Br. J. clin. Pharmac. (1983), **16**, 599–608

EXERCISE-INDUCED INCREMENTS IN PLASMA LEVELS OF PROPRANOLOL AND NORADRENALINE

G.A. HURWITZ*, J.G. WEBB, T. WALLE, S.A. BAI, H.B. DANIELL, LYNN GOURLEY, C. BOYD LOADHOLT & T.E. GAFFNEY

Departments of Pharmacology, Biometry and Medicine, Medical University of South Carolina, Charleston, S.C., USA.

1 Exercise-induced changes in the plasma levels of propranolol and noradrenaline were determined in nine volunteers.

2 Total plasma propranolol levels were increased during submaximal treadmill exercise, with exercise-induced increments of 13 ± 4% at 4 h after the last dose, 18 ± 7% at 9 h and 41 ± 5% at 16 h. Exercise-induced increments in plasma propranolol were observed after single as well as repeated doses.

3 During exercise, increments in plasma propranolol were correlated temporally with changes in plasma noradrenaline.

4 Exercise-induced increments in plasma noradrenaline were greater during propranolol administration than during placebo periods.

5 The changes in plasma propranolol concentration during exercise may reflect a redistribution of propranolol at its site(s) of action.

Keywords propranolol noradrenaline exercise plasma levels

Introduction

The interaction of propranolol and other β-adrenoreceptor antagonists with the sympathetic nervous system has been the subject of numerous investigations (Ljung *et al.*, 1975; Adler-Graschinsky & Langer, 1975; Saelens *et al.*, 1977; Rahn *et al.*, 1978). Recent evidence has suggested that sympathetic stimulation may alter the levels of propranolol in plasma. Powis and Snow (1978) injected horses intravenously with propranolol and found that exercise, a physiological state associated with intense stimulation of the sympathetic nervous system, produced a transient elevation in plasma propranolol concentration. Infusion of adrenaline produced a similar increase in propranolol levels. Henry *et al.* (1981) subsequently found that plasma concentrations of propranolol or acebutolol, another β-adrenoceptor blocker, were increased by brief periods of submaximal exercise in human volunteers who received single doses of (±)-propranolol, (±)-acebutolol and indomethacin simultaneously. Comparable levels of exercise in these and other studies did not affect the plasma levels of antipyrine (Powis & Snow, 1978), indomethacin (Henry *et al.*, 1981) or diazepam (Klotz & Lücke, 1977).

In studies in our laboratories we have investigated the effect of direct sympathetic nerve stimulation on the release of propranolol. In dogs pre-treated for 1 week with (±)-propranolol, stimulation of the cardio-accelerator nerve produced a parallel efflux of propranolol and noradrenaline from the heart, as determined by measurement of gradients of these substances between aortic and coronary-sinus blood; a parallel overflow of propranolol and noradrenaline was also observed when tyramine was injected (Daniell *et al.*, 1979). Similar observations were made in other vascular beds, indicating the more general nature of this phenomenon; thus, sympathetic stimulation of the perfused canine hindlimb also produced a parallel efflux of propranolol and noradrenaline into the venous effluent (Russell *et al.*, 1983). These studies have led to the development of the hypothesis that propranolol may be accumulated by adrenergic neurones and possibly by postsynaptic tissues as well, for release along with neurotransmitter during periods of increased sympathetic activity. Such a mechanism could conceivably influence noradrenaline release or some other aspect of adrenergic neurotransmission, and thereby contribute to the pharmacological actions of the drug.

It was the aim of the present study to extend the

*Present address: Division of Nuclear Medicine, Mallinckrodt Institute of Radiology, 510 S. Kingshighway, St. Louis, Missouri 63110, USA.

previous observations in animals and man, by measuring simultaneous increments in plasma propranolol and plasma noradrenaline during exercise performed after single and repeated oral doses of (±)-propranolol in normal volunteers and patients with essential hypertension. The effect of graded exercise on plasma levels of propranolol was assessed at 4–33 h after administration of drug.

Methods

Subjects

The subjects consisted of nine volunteers who were hospitalized in the General Clinical Research Centre for up to 17 days. On admission, a complete medical evaluation including history, physical examination, chest film, electrocardiogram, complete blood count and routine blood chemistries were done. Seven subjects were normal volunteers; two subjects were patients with essential hypertension with no evidence of target organ damage who had been withdrawn from medication at least 2 weeks prior to the study. All subjects were non-obese and of above average physical condition. Approval was obtained from the Institutional Review Board for Human Research of the Medical University of South Carolina and informed consent was obtained from each subject prior to initiation of the protocol.

Repeated dose study

The study design involved measurements of plasma levels of noradrenaline and propranolol before, during and after exercise stress. Subjects were placed on a standardized isocaloric diet containing 109 meq Na^+ and 80 meq K^+. Smoking and caffeine-containing beverages were prohibited for 3 h prior to any procedure. Subjects were initially acclimated to exercise laboratory procedures and then underwent identical submaximal exercise procedures each day. Following the initial 3 days of the protocol, subjects were optimally conditioned to the exercise procedure; this was indicated by identical heart rate responses during exercise on days 4 and 16 of the protocol.

Propranolol therapy was administered in single-blind, placebo-controlled fashion. Following an initial 4 days of placebo treatment, propranolol was administered every 12 h at a beginning daily dose of 80 mg. After 4 days at 80 mg the daily dose was increased to 320 mg and maintained for 4 days, after which placebo administration was reinstituted for an additional 4 days. Plasma propranolol and plasma noradrenaline were measured in relation to randomly assigned exercise or sham procedures at 4 and 9 h after dosing with the high dose of propranolol on the last 2 days of propranolol treatment. In six subjects, additional measurements of these parameters were made in relation to exercise at 16 and/or 33 h after discontinuation of the drug. Plasma noradrenaline was measured in relation to exercise on the last day of each placebo period in all subjects.

