Br. J. Pharmac. (1981), **74**, 643–649

THE ACTION OF PROPRANOLOL ON FACTORS CONCERNED WITH THE DELIVERY OF OXYGEN TO THE TISSUES

JOHN M. LEDINGHAM & DOUGLAS C.P. LEES

The Medical Unit, The London Hospital Medical College, London E1 1BB

1 In a previous study in conscious rats, orally administered propranolol acutely lowered cardiac output by 30.5% and oxygen uptake by 12.2%, while oxygen extraction rose by 31.5%. The present study is concerned with the way in which the rat meets its oxygen requirements against such a fall in perfusion.

2 The effect of known concentrations of propranolol on haemoglobin-oxygen affinity was studied *in vitro*. The effect of orally administered propranolol (given acutely and chronically) was then determined and this was related to the concentration of the drug in plasma and red cells. Further studies were made on the action of propranolol on the Bohr effect and on arterial oxygen carriage.

3 *In vitro*, high concentrations of propranolol (1×10^{-4} M) influenced haemoglobin-oxygen affinity in a biphasic manner and this was associated with changes in haematocrit and red cell and plasma pH. No change occurred in affinity after acute or chronic oral administration of the drug due to insufficient concentration in the blood. No direct action on the Bohr effect was detected.

4 Arterial oxygen content rose acutely due to an increase in haemoglobin concentration.

5 It is concluded that increased oxygen extraction in propranolol-treated rats is not explained by the actions of the drug on haemoglobin-oxygen affinity.

Introduction

The major haemodynamic change induced by propranolol is a lowering of cardiac output both at rest (Hansson, Zweifler, Julius & Hunyor, 1974) and during exercise (Brundin, Edhag & Lundman, 1976). This action occurs acutely and persists for as long as a year during continued drug administration in man (Frohlich, Tarazi, Dustan & Page, 1968). Oxygen uptake has also been reported to be depressed after acute administration of propranolol although this action becomes less marked during chronic administration (Brundin *et al.*, 1976). In both acute and chronic cases the reduction in cardiac output exceeds that in oxygen uptake; in a recent study, in unanaesthetized rats, propranolol, on average, acutely reduced cardiac output by 30.5% and oxygen uptake by 12.2% (Ledingham & Lees, 1981). This disproportionate change directs attention to ways in which propranolol could improve oxygen delivery to offset the reduction in tissue perfusion. One possible way could be a reduction in haemoglobin-oxygen affinity: several workers have observed a shift to the right of the haemoglobin-oxygen dissociation curve (Pendleton, Newman, Sherman, Brann & Maya, 1972; Oski, Miller, Delivoria-Papadopoulos, Manchester & Shelbourne, 1972; Agostoni, Berfasconi, Gerli, Luzzana & Rossi-Bernardi, 1973; Lichtman, Cohen, Murphy, Kearney & Whitbeck, 1974) but although this action appears to be well-established at high concentrations *in vitro* in man, there have been contradictory findings when the drug has been administered *in vivo*: Lichtman *et al.* (1974) could find no action in the short term but others have found a rightward shift in the dissociation curve after three months propranolol therapy in man (Schrumpf, Sheps, Wolfson, Aronson & Cohen, 1977).

Less attention has been directed to other ways in which propranolol might improve oxygen delivery. These include actions on the Bohr effect, on the rate of haemoglobin desaturation, on haemoglobin concentration, on pulmonary function (the possibility of marginal improvement in alveolar ventilation and ventilation-perfusion relationships) and on the density of the capillary network and hence on capillary diffusing capacity.

The present work was undertaken to explore some of the above possibilities using the rat for the *in vivo* studies.

Methods

Experimental animals

Female rats of approximately 200 g from a Glaxo Wistar stock were used in all experiments. Blood was removed in long-term experiments from the jugular

0007-1188/81/110643-07 $01.00

vein under light ether anaesthesia and in acute experiments either by this method or in the unanaesthetized state from previously implanted cannulae.

