying a 0.5% positive or 0.3% negative deviation from the original pharmacodynamic profile during steady-state levels. A similar change in \( K_{D,\text{Prop}} \) and \( K_T \) affected the steady-state level, resulting in a 2.4% positive or 1.7% negative deviation from the original pharmacodynamic profile for changes in \( K_{D,\text{Prop}} \), the equilibrium dissociation constant for \( l \)-propranolol, and a 2.0 or 0.9% positive deviations for changes in \( K_T \).

Among the two empirical model tested for describing the blood pressure data, the effect compartment model was preferable to the transit compartment model. The objective function value increased by 24 to 49 units for all blood pressures except systolic measured in the wheel, where an increase of 7 units was observed, when using the transit compartment model. In three of the four data sets, the better fit of the link model was also evident from goodness-of-fit graphics. In the effect compartment model, the relationships between plasma concentrations and reduction in blood pressures were described by an \( E_{\text{max}} \) model, which was relative to the baseline. The final population estimates for systolic and diastolic blood pressures are shown in Tables 2 and 3, respectively. Similar maximal effects were observed in both systolic and diastolic blood pressures during the two physiological conditions. The \( EC_{50} \) values during exercise are reduced when less concentration of an agonist is produced (postexercise data) in dia-

### Table 1

Population pharmacodynamic estimates of exercise-induced heart rate (CV%)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Heart Rate</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate (CV%)</td>
<td>IAV(^a)</td>
</tr>
<tr>
<td><strong>Placebo model</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base (morning) (beats/min)</td>
<td>438 (2)</td>
<td>5</td>
</tr>
<tr>
<td>Base (evening) (beats/min)</td>
<td>432 (2)</td>
<td>N.E.</td>
</tr>
<tr>
<td>Slope (beats/min/day)</td>
<td>0.00524 (14)</td>
<td>N.E.</td>
</tr>
<tr>
<td>Drug on slope (beats/min/day) (ng/ml)</td>
<td>0.00866 (10)</td>
<td>N.E.</td>
</tr>
<tr>
<td><strong>Drug effect model</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( E_{\text{max}} ) (%)</td>
<td>18.2 (7)</td>
<td>N.E.</td>
</tr>
<tr>
<td>( K_{D,\text{Prop}} ) (ng/ml)</td>
<td>1.71 N.E.</td>
<td>100(^a)</td>
</tr>
<tr>
<td>( C_{\text{max}}/K_{D,\text{NK}} )</td>
<td>0.157 (26)</td>
<td>N.E.</td>
</tr>
<tr>
<td>( SL )</td>
<td>0.193 (N.E.)</td>
<td>N.E.</td>
</tr>
<tr>
<td>( k_{\text{rec}} ) (day(^{-1}))</td>
<td>0.35 (N.E.)</td>
<td>N.E.</td>
</tr>
</tbody>
</table>

\(^a\) Interanimal variability (CV%).

\(^a\) N.E., not estimated, due to low interanimal variability.

\(^a\) Fixed values from Brynne et al. (1999).

\(^a\) Set at 100%.  

![Fig. 4. Mean \( \pm \) S.D. exercise systolic blood pressure. Observations over time for placebo (top) and the 4- (top middle), 8- (bottom middle), and 12- (bottom) day infusion groups. The fit of the effect-compartmental model, including circadian rhythm (solid line) and the linear placebo model (dotted line), is also shown in all graphs.](image-url)
TABLE 2
Population pharmacodynamic estimates of systolic blood pressure (CV%) during exercise and postexercise

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Exercise</th>
<th>Postexercise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate (CV%)</td>
<td>IAV</td>
</tr>
<tr>
<td>Placebo model</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base (morning) (mm Hg)</td>
<td>215 (2)</td>
<td>5</td>
</tr>
<tr>
<td>Base (evening) (mm Hg)</td>
<td>207 (2)</td>
<td>5</td>
</tr>
<tr>
<td>Slope (mm Hg/day)</td>
<td>0.15 (130)</td>
<td>47</td>
</tr>
<tr>
<td>Drug effect model</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{max}$ (%)</td>
<td>17.8 (31)</td>
<td>N.E.</td>
</tr>
<tr>
<td>$EC_{50}$ (ng/ml)</td>
<td>48.2 (79)</td>
<td>39</td>
</tr>
<tr>
<td>$k_{in}$ (day$^{-1}$)</td>
<td>0.21 (22)</td>
<td>170</td>
</tr>
</tbody>
</table>

* Interanimal variability (CV%).