Blood samples during exercise and sham procedures were drawn from a forearm vein through an indwelling cannula. Cannulas were inserted at least 1 h prior to each procedure and were kept patent with heparinized saline (50 U/ml). Subjects were kept quietly standing for 1 h prior to beginning each procedure.

Exercise laboratory procedures

A two-stage submaximal treadmill exercise lasting 12 min was used for each procedure. The conditions were individualized for each subject at the outset of the protocol and then kept constant throughout the 16 days of the repeated dose study, and subsequently for the 6 days of the single-dose study. A walking speed of 3.5 mph or 4.0 mph was used, and the treadmill slope was raised according to the heart rate response, to obtain heart rates equal to 65% of estimated maximum at stage I and 85–90% of estimated maximum at stage II (Astrand & Rodahl, 1971). Following the second stage of exercise, there was a 3 min walkdown and then the subjects were kept standing for another 12 min. Measurement of blood pressure and heart rate were made during a 10 min standing pre-exercise period, during the treadmill procedure, and during the 15 min post-exercise period. Heart rate and ECG lead II was monitored with an Avionics cardiac monitor and blood pressure was taken each minute with a Narco ® sphygmomanometer. Heart rate and blood pressure results were recorded as the average of four measurements made at 1 min intervals at the end of each stage of the exercise procedure.

Exercise at 4 and 9 h after propranolol on the last 2 days of therapy was carried out in a double-blind randomized fashion. Immediately prior to exercise and following drawing of the pre-exercise sample the subject was randomly allocated to either his usual treadmill exercise or a 'sham' procedure. During the 'sham' procedure, the subjects continued to stand quietly on the treadmill, and physiological measurements and blood sampling were carried out in identical fashion to the exercise procedure.

Heart rate responses are shown in Figure 1 for exercise procedures carried out on the fourth placebo day and 4 h after repeated dosing with propranolol 160 mg. Data are also shown for the corresponding sham procedure carried out 4 h after propranolol.

Blood sampling for propranolol and noradrenaline was carried out during the last minute of each stage of the procedure. Thus plasma levels of propranolol

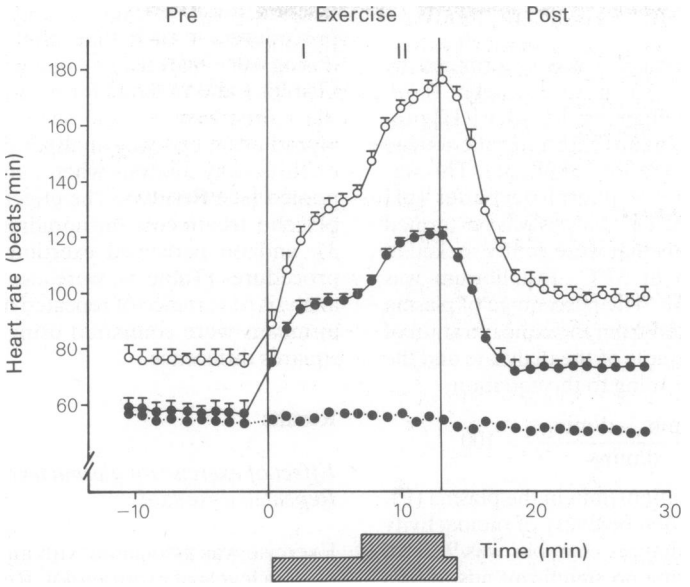

Figure 1 Heart rate (mean ± s.e. mean) in response to treadmill exercise procedure during administration of placebo (○) and propranolol (●). The exercise workloads were individualized for each subject during the placebo phase of the study to reach 65% and 90% of maximal heart rate at stage I and stage II of exercise respectively. The observations were made after 4 days of placebo administration or 4 h after a 160 mg dose of propranolol during the repeated dose study.

were generally determined during the last minute of the standing pre-exercise period (Pre), during the last minute of submaximal exercise (Exercise), and at 12 min after the end of treadmill exercise (Post), as tabulated in Table 1. During the sham procedures, blood samples were drawn during the corresponding times. To determine more precisely the time course of changes in plasma propranolol and plasma noradrenaline, blood was sampled more frequently in seven subjects at 4 h after repeated dosing, as demonstrated in Figure 2.

Single dose study

In four subjects who had performed the repeated dose study, responses to exercise were assessed following a single large oral dose of propranolol. These subjects were hospitalized in the GCRC and performed standardized submaximal exercise procedures twice daily at 09.00 h and 16.00 h. A dose of 240 mg was selected to attain plasma levels of propranolol similar to those obtained with the repeated dose study. Placebo or propranolol was administered in single-blind fashion. On the fifth day, plasma samples were drawn for measurement of plasma propranolol during treadmill exercise performed at 4 and 9 h after the dose; no sham procedures were performed. Exercise tolerance was established initially as for the previous study, and exercise procedures and blood sampling carried out in identical fashion.

During the single dose study, the subjective impression of exercise-induced fatigue was assessed using the rating scale described by Borg (1970). The severity of exercise was rated by each subject immediately following completion of the exercise procedures at 09.00 h and 16.00 h on the last day of placebo and for 2 days following administration of the drug.