Propranolol

The I.C.I. racemic mixture of propranolol hydrochloride was used in all experiments. In acute experiments 20 mg propranolol, dissolved in 0.6 ml water, was administered by gavage to give a concentration of 100 mg/kg in the rat (control rats received water alone). This dose was chosen on the basis of dose-response curves of heart rate which revealed that a maximum reduction in heart rate occurred with between 10 and 20 mg of orally administered propranolol. In long-term experiments on haemoglobin-oxygen affinity, rats were maintained for up to 12 weeks on standard rat cake to which had been added either 0.5 or 1.0 g propranolol per kg. Estimated doses based on a daily intake of 15 g diet per rat were 37.5 and 75 mg/kg respectively. The weight gain in rats fed the standard diet and in those fed diet plus propranolol was similar. In the first two series of rats, heart rates were measured by ECG (Hewlett Packard model 1500B) to monitor the drug's effectiveness.

Haemoglobin-oxygen affinity

The mixing technique of Edwards & Martin (1966) was employed. Approximately 5 ml rat blood was mixed and equal amounts were placed in two tonometers (Instrumentation Laboratory 237) where they were equilibrated for at least 15 min with gas mixtures containing either N_2 94.6%, CO_2 5.4% or O_2 94.6%, CO_2 5.4% (B.O.C. certificated special gases). Different saturations were prepared and analysed in a blood gas meter (Instrumentation Laboratory 213). All operations were conducted at 37°C and duplicate readings of P_{O_2} were made for each mixture. The pH of each mixture was measured and the P_{O_2} was corrected to pH 7.4 using a standard correction factor. This technique was used to derive the haemoglobin-oxygen curve, over the range between 30 and 70% oxygen saturation, in long-term dietary studies. The acute effect of a 20 mg dose of propranolol per rat, given by gavage in 0.6 ml water, was also studied. In order to have sufficient blood for analysis in acute studies, a group of rats served as a pool, each group being used as its own control. One ml of blood, replaced from a donor, was taken from each rat in the group under ether anaesthesia, well-mixed and the dissociation curve determined over the range 30–70% saturation. Each rat then received propranolol and 40 to 60 min later blood was taken for a second determination. Five groups were studied.

In *in vitro* studies, the effect of propranolol concentration on haemoglobin-oxygen dissociation was investigated and a dose-response curve, relating propranolol concentration to the partial pressure of oxygen at 50% saturation (P_{50}), was constructed.

Haematocrit

A range of blood samples of varying propranolol concentration was prepared. Several estimations of haematocrit were made on each sample (centrifuged for 6 min at 1500 *g*).

pH changes

Blood samples of varying propranolol concentration were tonometered with 5% CO_2. Samples were spun down and plasma pH measured. In order to obtain an index of intracellular pH, the pH was measured in the haemolysed solution obtained after resuspending red cells in twice their volume of water.

Observations on the Bohr effect

The mixing technique was employed to study the Bohr effect. A gas mixing pump (Wosthoff 1 SA 27/3F) was used to produce gas mixtures of varying CO_2 content in either O_2 or N_2. After tonometering blood with these gas mixtures, samples from each tonometer were mixed and P_{O_2} and pH measured at selected levels of O_2 saturation (between 30 and 70%). The pH-P_{O_2} curve, at about 50% saturation, was compared in blood from 4 control rats and pooled blood from 3 rats given two oral doses of 7.5 mg over the previous 24 h.

Oxygen saturation and haemoglobin levels

Experiments on the effect of propranolol on oxygen carrying capacity were performed in rats which had already been chronically implanted with abdominal aortic cannulae (Weeks & Jones, 1960). Blood was withdrawn before, and 40–60 min after, the administration of 20 mg propranolol by gavage. A control group received water alone. The rat was conscious and breathing air during sampling. Estimations of haemoglobin concentration and oxygen saturation were made in a hemoximeter (Radiometer OSM2). The long-term effect of propranolol was examined in rats that received dietary propranolol and compared to rats on the standard diet. In these, blood was withdrawn either from an indwelling cannula in conscious rats or from the jugular vein under light ether anaesthesia.

The hemoximeter estimation of haemoglobin was validated against the cyanmethaemoglobin method and in some of the experiments the latter method was employed.

Propranolol concentration in blood

The method was based on standard procedures involving solvent extraction, formation of a fluorinated derivative and gas-liquid chromatography with electron capture detection (Obel, 1978). The solvent was a 5:1 ether/chloroform mixture and the fluorination was performed in *n*-hexane with trifluoroacetic-anhydride. The derivative, dissolved in ethyl acetate, was injected into a Pye Unicam model 104 chromatograph with a 10 mCi ^{63}Ni electron capture detector. Blood to be analysed was spun down and estimations were made on plasma and on a preparation of red cells resuspended in three times their volume of water to cause haemolysis. Measurements were made in rats receiving both the low and the high dietary dose of propranolol for a period of 12 weeks. Estimations were also made in blood withdrawn from rats 50 min after administration of 20 mg propranolol by gavage.

