OXYHAEMOGLOBIN EQUILIBRIUM AND CHRONIC β -ADRENOCEPTOR BLOCKADE IN CORONARY HEART DISEASE

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1 In 20 patients with coronary heart disease the effect of long-term β -adrenergic receptor blockade on the haemoglobin oxygen equilibrium was investigated. Study patients received alprenolol 200 mg twice daily for 12–41 months (mean: 24 months) as a secondary preventive measure following a myocardial infarction. While on and again following gradual withdrawal of alprenolol, the patients performed a maximum bicycle ergometer test. Haemoglobin oxygen affinity as expressed by the P_{50} value, 2,3-diphosphoglycerate (2,3-DPG) and carbon monoxide haemoglobin were measured before and following exercise. Pre-exercise P_{50} decreased from 25.2 ± 0.3 mm Hg (mean $\pm$ s.e. mean) while on β -adrenoceptor blocker to 24.6 ± 0.4 mm Hg in the off-treatment state ($P < 0.05$). Five minutes after stopping exercise P_{50} was 25.1 ± 0.3 in patients taking alprenolol as compared to 24.7 ± 0.3 after withdrawal of the drug ($P < 0.01$).

2 It is concluded that the slight decrease in haemoglobin oxygen affinity in long-term treatment with alprenolol, which is observed in the present study probably is without clinical bearing. The question should be further elucidated by analysis of coronary sinus blood samples.

Keywords β -adrenoceptor blockade coronary heart disease oxyhaemoglobin equilibrium

Introduction

β -adrenoceptor blocking drugs are widely used to protect the ischaemic myocardium in angina pectoris (Prichard, 1974; Comerford & Besterman, 1976; Aronow *et al.*, 1980) and in myocardial infarction (MI) (Vedin *et al.*, 1975; Andersen *et al.*, 1979; The Norwegian Multicenter Study Group, 1981; Jürgensen *et al.*, 1981; Hjalmarson *et al.*, 1981; Beta-blocker Heart Attack Study Group, 1981). The main mechanism of action of these agents probably is to decrease myocardial oxygen consumption by slowing heart rate and by reducing myocardial contractility (Mueller *et al.*, 1974). To a minor degree oxygen delivery may also be beneficially influenced by β -adrenergic receptor blockade: Firstly, experimental data suggest that following β -adrenoceptor blockade myocardial blood flow is appropriately redistributed in favour of the subendocardium (Warltier *et al.*, 1976). Secondly, in human studies propranolol has been reported to enhance tissue oxygenation by reducing the haemoglobin affinity for oxygen as reflected by a rightward shift of the oxyhaemoglobin equilibrium curve (Osaki *et al.*, 1972; Pendleton *et al.*, 1972; Agostoni *et al.*, 1973; Lichtman *et al.*, 1974; Frishman *et al.*, 1976; Schrumph *et al.*, 1977). However, conflicting data regarding this specific

pharmacological action of propranolol have been published (Brain *et al.*, 1974; Berntorp *et al.*, 1979).

The present investigation was therefore designed to elucidate further the effect of long-term β -adrenergic receptor blockade on the haemoglobin oxygen affinity in patients with coronary heart disease (CHD).

Study population

The subjects included in the present investigation all attended an out-patient clinic for follow-up of post-MI patients. β -adrenergic receptor blockade with alprenolol originally had been initiated on admission to our Coronary Care Unit for an acute MI, and alprenolol had been continued during the post-hospital period as a secondary preventive measure (Andersen *et al.*, 1979). Fifteen men and five women aged 43–74 years (mean: 61 years), who met the following criteria entered the present study: (1) CHD as evidenced by a prior MI, the diagnosis having been established according to W.H.O. criteria (1971). (2) Long-term treatment with alprenolol, i.e. 12–41 months (mean: 24 months) covering the period from the qualifying MI to the present investigation. Dose

of alprenolol was 200 mg twice daily in a form of a sustained-release preparation (Aptin Durules®). (3) Functional class I or II according to New York Heart Association (Criteria Committee, 1964).

Patients with chronic respiratory disease, diabetes mellitus or other endocrinological disorders, haematological disease, known hyperlipidaemia, and impaired renal function were excluded from the study. Informed consent was obtained from all patients.