Plasma analyses

Blood was collected in heparinized glass syringes and transferred immediately to heparinized polypropylene tubes for propranolol assay and to tubes containing EDTA for noradrenaline assay. Plasma was separated by centrifugation at 4°C and frozen (−70°C) until assayed. Plasma noradrenaline was measured using the radioenzymatic method of Henry *et al.* (1975). Total plasma propranolol was determined by gas chromatography-mass spectrometry using an internal standard labelled with stable isotope (Walle *et al.*, 1978). The coefficients of variation for the propranolol and noradrenaline assays were 2% and 6%, respectively. Plasma samples were coded and determinations of propranolol and noradrenaline were made in blind fashion.

Plasma binding of propranolol

Plasma binding of propranolol was determined by equilibrium dialysis according to Ehrnebo *et al.* (1971). Buffer (5 μl) containing 1.0 μCi [^{3}H]-propranolol (10.8 ng) (Amersham) with a specific activity of 24 Ci/mmol was added to 1 ml of plasma. This was dialyzed against 1.00 ml of phosphate buffer (pH 7.38; ionic strength 0.11). The dialysis was performed in lucite dialysis chambers that were gently rocked in a water bath for 14 h at 37°C. Equilibrium was attained at the end of 10 h. The percentage of plasma bound drug was calculated from the concentration of radioactivity in 50 μl aliquots of the dialysate and the plasma after dialysis according to the equation:

$$\% \text{ bound} = \frac{\text{d/min}_P - \text{d/min}_D}{\text{d/min}_P} \times 100$$

where d/min is disintegrations/min in the plasma (P), and dialysate (D). The total recovery of radioactivity from both sides of the dialysis chamber was 98% of the added counts, showing no significant adsorption of radioactive propranolol. In three patients duplicate plasma samples were dialyzed to determine the reproducibility of the measurements. The coefficient of variation was about 1%.

Statistical analysis

All data are given as the mean ± s.e. mean. Exercise-induced changes in drug levels were analyzed by several different techniques. Percentage change from Pre to Exercise values were analyzed by Student's *t*-test. Analysis of sequential changes during exercise and sham procedures was performed using analysis of variance and covariance of repeated measures (Snedecor & Cochran, 1967). Values of total plasma propranolol, free (unbound) propranolol and bound fraction for Pre, Exercise and Post during exercise procedures at each time after single and repeated dosing were analyzed by a one-way analysis of variance (Tables 1 and 2). Data for plasma propranolol during the paired exercise and sham procedures were analyzed using two-way analyses of variance (Figure 2) or three-way analysis when data for 4 and 9 h were pooled (see **Results**). The effects of propranolol and placebo treatments on noradrenaline levels (Figure 3), and on perceived exertion during the exercise procedures (Table 3), were also assessed by two-way analysis of variance of repeated measures. Differences in means were compared using the method of least squares difference.

Results

Effect of exercise on plasma levels of propranolol: Repeated dose study

Exercise was associated with an acute increase in the plasma levels of propranolol. Results obtained during exercise procedures 4–33 h after the repeated 160 mg dose of propranolol are shown in Table 1. Baseline plasma level of propranolol prior to the procedures varied three to four-fold among the subjects. Prior to exercise 4 h after the dose, plasma levels of propranolol were 168 ± 27 ng/ml; at 9 h the comparable levels in the same nine subjects had fallen to 81 ± 15 ng/ml, concordant with the known 4 h half-life for propranolol (Routledge & Shand, 1979). Exercise increased plasma levels of propranolol by 21 ng/ml at 4 h and 11 ng/ml at 9 h. This effect of exercise on plasma propranolol was quickly reversed; thus, 12 min after exercise, plasma levels of propranolol returned to pre-exercise levels. On the sham days, basal plasma levels of propranolol were indistinguishable from those on the exercise day. Plasma propranolol

Table 1 Effect of exercise on plasma propranolol levels after repeated dose and single dose studies. Values are means ± s.e. mean.

Time after last dose (h)	*Plasma propranolol (ng/ml)*			*Exercise-induced increment*
	Pre	*Exercise*	*Post*	*% increase*
Repeated dose				
4 (n=9)	168 ± 27	189 ± 32*	165 ± 21	13 ± 4††
9 (n=9)	81 ± 15	92 ± 15**	78 ± 13	18 ± 7††
16 (n=6)	19 ± 4	27 ± 5***	20 ± 5	41 ± 5†††
33 (n=4)	3.6 ± 1.3	3.6 ± 1.4	3.0 ± 0.7	3 ± 7
Single dose				
4 (n=4)	129 ± 41	157 ± 58	122 ± 34	17 ± 7†
9 (n=4)	67 ± 20	74 ± 20**	57 ± 16	18 ± 14

Exercise value different from both Pre and Post values by analysis of variance; * $P < 0.1$, ** $P < 0.05$, *** $P < 0.01$.
Percent increase from Pre to Exercise value, by *t*-test;
† $P < 0.1$, †† $P < 0.05$, ††† $P < 0.01$.

measurements made repeatedly during the sham procedure showed no change, at 9 h, or a decrease at 4 h, presumably due to metabolism of the drug. Data was pooled for exercise and sham procedures at 4 and 9 h; analysis of variance verified that the levels of propranolol during exercise were significantly different from both pre-exercise and post-exercise levels ($P < 0.01$ for data at 4 h, $P = 0.05$ for 9 h) and that the effect of exercise at 4 and 9 h was different from the sham procedure ($P < 0.03$).