Results

Haemoglobin-oxygen dissociation

Three series of rats were studied to determine the effect of dietary propranolol. Controls had free access to standard rat cake and water, test animals received the propranolol diet (see Methods). In the first series of animals monitored by ECG, the heart rates of the control and test groups were significantly different from the first week onwards, being lower in the propranolol-treated group by 15.2% ($P<0.05$) after one week and by 23.1% ($P<0.0005$) after six weeks. In the second series, when heart rates were measured after two weeks, the propranolol-treated group had heart rates that were lower than the controls by 12.8% ($P<0.0005$).

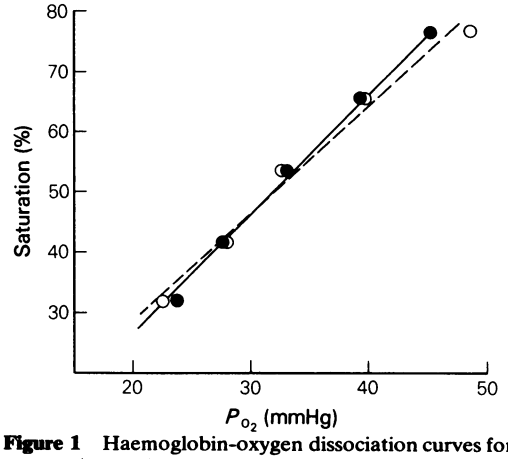

Figure 1 Haemoglobin-oxygen dissociation curves for treated (O---O) and control (●——●) rats. Regression line for treated group: $r=0.992$; $P_{50}=32.3$ mmHg (s.d. = 2.5). For the controls: $r=0.998$; $P_{50}=32.0$ mmHg (s.d. = 2.8).

In the first series, 4 control and 4 propranolol-treated (0.5 g/kg diet) rats were maintained on their diets for 8 weeks. Haemoglobin-oxygen curves were then constructed (30–70% saturation, Figure 1). In the second series, 6 control and 6 propranolol-treated rats were maintained on their diets for 17 to 23 days and similar curves were then constructed.

There was very good agreement between the control curves and those of propranolol-treated rats in each of the two studies. There was no significant difference in P_{O_2} (by Student's *t* test) between the groups within each series, at any level of saturation. In the first two series, regression lines were derived from the data and P_{50} thereby obtained. Values of P_{50} for controls and propranolol-treated rats, respectively, were 32.0 mmHg (s.d. = 2.8) and 32.3 mmHg (s.d. = 2.5) in the first series ($P<0.475$), and 31.9 mmHg (s.d. = 0.8) and 31.4 mmHg (s.d. = 1.2) in the second series ($P<0.2$).

The third study, in which the dietary concentration of propranolol was 1 g/kg and which was maintained for 12 weeks, revealed a small shift to the right of the mean dissociation curve of the propranolol-treated group. This was, however, not significant. Values of P_{50} in the control and treated groups were, respectively, 30.4 mmHg (s.d. = 2.3) and 31.3 mmHg (s.d. = 1.9), ($P<0.35$).

In the acute study (20 mg propranolol by gavage) there was a small increase in P_{50}, 40 to 60 min after propranolol administration: P_{50} rose from 30.1 mmHg (s.d. = 1.4) to 30.6 mmHg (s.d. = 1.4) which was not significant ($P<0.2$).

Propranolol was shown to have a biphasic effect on P_{50} *in vitro*. As the concentration of the drug increased there was an initial rise in P_{50} and a subsequent fall (Figure 2). Peak elevation of P_{50} occurred at about 1×10^{-3} M propranolol.