The standard deviation of the residual variability was estimated to be within 8 to 11 mm Hg in all blood pressure analyses and 23 beats/min in the heart rate analysis.

**Discussion**

The purpose of this was to elucidate whether there is a time dependence in the development of the rebound effect, which has been shown to occur after abrupt cessation of chronic $l$-propranolol infusion in SHR (Brynne et al., 1999). A mechanism-based model has previously been developed to describe the pharmacological development of the rebound effect with $l$-propranolol (Brynne et al., 1999). This model includes both competitive antagonism and spare receptors, and it was able to describe the present data, although no rebound effect did occur. Instead, a pronounced decrease in exercise heart rate over time was observed in the placebo animals. This positive influence of exercise on heart rate was also shown in drug-treated animals, although to a less marked extent.

Rebound effect was expected in this study, but instead a decrease in exercise heart rate was observed in the placebo group. A possible explanation for both the absence of a rebound effect and the time-dependent decrease in heart rate could be the current 3-fold increase in the number of exercise occasions compared with an earlier study (Brynne et al., 1999). Exercise is associated with the release of catecholamines, and recurrent exposure to catecholamines decreases the density and sensitivity of $\beta$-adrenoceptors (Galant et al., 1978; Fitzgerald et al., 1981). The opposite effect occurs during chronic exposure of $\beta$-antagonists, resulting in an increase in both the receptor density and the sensitivity of the transducer complex (Brodde et al., 1986; van den Meiracker et al., 1989). Furthermore, exercise increases skeletal muscle oxidative enzymes and capillary density, resulting in improved extraction and utilization of oxygen and metabolic substrates (Varnauskas et al., 1970; Salmons and Henriksson, 1981). These morphological and enzymatic adaptations have been reported as being absent in trained rats that have received $\beta$-antagonists (Harri, 1980; Ji et al., 1986; Favier et al., 1989) and thus explains the less marked decrease in heart rate in the drug treatment groups in our study. The impact of long-term treatment with $\beta$-antagonists on the oxygen uptake in skeletal muscles has been evaluated in many reports (see Shepherd, 1985, for a review). The absence of rebound effect and the positive influence of exercise on heart rate may suggest that moderate exercise can avoid rebound effects and possibly the induction of heart rate progression. However, assuming that the same amount of norepinephrine is released during each exercise occasion, it is still impossible to distinguish whether the positive influence on the heart rate is due to changes in receptor density or changes in oxygen uptake in skeletal muscles in the present data. Therefore, previous values describing changes in receptor density are used in the mechanistic model (Brynne et al., 1999).

The $\beta$-adrenoceptor density was estimated to have increased by 18%, which is lower than that previously reported value (32%; Brynne et al., 1999) and lower than values reported from studies in humans (25–51%) (Fitzgerald et al., 1981; Brodde et al., 1986; van den Meiracker et al., 1989). The lower value in the present study is probably due to increased norepinephrine exposure, because increased exposure of catecholamines decreases the receptor density (Brodde et al., 1986).

The maximal effect was 18.2% in the present study, which is similar to values reported in previous studies in SHR, where the maximal effects were 21.4 and 17.3% (Brynne et al., 1998, 1999). It is known that the amount of $\beta$-receptors, as well as cAMP, displays circadian rhythm (Witte et al., 1995); thus, the lower value for the maximal effect in the present study could be due to circadian variability in the receptor density and second messenger.