Exercise-induced increments in plasma propranolol were detectable for at least 16 h following the last dose of drug (Table 1). At 16 h plasma propranolol levels were 19 ± 4 ng/ml prior to exercise, increased to 27 ± 5 ng/ml during peak exercise, and again fell to baseline 12 min after exercise. In terms of percent change, the 41% increase in plasma propranolol observed at 16 h was significantly greater ($P < 0.05$) than that observed at either 4 h (13%), or 9 h (18%). At 33 h after dosing with propranolol, plasma propranolol values were extremely low although still measurable, and there was no detectable enhancement of plasma propranolol levels with exercise.

Effect of exercise on plasma propranolol levels: Single dose study

Four subjects were studied after a single dose of 240 mg propranolol. One subject was unable to complete the exercise 4 h after the drug due to extreme fatigue; in this subject the study was repeated with a dose of 160 mg. The single dose of propranolol achieved plasma levels of drug similar to those obtained with the multiple dose protocol (Table 1). In the four subjects, exercise after the single dose produced increments in plasma propranolol of comparable magnitude to those seen during the repeated dose study; these increments were again reversed by 12 min after exercise. Although the number of subjects was small, the exercise-induced elevation in plasma propranolol 9 h after the single dose was significant ($P < 0.05$ by analysis of variance).

Comparative effects of exercise on plasma propranolol and plasma noradrenaline

In seven subjects receiving repeated doses of propranolol, samples were drawn at frequent intervals during the exercise and sham procedures for plasma propranolol and plasma noradrenaline. This enabled us to establish the time-course of exercise-induced changes in plasma propranolol and to compare these to exercise-induced changes in noradrenaline. Successive changes in plasma propranolol and noradrenaline concentrations (Figure 2) were assessed during the exercise and randomized sham procedures 4 h after the dose; these were compared to the concentration observed immediately prior to exercise or the corresponding value during the sham procedure. Analysis of variance again showed that the changes in plasma propranolol induced by exercise were different from those occurring during the sham procedure ($P < 0.005$). Specifically, plasma propranolol levels were falling prior to exercise, then rose stepwise by over 30 ng/ml, reaching a maximum at peak exercise, then fell progressively after exercise. The onset and peak of the changes in plasma propranolol paralleled the exercise-induced changes in noradrenaline. In

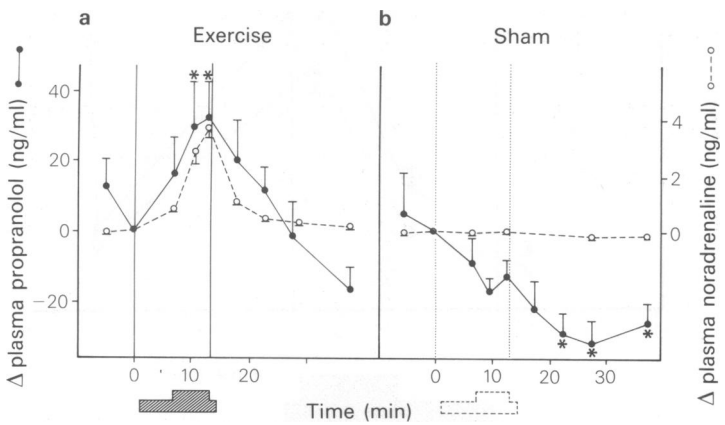

Figure 2 Exercise-induced changes in plasma levels of propranolol (●) and noradrenaline (○). Experiments were conducted 4 h following a 160 mg dose of propranolol (repeated dose study). Each value represents the mean ± s.e. mean for seven subjects. (a) Exercise. (b) Sham procedure. Plasma levels are compared to the point immediately prior to exercise, and the corresponding time during the sham procedure. Significant differences from the 'zero point' in each panel are shown for plasma propranolol levels * $P < 0.05$.

Table 2 Effect of exercise on free (unbound) propranolol in five subjects following the repeated dose study. Values are means ± s.e. mean.

Time after last dose (h)	*Pre*	*Exercise*	*Post*	*Exercise-induced increment (% increase)*
Free propranolol concentration (ng/ml)				
4 h (n=5)	16.1 ± 3.0	19.9 ± 2.7**	16.5 ± 2.2	29.9 ± 9††
9 h (n=5)	8.7 ± 1.7	10.2 ± 1.6*	8.6 ± 1.5	22 ± 10†
Bound fraction (% of total drug)				
4 h (n=5)	86.4 ± 2.4	85.0 ± 1.6	86.2 ± 1.8	—
9 h (n=5)	85.5 ± 2.7	85.4 ± 2.0	85.6 ± 1.7	—

Exercise values different from both Pre and Post values by analysis of variance; * $P < 0.1$; ** $P < 0.01$.
Percent increase from Pre to Exercise value, by t-test; † $P < 0.1$; †† $P < 0.01$.

contrast, during the sham procedure plasma propranolol fell progressively, whereas plasma noradrenaline remained unchanged.

Effect of exercise on free (unbound) propranolol levels

An increase in plasma binding of propranolol could have contributed to the observed enhancement of total plasma propranolol concentrations induced by exercise; consequently the effect of exercise on plasma binding of propranolol was investigated in five of the subjects studied 4 and 9 h after the last propranolol dose in the repeated dose protocol (Table 2). The concentrations of free propranolol in these five subjects demonstrated a significant increase during exercise at 4 h (29 ± 9%; $P < 0.01$) but the change did not reach statistical significance at 9 h (22 ± 10%, $P = 0.1$). However, when data from 4 and 9 h were pooled, exercise-induced changes were significant with $P < 0.005$. The plasma binding in these subjects averaged 86% prior to exercise. With exercise at either 4 or 9 h the binding was unchanged, averaging 85%.