Experimental design

Studies took place between 09.00 h and 14.00 h. Patients sustained a fasting and non-smoking period of 4 h before the first blood sample was obtained. This sample was drawn exactly 2 h following the final dose of alprenolol. A 17-gauge needle was inserted into an antecubital vein and slow infusion of physiological saline begun. After 15 min rest in the supine position 15 ml of venous blood was sampled in heparinized plastic syringes. A two-syringe technique was used and the first 5 ml of blood discarded. A maximum multistage graded exercise test was then performed using a bicycle ergometer (Elma Schönander). The starting work load was 200 kpm/min (33 watt), and this load was augmented by 200 kpm/min (33 watt) every 4 min until fatigue or chest pain. A 6-lead ECG was recorded continuously. Immediately after the patient stopped pedalling a second blood sample was drawn. The final two samples were drawn after 5 and 10 min rest in the supine position. Following sampling, blood specimens were kept at 4°C. All laboratory analyses except for the 2,3-DPG determinations were completed within 3 h. Alprenolol was gradually withdrawn over a 2-week period, and following another 2 weeks the above procedure was repeated.

Laboratory analyses

All analyses were done in duplicate and the mean value employed.

Haemoglobin concentration (mmol/l) and oxyhaemoglobin saturation (%) were determined by use of OSM2 Hemoxymeter (Radiometer, Copenhagen). Carbon monoxide haemoglobin was measured after reduction of the oxyhaemoglobin in glass capillary tubes containing a sodium dithionite solution (40 mmol/l with tris base 40 mmol/l) and subsequent transfer to the OMS2. Blood pH was measured at 37°C using a BMS2 Mk II blood micro system (Radiometer, Copenhagen).

P₅₀ determinations

P₅₀ i.e. the P_{O₂} (mm Hg) necessary to produce 50% saturation of haemoglobin with oxygen at P_{CO₂} 40

mm Hg and pH 7.40 was determined as follows: Two blood specimens—each about 70 µl of whole blood—were equilibrated for 10 min at 37°C in the BMS2 microtonometer. The one sample was equilibrated to a P_{O₂} of about 23 mm Hg and a P_{CO₂} of 40 mm Hg, the other to a P_{O₂} of about 33 mm Hg and a P_{CO₂} of 40 mm Hg, yielding haemoglobin oxygen saturations of about 40% and 60%, respectively. By use of the Hill-equation (Hill, 1910) P₅₀ was then calculated and corrected to pH 7.40 using a Bohr coefficient: -0.48. An increase in P₅₀ indicates a rightward shift of the oxyhaemoglobin dissociation curve and a decrease in haemoglobin oxygen affinity.

Erythrocyte 2,3-DPG analyses

Protein-free extracts of blood were obtained by adding 3 ml to 9 ml of trichloroacetic acid, 0.5 mol/l, the mixture was centrifuged at 1500 g for 20 min and the supernatant stored at -20°C. A manual spectrophotometric, enzymatic endpoint procedure was used (detailed procedure is given on request). The principle is given by Keitt (1971). Reagents were obtained from Sigma Chemical Company, St. Louis, USA. Results were corrected for volume of precipitated protein (Ericson & de Verdier, 1972) and blood 2,3-DPG concentrations were calculated per mol of tetramer haemoglobin. Analytical precision, within series CV, was 6.0%. Accuracy was secured by the use of Sigma Metabolite Control.

Statistical methods

For all variables the arithmetic mean with the s.e. mean is presented. For tests of significance the Wilcoxon matched pairs signed rank sum test was employed. Differences yielding *P* values < 0.05 were considered significant.

Results

In patients taking alprenolol a significant reduction in the resting as well as the maximum exercise heart rate was recorded (Table 1). The maximum working capacity increased from 4261 ± 622 to 4526 ± 661 kpm/min (710→754 watt) following cessation of β-adrenoceptor blockade (*P* < 0.01). Blood haemoglobin concentration was not significantly influenced by alprenolol. Carbon monoxide haemoglobin, on the other hand, increased from 2.2 ± 0.5 to 3.2 ± 0.7% following withdrawal of the drug (*P* < 0.01; Table 1). Neither in patients taking alprenolol nor in the post-treatment state did physical exercise significantly influence P₅₀ values referred to pH 7.40 (Figure 1). In comparing the alprenolol and the post-alprenolol state, pre-exercise P₅₀ values decreased from 25.2 ± 0.3 to 24.6 ± 0.4 mm Hg (*P* < 0.05), and

Table 1 Heart rate, maximum working capacity, blood haemoglobin concentration and carbon monoxide haemoglobin in patients on long-term β -adrenoceptor blockade and after withdrawal of treatment.