No time-dependent rebound effects were observed in either systolic or diastolic blood pressure. It has been reported in both human and animal studies that exercise attenuates hypertension, and thus exercise has been suggested as an alternative to pharmacological treatment (Westheim et al., 1985). Although exercise acutely raises blood pressure, there is growing evidence that prolonged physical training reduces blood pressure in both normotensive and hypertensive humans (Bjorntorp, 1982; Tipton, 1984) and animals (Tipton et al., 1983; Véras-Silva et al., 1997), but the mechanism is still...
unclear. The explanations are different depending on the age at which exercise is initiated and the intensity of exercise used in animal studies (Tipton et al., 1983). A low intensity of exercise (16–20 m/min) has been shown to attenuate hypertension, but high intensity (25–30 m/min) or sedentary states in trained SHR have not (Véras-Silva et al., 1997). Exercise training in young SHR (2–3 weeks old) at moderate intensity has been shown to lower resting blood pressure within 4 to 6 weeks after the initiation of training, but it is not able to normalize the resting blood pressure (Tipton et al., 1983). Within the present 4-week study, a small increase in baseline values was observed in both the systolic and diastolic blood pressures. Because of the present study design, we were not able to determine whether these small increases in blood pressure baselines are due to progression of the disease or whether they are smaller in comparison with the natural history.

The two blood pressure parameters gradually decrease after the start of infusion and then gradually increase after its termination. These slow changes in blood pressure indicate that the hypotensive effect is under rather complex homeostatic control and is not merely related to the direct activation of the β-adrenoceptor. Different concentration-effect relationships were tested, and based on changes in the objective function value, an Emax model that was related to a baseline with circadian variation could describe all blood pressure data. A profound effect delay, and consequently also a long duration, was observed between the plasma concentration and the reduction in systolic and diastolic blood pressure, in both exercise and postexercise data. Two different models were tested for describing the effect delay in blood pressure data, a transit and an effect compartment model, and the latter best described the data. Because both models can be considered empirical for a multifactorial response such as blood pressure, mechanistic interpretations based on this difference seem inappropriate. The rate constant estimating the effect delay, k ef, differed between exercise and postexercise data, where longer effect delays were found during exercise. The rate constant estimating the effect delay, k ef, was found to be longer in the exercise data than in postexercise data. One possible mechanistic explanation is that the cardiovascular homeostatic mechanisms, which includes neural reflexes, structural adaptations, and endocrine or renal mechanisms, are triggered during different time domains and gains, which may cause the time-dependent discrepancies between drug concentration and cardiovascular effects (Struyker Boudier, 1992). The resting level of blood pressure appears to be controlled primarily by the neuroendocrine system, but the mechanism of this control is currently unknown.

No differences in maximal response were observed in systolic and diastolic blood pressure during exercise and postexercise. Because β-antagonists competitively inhibit norepinephrine, the concentration-effect relationship is shifted to the right with increasing agonist concentration in the diastolic blood pressure. The poor precision in the systolic E C50 value during exercise could explain why no such shift is observed in the systolic blood pressure data. A difference in the concentration-effect relationship for cardiovascular drugs is not surprising, due to homeostatic mechanisms. One example is nifedipine, where a rapid infusion of nifedipine resulted in a significant increase in heart rate and only a modest decrease in blood pressure. A relatively slow administration, on the other hand, resulted in a marked drop in blood pressure with no increase in heart rate (Kleinbloesem et al., 1987). The difference in the concentration-effect relationship was explained on the basis of a difference in baroreceptor reflex activation.

In summary, no time-dependent rebound effects were observed in exercise heart rate or blood pressure data. The effect of l-propranolol on heart rate could be quantified by the mechanism-based model in the presence of a positive influence of exercise; the influence was less marked in drug-treated animals. The effects of l-propranolol on systolic and diastolic blood pressures, both during exercise and postexercise, were characterized by an effect-compartment model and an Emax model. All cardiovascular effects were modeled in the presence of circadian variation.

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