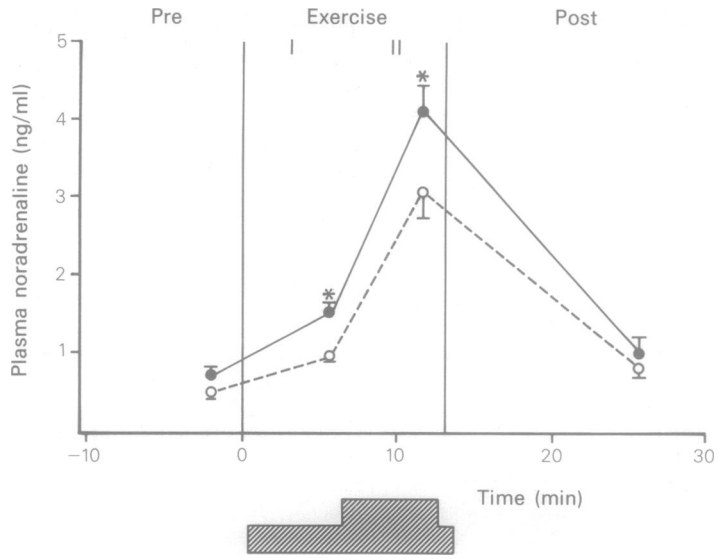

Figure 3 Plasma noradrenaline in relation to exercise during propranolol treatment and placebo. Each point represents the plasma noradrenaline values (mean ± s.e. mean) in nine subjects during the last minute of each stage of the exercise procedure. Placebo values (○) are the average of the initial and final placebo periods. Values on propranolol (●) are shown for the exercise procedures performed 4 h after a 160 mg dose. Differences between propranolol and placebo values were assessed by analysis of variance. *, $P < 0.01$.

Table 3 Effect of propranolol on perceived exertion of subjects in the single dose study. Values are means ± s.e. mean.

Condition	*Time*	*Time after drug (h)*	*Perceived exertion*
Placebo	9:00	—	10.5 ± 2.4
	16:00	—	11.5 ± 3.1
Propranolol	9:00	4	16.5 ± 4.1*
Day 1	16:00	9	14.4 ± 3.0
Propranolol	9:00	28	11.6 ± 1.9
Day 2	16:00	33	11.9 ± 2.8

* $P < 0.05$ by analysis of variance of repeated measures.

Effect of propranolol on plasma noradrenaline concentrations before and during exercise

The effect of exercise on plasma noradrenaline during propranolol therapy and during placebo therapy 4 h after dosing is shown in Figure 3. Exercise increased plasma noradrenaline during both treatment periods ($P < 0.0001$). However, the concentrations of noradrenaline reached higher levels at both stages of exercise during propranolol administration than during the placebo periods ($P < 0.01$). Four hours after a dose of propranolol, plasma noradrenaline levels at stages I and II of exercise averaged 1.5 and 4.1 ng/ml as compared with a value of 0.95 and 3.1 ng/ml respectively while on placebo. This enhancement in plasma noradrenaline was related to time after dosing. At 9 h after the drug, noradrenaline levels at stages I and II were 1.2 and 3.4 ng/ml, whereas at 16 h after the drug there was no difference from placebo values. Plasma concentrations of noradrenaline during quiet standing were not significantly altered by propranolol treatment at 4, 9 or 16 h after the drug.

Effect of propranolol on subjective assessment of intensity of exercise

The ratings of exercise intensity (Borg, 1970) by the four subjects receiving single doses are shown in Table 3. Administration of propranolol resulted in an increase in perceived exertion which was most marked 4 h after the single dose, yet still present at 9 h. The subjects' increased fatigue was also evident to the physician monitoring the procedures; in fact, one subject was unable to complete the 12 min of exercise at 4 h after 240 mg of propranolol. On the day following propranolol administration at 28 and 33 h after the drug, perceived exertion was the same as on the placebo day.

Discussion

In the present study, submaximal treadmill exercise was found to increase the concentration of propranolol in plasma with a time-course that closely paralleled exercise-induced increments in plasma noradrenaline. Propranolol levels were increased by over 20 ng/ml when exercise was performed 4 h after the last dose of drug, and significant exercise-induced increments could be demonstrated for at least 16 h after drug dosing. Increments of similar magnitude in plasma propranolol concentration occurred when exercise was performed following single doses of the drug or following repeated dosing. These observations of exercise-induced elevations of propranolol levels in human volunteers confirm a previous report of a similar phenomenon in experimental animals (Powis & Snow, 1978). Moreover, they raise questions concerning the mechanism and significance of these changes.

Although the parallel increase in the plasma concentrations of propranolol and noradrenaline during exercise is consistent with a simultaneous release of noradrenaline and propranolol from excitable tissues, alternative explanations need to be considered. Exercise is a complex phenomenon which is associated with haemodynamic and metabolic changes as well as sympathetic stimulation; several of these changes might contribute to exercise-induced alterations in drug levels.