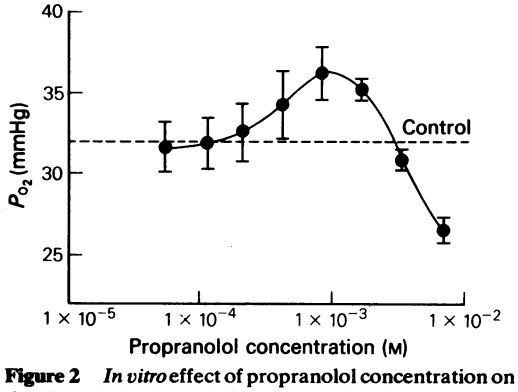

Figure 2 *In vitro* effect of propranolol concentration on the P_{O_2} of blood at 50% saturation (P_{50}) in rats. Each bar represents 1 s.d. ($n=3$).

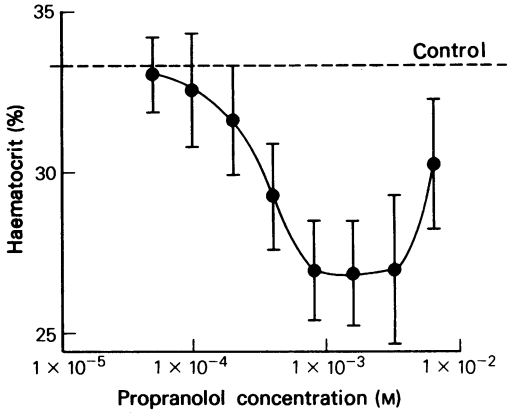

Figure 3 *In vitro* effect of propranolol concentration on haematocrit of the rat. Each bar represents 1 s.d. ($n = 5$).

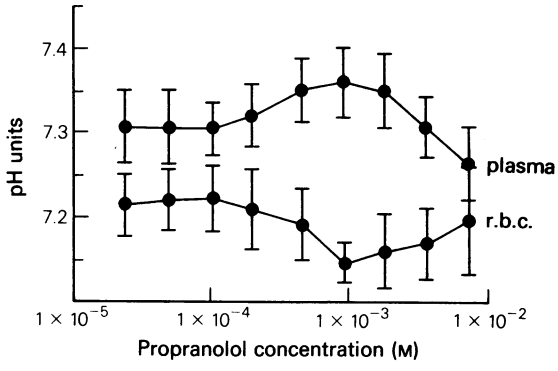

Figure 4 *In vitro* effect of propranolol concentration on the pH of plasma and a solution of haemolysed red cells of the rat. Each bar represents 1 s.d. ($n = 6$).

Propranolol was also shown to affect the haematocrit in a biphasic fashion (Figure 3). Peak depression of haematocrit occurred at about the same concentration which caused peak elevation of P_{50}.

Propranolol also affected plasma and red cell pH *in vitro* and these changes were, again, biphasic (Figure 4). The plasma and red cell fractions, however, gave mirror image responses, suggesting a relative shift of hydrogen ions. The peak fall in red cell pH occurred at that concentration of propranolol which caused peak elevation of plasma pH and of P_{50}.

The Bohr effect

In a group of 4 control rats, the slope of the curve relating P_{O_2} to pH at 50% saturation was −5.3 mmHg/0.1 pH units (s.d. = 0.7); this compared with a value of −5.1 in a pooled sample from 3 rats that received 15 mg of propranolol by gavage, in two doses at 24 and 2 h before the measurement.

Oxygen saturation

In the acute study, 40 to 60 min after the administration of 20 mg propranolol by gavage to 10 rats, mean arterial saturation in blood samples from indwelling cannulae in conscious animals, was 97.1% (s.d. = 3.1%) compared with 95.7% (s.d. = 3.9%) before. This difference was not significant ($P < 0.15$).

The effect of propranolol in the long-term on arterial oxygen saturation *in vivo* and on the ability of blood to saturate *in vitro* is shown in Table 1. No significant differences arose between the treated and the control rats.

Haemoglobin levels

In acute experiments, 40 to 60 min after the administration of 20 mg propranolol by gavage, arterial haemoglobin rose by a mean 6.3% (s.d. = 3.6%) in 10 rats. The change was significant ($P < 0.025$) when

Table 1 Arterial oxygen saturation *in vivo* and *in vitro* and P_{O_2} *in vivo*, in propranolol-treated (for 14 to 17 days) and control rats

	Propranolol-treated	*Controls*	P *for difference between groups*
In vivo			
Saturation (%)	96.4 ± 1.6 ($n = 5$)	95.7 ± 3.2 ($n = 3$)	0.4
P_{O_2} (mmHg)	90.6 ± 4.8 ($n = 5$)	89.0 ± 5.0 ($n = 3$)	0.35
In vitro			
Tonometry at high P_{O_2} (> 140 mmHg)	97.8 ± 2.3 ($n = 5$)	97.7 ± 2.2 ($n = 5$)	0.475

Values are given ± s.d.