	Alprenolol (n = 20)	Post-alprenolol (n = 20)	P value
Resting heart rate (beats/min)	68 $\pm$ 2	73 $\pm$ 2	$P < 0.05$
Maximum heart rate during exercise (beats/min)	113 $\pm$ 4	139 $\pm$ 4	$P < 0.01$
Maximum working capacity (kpm/min)	4261 $\pm$ 623	4526 $\pm$ 661	$P < 0.01$
Blood haemoglobin concentration (mmol/l)	8.0 $\pm$ 0.1	7.7 $\pm$ 0.1	NS
Carbon monoxide haemoglobin (%)	2.2 $\pm$ 0.5	3.2 $\pm$ 0.7	$P < 0.01$

5 min post-exercise P_{50} dropped from 25.1 ± 0.3 to 24.7 ± 0.3 mm Hg ($P < 0.01$). Comparing plasma pH and erythrocyte 2,3-DPG values obtained in patients on chronic β -adrenergic receptor blockade to values that came out after withdrawal of treatment

Discussion

A decrease in haemoglobin oxygen affinity conceivably improves the balance between tissue oxygen supply and demand and may contribute to relieve pain in angina pectoris and to save ischaemic cells in acute MI. The present study demonstrates a slight, yet significant, rightward shift of the dissociation curve in post-MI patients on long-term β -adrenoceptor blockade with alprenolol. Other pharmacological agents such as ortho-iodo-sodium benzoate (Gross *et al.*, 1977), testosterone (Parker *et al.*, 1972), and acetazolamide (Eliot *et al.*, 1972) have been reported to shift the equilibrium curve to the right. Anti-anginal medication with nitroglycerin, however, does not seem to influence haemoglobin oxygen affinity in humans (Sugimoto & Ishikawa, 1972; Clerbaux *et al.*, 1979).

In a variety of clinical conditions including respiratory and heart disease a change in haemoglobin oxygen affinity has been observed (Eliot & Mizukami, 1966; Edwards *et al.*, 1968; Metcalfe *et al.*, 1969; Woodson *et al.*, 1970; Shappell *et al.*, 1970; Kostuk *et al.*, 1973). The patients included in the present study form a diagnostically homogenous group: all having sustained a previous MI, all belonging to the New York Heart Association class I or II

Figure 1 P_{50} values before and following exercise in patients on long-term β -adrenoceptor blockade with alprenolol ($\square$, $n = 20$) and after withdrawal of treatment ($\square$ $n = 20$) (mean $\pm$ s.e. mean). * $P < 0.05$, ** $P < 0.01$.

demonstrated no significant differences (Table 2). However, in the alprenolol state physical exercise was followed by a 26% increase of erythrocyte 2,3-DPG ($P < 0.01$) as compared with a non-significant 8% increase in the post-alprenolol state.

Table 2 Erythrocyte 2,3-DPG and plasma pH before and following exercise in patients on long-term β -adrenoceptor blockade and after withdrawal of treatment.

	Plasma-pH		P value	Erythrocyte 2,3-DPG (mol/mol Hb (Fe ₄))		P value
	Alprenolol (n = 20)	Post-alprenolol (n = 20)		Alprenolol (n = 18) ¹⁾	Post-alprenolol (n = 18) ¹⁾	
Pre-exercise	7.407 $\pm$ 0.004	7.409 $\pm$ 0.006	NS	0.77 $\pm$ 0.04	0.88 $\pm$ 0.05	NS
At maximum exercise	7.353 $\pm$ 0.008	7.355 $\pm$ 0.009	NS	0.86 $\pm$ 0.05	0.92 $\pm$ 0.06	NS
5 min post-exercise	7.349 $\pm$ 0.010	7.346 $\pm$ 0.011	NS	0.97 $\pm$ 0.03	0.95 $\pm$ 0.04	NS
10 min post-exercise	7.357 $\pm$ 0.009	7.352 $\pm$ 0.011	NS	0.82 $\pm$ 0.04	0.87 $\pm$ 0.04	NS

¹⁾2,3-analyses failed in two patients due to technical difficulties in blood sampling.

and not suffering from respiratory disease. Tablet intake was not controlled by measuring the plasma alprenolol level, but, as expected, a significant decrease in heart rate before and following exercise was observed in patients on β -adrenoceptor blocker.