Decreased renal (Ylitalo *et al.*, 1977; Mason *et al.*, 1980; Swartz & Sidell, 1973) as well as hepatic (Rowell *et al.*, 1964; Swartz *et al.*, 1974) clearance of several drugs have been reported during moderately prolonged periods of exercise, presumably as a result of decreased blood flow to these organs. Such altered haemodynamics would, however, not be expected to lead to an increase in plasma concentrations of drug, but rather to a slower decline during the period of exercise (Powis & Snow, 1978). In fact, this change in pharmacokinetics has been observed for indocyanine green (Rowell *et al.*, 1964), a drug whose elimination, like that of propranolol is determined primarily by hepatic blood flow (Nies *et al.*, 1973). Powis & Snow (1978) studied the elimination of propranolol in exercising horses to determine if the rate of propranolol clearance was reduced as a result of decreased hepatic

blood flow; instead, they found exercise-induced elevations in propranolol concentrations which they were unable to explain.

A 12% decrease in plasma volume has been reported to occur shortly after the onset of moderate exercise (Costill & Fink, 1974; Convertino *et al.*, 1981) and such an alteration might perhaps affect the plasma concentration of propranolol or other drugs. However, the magnitude of the increments in total propranolol levels seen with exercise (e.g. 40%, at 16 h) make it unlikely that haemoconcentration alone is responsible. Additionally, the exercise-mediated decrease in plasma volume should be associated with an increased plasma concentration of total protein (Costill & Fink, 1974), which might lead to increased plasma binding of drug. In this study we found no change in plasma binding of propranolol during exercise. The fact that plasma binding of drug did not increase, and that free (unbound) concentrations of drug were increased by exercise, suggests that drug was being released during exercise as a consequence of some process occurring in tissues.

As propranolol is extensively bound to tissues including heart, kidney, brain and skeletal muscle (Schneck *et al.*, 1977; Bianchetti *et al.*, 1980), a small change in binding to these tissues could result in substantial increments in plasma concentrations of drug. Because of the weakly basic nature of propranolol, its distribution across cell membranes might be influenced by pH gradients (Taylor *et al.*, 1981). Exercise is accompanied by sharp parallel decreases in pH of plasma and working muscle cells (Hermansen & Osnes, 1972); the net effect of these alterations on movement of propranolol would be difficult to predict. Also, the increase of blood flow which accompanies exercise could give rise to transiently enhanced plasma concentrations of drug, if one assumes that there are underperfused organs (e.g. spleen) or tissues (e.g. skeletal muscle) in which the drug is sequestered; however, exercise did not change plasma levels of diazepam (Klotz & Lücke, 1977), a neutral drug which, like propranolol, is strongly bound to tissues, though its volume of distribution is significantly less than that of propranolol. It is therefore unknown at present to what extent these mechanisms may contribute to an overflow of propranolol from tissues into plasma during exercise.

Another possible mechanism for the observed increments in venous plasma propranolol levels is that propranolol may be released from adrenergic neurones following membrane depolarization. Our observation that exercise was associated with a parallel rise and fall in plasma noradrenaline and propranolol in man, is not unlike the sequential and parallel changes in plasma levels of neurotransmitter and drug observed during sympathetic nerve stimulation of heart, and hindlimb (Daniell *et al.*, 1979; Russell *et al.*, 1983). Daniell *et al.* (1979) showed that in dogs pretreated with propranolol for 1 week that either tyramine or stimulation of the cardioaccelerator nerve resulted in a parallel efflux of noradrenaline and propranolol from the heart. Isoprenaline however, in doses producing comparable cardiac stimulation failed to produce a similar release, suggesting that an efflux of propranolol from presynaptic neurons underlay the phenomenon. The potential contribution of presynaptic sites to this phenomenon was also suggested by experiments in which tyramine and veratridine released tritiated propranolol from cultured rat sympathetic ganglia (Daniell *et al.*, 1979) and in which potassium and veratridine induced the release of tritiated propranolol from rat brain synaptosomes (Bright *et al.*, 1982). Thus, activation of the sympathetic nervous system may result in a significant efflux of propranolol from tissue storage sites and thereby contribute to the increments in venous propranolol observed during exercise in the present study.

In summary, a number of possible mechanisms may contribute to the observed exercise-induced alterations in the pharmacokinetics of propranolol. Thus, superimposed on a background of decreased hepatic blood flow and haemoconcentration, an efflux of propranolol from tissues could conceivably occur because of 'washout' from underperfused sites, passive movement of propranolol in response to pH gradients, or the release of propranolol from adrenergic or other tissues following membrane depolarization.

The observed changes in plasma propranolol levels could provide insight into factors which govern the distribution of propranolol at its sites of action. The magnitude of the increments in free and total plasma propranolol is relatively small and might be presumed to have only a small influence on the net actions of the drug. However, these increments in venous plasma levels, if they are indeed derived from tissue stores of the drug, would reflect only the overflow of propranolol into the blood from its site(s) of release, and therefore represent only a small fraction of the total drug released from tissues before its removal by diffusion, redistribution or other processes. If activation of adrenergic neurones induces the release of propranolol, as suggested by our previous laboratory experiments, the possibility exists that higher than anticipated levels of propranolol occur in the synapses of neuroeffector junctions. Such concentrations might not only reinforce β-adrenoceptor blockade at pre- or post-synaptic receptors, but might also be sufficient to exert pharmacological actions (Shanks, 1967; Noack *et al.*, 1978; Jackson & Campbell, 1981) not generally expected from the concentrations of propranolol observed in plasma.