Table 2 Haematocrits and haemoglobin using the cyanmethaemoglobin method, in rats measured 17 to 23 days after starting the propranolol diets

	No. of animals	*Propranolol-treated*	*Controls*	P
Mean haematocrit (%)	6	44.8 ± 1.7	45.7 ± 1.6	0.15
Mean Hb transmittance (%)	6	41.8 ± 4.6	41.0 ± 2.6	0.48

Values are given ± s.d.

compared, by an unpaired *t* test, with the effect of water on a group of 7 controls. The long-term effect of propranolol on haemoglobin is shown in Table 2. This compares 6 propranolol-treated and 6 control rats with respect to haematocrit and haemoglobin transmission measurements made on jugular blood samples. No significant difference was found. A separate series involved hemoximeter measurements on blood samples taken from indwelling aortic cannulae in conscious rats; after 14 days, in 5 propranolol-treated rats the mean haemoglobin concentration was $13.3 \pm 1.0\ g\ dl^{-1}$ compared to $13.6 \pm 0.7\ g\ dl^{-1}$ in 3 control rats, the difference being insignificant ($P < 0.4$).

Propranolol concentration in the blood

After 12 weeks, 3 rats that received a dietary propranolol concentration of 0.5 g/kg had a mean plasma concentration of 2.52×10^{-6} M (s.d. = 0.8×10^{-6} M) and a mean red cell concentration of 2.54×10^{-6} M (s.d. = 1.1×10^{-6} M). In 4 rats that received twice this dose over the same period, plasma concentration was 2.05×10^{-6} M (s.d. = 1.1×10^{-6} M) and mean red cell concentration was 5.17×10^{-6} M (s.d. = 2.1×10^{-6} M).

In the acute study, 50 min after a single dose of propranolol, by gavage, the mean concentration in 3 rats was 6.6×10^{-6} M (s.d. = 0.3×10^{-6} M) in the plasma and 1.37×10^{-5} M (s.d. = 0.1×10^{-5} M) in the red cells.

The extent of protein binding in both plasma and red cells, which could interfere with measurements made by the present technique, has been subjected to preliminary studies. There is evidence that plasma propranolol was only slightly underestimated (7%) whereas in the red cell the underestimate was likely to have been considerably larger (possibly 50%).

Discussion

Haemoglobin-oxygen dissociation

No rightward shift in the dissociation curve *in vivo* was found, either acutely or after propranolol treatment lasting as long as 12 weeks. Schrumpf *et al.* (1977) reported a fall in P_{50} in man 4 days after discontinuing propranolol therapy of 12 weeks duration; they concluded that the failure of others to detect such shifts was due to too short a duration of treatment. However, the present studies in rats were of a similar duration and employed doses equivalent to 2.5 g per day in man which exceeded those used by these authors (152 mg).

Oski *et al.* (1972) detected an increase in P_{50} in human blood with 1×10^{-5} M propranolol, *in vitro*, which was attributed to a release of 2,3 DPG from the red cell membrane; *in vivo* studies revealed unbinding of 2,3 DPG and an increase in P_{50} with as little as 40 mg in man but no estimations of blood levels of the drug were made. Pendleton *et al.* (1972) found large increases in P_{50} of between 6 and 8 mmHg, *in vitro*, using concentrations of propranolol of about 1.5×10^{-4} M and these were linked to membrane changes in the erythrocyte: 2×10^{-5} M was found to be ineffective. A rise in P_{50} was also reported by Agostoni *et al.* (1973) with 5×10^{-4} M propranolol, *in vitro*, and they suggested that the Bohr effect was involved since there was ionic redistribution across the red cell wall. Lichtman *et al.* (1974) found that P_{50} rose and red cell pH fell when propranolol was added, *in vitro*, to blood, but 24 h after doses of between 10 and 360 mg in man, no change in P_{50} was found.

The present studies in rat blood, *in vitro*, confirm that propranolol increases P_{50} but as part of a biphasic response which is dose-dependent. No increase was found below 1×10^{-4} M, a peak rise occurred at 1×10^{-3} M and at higher concentrations, P_{50} fell, declining below control values. These changes were associated with changes in haematocrit and in plasma and red cell pH. These results strongly suggest that the Bohr effect brings about the change in P_{50} and that the pH changes, themselves, are a result of ionic shifts which are also responsible for the red cell volume changes. Some membrane action of propranolol is the likely underlying mechanism; a propranolol-induced efflux of cellular potassium has been the suggested mechanism behind a 20% fall in cell volume at a 5.5×10^{-4} M concentration of the drug (Ekman, Manninen & Salminen, 1969). The

importance of the red cell membrane in the action of propranolol on P_{50} was demonstrated by Pendleton *et al.* (1972) who found no action in haemoglobin solutions; with concentrations of the drug which affected P_{50} red cells shrank and the cell membrane folded; when the shrinkage was selectively blocked with chlorbutanol, the P_{50} changes were also inhibited.

Failure to detect an increase in P_{50} after acute and chronic propranolol administration in rats may have been due to insufficient concentration being achieved in the blood. The highest value which was found was 1.37×10^{-5} M, after acute administration, which was less than one tenth that which increased P_{50} *in vitro*. Yet in these acute studies, if the drug were distributed uniformly throughout the body water, a concentration of 3×10^{-4} M would be achieved. Such a concentration would, then, affect P_{50} and although a small shift of 0.5 mmHg occurred, it was not significant. It remains possible, however, that the present technique of estimating red cell propranolol gives a low result in consequence of binding to red cell matrix.

The Bohr effect

No change in the Bohr effect under the influence of propranolol seems likely; there was close agreement in the P_{O_2}-pH slope in propranolol-treated and control rats.

Oxygen saturation

Arterial oxygen saturation was not significantly changed in the acute phase or in the long-term although there was a small rise in the propranolol-treated rats with respect to the controls in both cases. Similarly, in the long-term, P_{O_2} was slightly higher in the treated group, but not significantly so. The ability of blood withdrawn from chronically treated rats, to saturate *in vitro*, was almost identical with that of the controls.

Haemoglobin levels

There was a significant acute rise in arterial haemoglobin after propranolol. This action was short-lived since no change was detected in haematocrit or haemoglobin in the long-term. The acute rise was more likely to have been due to a fall in plasma volume than to an increase in circulating red cell mass (Ledingham & Lees, 1981). Acute falls in plasma volume were also found by Julius, Pascual, Abbrecht & London (1972).

Propranolol measurement

Rats that received the standard dietary dose of propranolol (0.5 g/kg) for 12 weeks had similar concentrations of the drug in plasma and red cells (2.5×10^{-6} M). No cellular accumulation of the drug had occurred as has been reported by Potter (1967), who found the cell/medium ratio to be 30 in guinea-pig isolated atria. However, in rats receiving twice the standard dose for 12 weeks, the plasma levels were lower (2.05×10^{-6} M) than at the lesser dose but red cell levels were higher (5.17×10^{-6} M), suggesting some accumulation by the cells. In the acute study, involving a single large dose by gavage, plasma levels were higher than in the dietary studies (6.6×10^{-6} M) and the red cell levels were greater again (1.37×10^{-5} M). These results indicate some cellular accumulation of propranolol but not enough to reach those concentrations which influenced P_{50}, *in vitro*. Further studies on the technique revealed that red cell propranolol may have been underestimated by 50% but even if the measurements were doubled in the present studies they would still remain well below effective concentration. Hayes & Cooper (1971) found high tissue/blood ratios in animals but their studies involved the analysis of whole blood, i.e. plasma and red cells; this may indicate that accumulation of propranolol is less a feature of red cells than of cells of other tissues. It is possible, therefore that some tissues may have had much higher concentrations of the drug than were estimated in blood. This would account for the concentration in blood being lower than estimated when uniform distribution is assumed.

In conclusion, in rats treated with propranolol, a reduction in haemoglobin-oxygen affinity is unlikely in view of the high concentrations of the drug required to affect it *in vitro*. This was borne out in both acute and long-term experiments: affinity was unchanged, blood levels being too low. There was no effect of propranolol on the Bohr effect or on arterial oxygen saturation. Acutely, but not chronically, arterial oxygen content was elevated due to a rise in haemoglobin concentration which was attributed to a fall in plasma volume. Such haemoconcentration may have aided oxygen delivery to the tissues. The greater extraction of oxygen from blood during propranolol treatment is possibly due to a slower passage of blood through the capillaries, permitting a greater degree of desaturation, combined with the Bohr action due to local pH changes.