A further consequence of the interaction of propranolol with adrenergic nerve endings could be altered storage and release of noradrenaline. In

animal studies, propranolol has been suggested to decrease noradrenaline release, whether by blockade of presynaptic β-receptors (Adler-Graschinsky & Langer, 1975), a neuronal blocking effect (Saelens *et al.*, 1977; Kaiho *et al.*, 1981), or a central mechanism (Privitera *et al.*, 1979). Insofar as plasma noradrenaline levels reflect the release of neurotransmitter, the data from the present study do not support diminished neurotransmitter release during treatment with propranolol. Plasma levels of noradrenaline were not decreased during standing or exercise following 8 days of propranolol administration; in fact, plasma noradrenaline levels achieved with exercise were significantly greater during propranolol therapy than during the placebo periods. This observation is consistent with other clinical reports of the effects of propranolol on plasma catecholamines (Rahn *et al.*, 1978; Planz & Planz, 1981). However, extrapolation of these findings to effects on neurotransmitter release is complex, and assumes a reproducible degree of sympathetic nervous system activation in response to exercise during treatment with propranolol and placebo. This assumption may not be justified as observations in this study confirm other reports (Pearson *et al.*, 1979) that the sense of fatigue accompanying exercise is increased by propranolol. Additionally, propranolol has been reported to alter the clearance of catecholamines from plasma (Clutter *et al.*, 1980), an observation which may complicate the use of plasma noradrenaline to reflect neurotransmitter release in this circumstance. Thus, the influence of propranolol on the neuronal release of noradrenaline in man remains an unresolved question.

In summary, we have observed exercise-induced increments in total and free levels of propranolol in the plasma of normal and hypertensive volunteers during acute and chronic propranolol therapy. In association with studies in laboratory animals, these observations suggest that activation of the sympathetic nervous system may help determine the concentration of propranolol at synapses and other cell surfaces, by delivering higher concentrations of propranolol than would be anticipated by determination of peripheral plasma levels. This phenomenon represents an additional factor to be considered in evaluating in tissues and plasma concentration-effect relationships for propranolol. Whether exercise or other forms of sympathetic stimulation may alter plasma levels of the more hydrophilic β-adrenergic receptor blocking drugs is presently unknown. It also remains to be determined whether this phenomenon contributes in some way to the therapeutic actions of propranolol and other β-adrenoceptor blocking drugs (Nies & Gerber, 1980).

This work was supported by Grants GM-20387 and RR-01070 from the United States Public Health Service.

References

ADLER-GRASCHINSKY, E. & LANGER, S.Z. (1975). Possible role of a β-adrenoceptor in the regulation of noradrenaline release by nerve stimulation through a positive feed-back mechanism. *Br. J. Pharmac.*, **53**, 43–50.

ASTRAND, P-O. & RODAHL, K. (1971). *Textbook of Work Physiology*. New York: McGraw-Hill.

BIANCHETTI, G., ELGHOZI, J.L., GOMENI, R., MEYER, P. & MORSELLI, P.J. (1980). Kinetics of distribution of dl-propranolol in various organs and discrete brain areas of the rat. *J. Pharmac. exp. Ther.*, **214**, 682–687.

BORG, G. (1970). Perceived exertion as an indicator of somatic stress. *Scand. J. Rehabil. Med.*, **2–3**, 92–98.

BRIGHT, P., GAFFNEY, T.E., STREET, J.A., WALLE, T. & WEBB, J.G. (1983). Accumulation and depolarization-induced release of propranolol in synaptosomes from rat cerebral cortex. *Br. J. Pharmac.*, **78**, 111P.

CLUTTER, W.E., BIER, D.M., SHAH, S.D. & CRYER, P.E. (1980). Epinephrine plasma metabolic clearance rates and physiologic thresholds for metabolic and hemodynamic action in man. *J. clin. Invest.*, **66**, 94–101.

CONVERTINO, V.A., KEIL, L.C., BERNAUER, E.M. & GREENLEAF, J.E. (1981). Plasma volume, osmolality, vasopressin and renin activity during graded exercise in man. *J. appl. Physiol.*, **50**, 123–128.

COSTILL, D.L. & FINK, W.J. (1974). Plasma volume changes following exercise and thermal dehydration. *J. appl. Physiol.*, **37**, 521–525.

DANIELL, H.B., WALLE, T., GAFFNEY, T.E. & WEBB, J.G. (1979). Stimulation-induced release of propranolol and norepinephrine from adrenergic neurons. *J. Pharmac. exp. Ther.*, **208**, 354–359.

EHRNEBO, M., AGURELL, S., JALLING, B. & BOREUS, L.O. (1971). Age differences in drug binding by plasma proteins. Studies on human foetuses, neonates and adults. *Eur. J. clin. Pharmac.*, **3**, 189–193.

HENRY, D.P., STARMAN, B.S., JOHNSON, D.G. & WILLIAMS, R.H. (1975). A sensitive radioenzymatic assay for norepinephrine in tissue and plasma. *Life Sci.*, **16**, 375–384.

HENRY, J.A., ILIOPOULOU, A., KAYE, C.M., SANKEY, M.G. & TURNER, P. (1981). Changes in plasma concentrations of acebutolol, propranolol and indomethacin during physical exercise. *Life Sci.*, **28**, 1925–1929.

HERMANSEN, L. & OSNES, J.B. (1972). Blood and muscle pH after maximal exercise in man. *J. appl. Physiol.*, **32**, 304–308.

JACKSON, E.K. & CAMPBELL, W.B. (1981). Possible antihypertensive mechanisms of propranolol: Antagonism of angiotensin II enhancement of sympathetic nerve transmission through prostaglandins. *Hypertension*, **3**, 23–33.

KAIHO, M., KUBO, T. & MISU, Y. (1981). Comparative studies of (−) −, (±), (+) − propranolol, atenolol, guanethidine, bretylium and tetracaine on adrenergic transmission. *Br. J. Pharmac.*, **74**, 365–370.