Acknowledgement is made to Dr F.J. Conway of I.C.I. Ltd. for advice on the dosage of propranolol, for the provision of the pure preparation of propranolol and for helpful discussion. We are most grateful for excellent technical help in the cannulation of the rats by Miss Susan Gofford and for help in the design and construction of the equipment by Mr Clifford Browning.

Acknowledgement is also made for advice and help in the estimation of propranolol from Professor S.H. Curry and Dr R. Whelpton.

References

AGOSTONI, A., BERFASCONI, C., GERLI, G.C., LUZZANA, M. & ROSSI-BERNARDI, L. (1973). Oxygen affinity and electrolyte distribution of human blood: changes induced by propranolol. *Science*, **182**, 300–301.

BRUNDIN, T., EDHAG, O. & LUNDMAN, T. (1976). Effects remaining after withdrawal of long-term beta-receptor blockade. Reduced heart rate and altered haemodynamic response to acute propranolol administration. *Br. heart J.*, **38**, 1065–1072.

EDWARDS, M.J. & MARTIN, R.J. (1966). Mixing technique for the oxygen-haemoglobin equilibrium and Bohr effect. *J. appl. Physiol.*, **21**, 1898–1902.

EKMAN, A., MANNINEN, V. & SALMINEN, S. (1969). Ion movements in red cells treated with propranolol. *Acta physiol. scand.*, **75**, 333–344.

FROHLICH, E.D., TARAZI, R.C., DUSTAN, H.P. & PAGE, I.H. (1968). The paradox of beta-adrenergic blockade in hypertension. *Circulation*, **37**, 417–423.

HANSSON, L., ZWEIFLER, A.J., JULIUS, S. & HUNYOR, S.N. (1974). Haemodynamic effects of acute and prolonged beta-adrenergic blockade in essential hypertension. *Acta med. scand.*, **196**, 27–34.

HAYES, A. & COOPER, R.G. (1971). Studies on the absorption, distribution and excretion of propranolol in rat, dog and monkey. *J. Pharmac. exp. Ther.*, **176**, 302–311.

JULIUS, S., PASCUAL, A.V., ABBRECHT, P.H. & LONDON, R. (1972). Effect of beta-adrenergic blockade on plasma volume in human subjects. *Proc. Soc. exp. Biol. Med.*, **140**, 982–985.

LEDINGHAM, J.M. & LEES, D.C.P. (1981). Acute effects of propranolol on the circulation and on oxygen uptake in conscious rats. *Br. J. Pharmac.* **74**, 635–641.

LICHTMAN, M.A., COHEN, J., MURPHY, M.S., KEARNEY, E.A. & WHITBECK, A.A. (1974). Effect of propranolol on oxygen binding to haemoglobin in vitro and in vivo. *Circulation*, **59**, 881–886.

OBEL, A.O. (1978). A study of some factors which influence the disposition of propranolol, antipyrine and pyridopyridazine. *Thesis presented to London University.*

OSKI, F.A., MILLER, L.D., DELIVORIA-PAPADOPOULOS, M., MANCHESTER, J.H. & SHELBOURNE, J.C. (1972). Oxygen affinity in red cells: changes induced by propranolol. *Science*, **175**, 1372–1373.

PENDLETON, R.G., NEWMAN, D.J., SHERMAN, S.S., BRANN, E.G. & MAYA, W.E. (1972). Effect of propranolol upon the haemoglobin-oxygen dissociation curve. *J. Pharmac. exp. Ther.*, **180**, 647–656.

POTTER, L.T. (1967). Uptake of propranolol by isolated guinea-pig atria. *J. Pharmac. exp. Ther.*, **155**, 91–100.

SCHRUMPF, J.D., SHEPS, D.S., WOLFSON, S., ARONSON, A.L. & COHEN, L.S. (1977). Altered haemoglobin-oxygen affinity with long-term propranolol therapy in patients with coronary artery disease. *Am. J. Vardiol.*, **40**, 76–82.

WEEKS, J.R. & JONES, J.A. (1960). Routine direct measurements of arterial pressure in unanaesthetized rats. *Proc. Soc. exp. Biol. Med.*, **104**, 646–650.