KLOTZ, U. & LÜCKE, C. (1977). Physical exercise and disposition of diazepam. *Br. J. clin. Pharmac.*, **5**, 349–350.

LJUNG, B., ABLAD, B., DAHLÖF, C., HENNING, M. & HULTBERG, E. (1975). Impaired vasoconstrictor nerve function in spontaneously hypertensive rats after long-term treatment with propranolol and metoprolol. *Blood Vessels*, **12**, 311–315.

MASON, W.D., KOCHAK, G., WINER, N. & COHEN, I. (1980). Effect of exercise on renal clearance of atenolol. *J. pharm. Sci.*, **69**, 344–345.

NIES, A.S., EVANS, G.H. & SHAND, D.G. (1973). The hemodynamic effects of beta adrenergic blockade on the flow-dependent hepatic clearance of propranolol. *J. Pharmac. exp. Ther.*, **184**, 716–720.

NIES, A.S. & GERBER, J.G. (1980). Current status of β-adrenergic blocking drugs in the United States. In *Year Book of Drug Therapy*, ed. Hollister, L.E. pp. 9–26. Chicago: Year Book Medical Publishers.

NOACK, E., KURZMACK, M., VERJOVSKI-ALMEIDA, S. & INESI, G. (1978). The effect of propranolol and its analogs on Ca^{++} transport by sarcoplasmic reticulum. *J. Pharmac. exp. Ther.*, **206**, 281–288.

PEARSON, S.B., BANKS, D.C. & PATRICK, J.M. (1979). The effect of β-adrenoceptor blockade on factors affecting exercise tolerance in normal man. *Br. J. clin. Pharmac.*, **8**, 143–148.

PLANZ, G. & PLANZ, R. (1981). Dissociation between duration of plasma catecholamine and blood pressure responses to beta-adrenergic blockade in normotensive subjects during physical exercise. *Eur. J. clin. Pharmac.*, **19**, 83–88.

POWIS, G., & SNOW, D.H. (1978). The effects of exercise and adrenaline infusion upon the blood levels of propranolol and antipyrine in the horse. *J. Pharmac. exp. Ther.*, **205**, 725–731.

PRIVITERA, P.J., WEBB, J.G. & WALLE, T. (1979). Effects of centrally administered propranolol on plasma renin activity, plasma noradrenaline and arterial pressure. *Eur. J. Pharmac.*, **54**, 51–58.

RAHN, K.H., GIERLICHS, H.W., PLANZ, G., PLANZ, R., SCHOLS, M. & STEPHANY, W. (1978). Studies on the effect of propranolol on plasma catecholamine levels in patients with essential hypertension. *Eur. J. clin. Invest.*, **8**, 143–148.

ROUTLEDGE, P. & SHAND, D.G. (1979). Clinical pharmacokinetics of propranolol. *Clin. Pharmacokin.*, **4**, 73–90.

ROWELL, L.R., BLACKMAN, J.R. & BRUCE, R.A. (1964). Indocyanine green clearance and estimated hepatic flow during mild to maximal exercise in upright man. *J. clin. Invest.*, **43**, 1677–1689.

RUSSELL, M.P., WEBB, J.G., WALLE, T., DANIELL, H.B., PRIVITERA, P.J. & GAFFNEY, T.E. (1983). Adrenergic nerve stimulation-induced release of propranolol from the perfused hindlimb and spleen of the dog and associated changes in postjunctional response. *J. Pharmac. exp. Ther.*, **226**, 324–329.

SAELENS, D.A., DANIELL, H.B. & WEBB, J.G. (1977). Studies on the interactions of propranolol with adrenergic neurons. *J. Pharmac. exp. Ther.*, **202**, 635–645.

SCHNECK, D.W., PRITCHARD, J.F. & HAYES, A.H. (1977). Studies on the uptake and binding of propranolol by rat tissue. *J. Pharmac. exp. Ther.*, **203**, 621–629.

SHANKS, R.G. (1967) The peripheral vascular effects of propranolol and related compounds. *Br. J. Pharmac. Chemother.*, **29**, 204–217.

SNEDECOR, G.W. & COCHRAN, W.G. (1967). *Statistical methods.* 6th ed. Ames: Iowa State University Press.

SWARTZ, R.D. & SIDELL, F.R. (1973). Effects of heat and exercise on the elimination of pralidoxime in man. *Clin. Pharmac. Ther.*, **14**, 83–89.

SWARTZ, R.D., SIDELL, F.R. & CUCINELL, S.A. (1974). Effect of physical stress on the disposition of drugs eliminated by the liver in man. *J. Pharmac. exp. Ther.*, **188**, 1–7.

TAYLOR, E.A., JEFFERSON, D., CARROLL, J.D. & TURNER, P. (1981). Cerebrospinal fluid concentrations of propranolol, pindolol and atenolol in man: evidence for central actions of β-adrenoceptor antagonists. *Br. J. clin. Pharmac.*, **12**, 549–559.

WALLE, T., WALLE, U.K., BRIDGES, D.R., CONRADI, E.C. & GAFFNEY, T.E. (1978). Quantitative GC-MS of propranolol, a key to an improved understanding of the relationship between plasma concentrations and dose. *Clin. Chem.*, **24**, 991.

YLITALO, P., HINKKA, H. & NEUVONEN, P. (1977). Effect of exercise on the serum level and urinary excretion of tetracyline, doxycycline and sulphamethizole. *Eur. J. clin. Pharmac.*, **12**, 367–373.

(Received April 18, 1983, accepted August 15, 1983)