In interpreting the slight rightward shift of the dissociation curve seen in our patients on long-term β -adrenergic receptor blockade all well recognized determinants of the haemoglobin oxygen equilibrium should be considered: All P_{50} measurements were done at standard pH, P_{CO_2} and temperature conditions. Organic phosphates within erythrocytes, notably 2,3-DPG influence P_{50} greatly (Benesch *et al.*, 1968; Rörth, 1968). While study patients were on alprenolol a 26% increase in erythrocyte 2,3-DPG following exercise was recorded as compared to a 8% increase in the post- β -adrenoceptor blocker state. This difference was related to a low pre-exercise 2,3-DPG value while study patients were on the β -adrenoceptor blocker and is hardly relevant as it was unaccompanied by changes in P_{50} . Other workers observed an increase in 2,3-DPG following short-term exhaustive exercise (Klein *et al.*, 1980). The blood haemoglobin concentration also bears impact on P_{50} (Torrance *et al.*, 1970) and was not influenced by the β -adrenoceptor-blockade. Carbon monoxide shifts the equilibrium curve to the left (Astrup *et al.*, 1966). In our study the haemoglobin carbon monoxide binding was significantly increased in the post-alprenolol state. This finding may explain the lower P_{50} after withdrawal of alprenolol. An increment in haemoglobin carbon monoxide binding was recorded in 17 out of 20 study patients all of whom denied any change in their smoking status following withdrawal of alprenolol. The possibility that β -adrenoceptor blockade may influence haemoglobin carbon monoxide binding or endogenous carbon-monoxide production should be further studied. It has been suggested that smokers may derive special benefit from secondary prevention with a β -adrenoceptor blocker in MI (unpublished data from the Norwegian Timolol trial (The Norwegian Multicenter Study Group, 1981) and the Danish alprenolol study (Andersen *et al.*, 1979).

From *in vitro* (Pendleton *et al.*, 1972; Agostoni *et al.*, 1973; Lichtman *et al.*, 1974) and *in vivo* studies (Osiki *et al.*, 1972; Schruppf *et al.*, 1977) in humans there is evidence that propranolol might decrease haemoglobin oxygen affinity, a finding of obvious clinical relevance. To explain this effect essentially two theories were offered: (1) Propranolol might

increase the concentration of unbound 2,3-DPG in erythrocytes (Osiki *et al.*, 1972). This view was not supported by data from other workers, however, (Brann & Newman, 1973). (2) Alternatively propranolol might influence ion-fluxes across the erythrocyte membrane increasing efflux of potassium, chloride and water, thus producing a decrease in erythrocyte cell volume and a reduction of red cell pH due to influx of hydrogen ions (Lichtman *et al.*, 1904; the Bohr-effect then gives rise to a rightward shift of the equilibrium curve (Bohr *et al.*, 1904; Agostoni *et al.*, 1973). In the above *in vitro* studies unphysiologically high concentrations of propranolol were employed, however. In two *in vivo* studies demonstrating an effect of propranolol on P_{50} the carbon-monoxide haemoglobin binding was not investigated (Osiki *et al.*, 1972; Schruppf *et al.*, 1977). In addition, some negative *in vivo* studies have been published (Lichtman *et al.*, 1974; Frishman *et al.*, 1976; Brain *et al.*, 1974; Berntorp *et al.*, 1979). The effect of propranolol on ion-fluxes across the erythrocyte membrane is not blocked by adrenaline (Lichtman *et al.*, 1974). The view, that β -adrenoceptors are not involved in this specific membrane action of propranolol is further supported by the fact, that the (+)-isomer of the drug, lacking β -adrenoceptor blocking properties, influences red cell volume and ion-permeability to a higher degree than the racemic ($\pm$)-propranolol (Pendleton *et al.*, 1972; Lichtman *et al.*, 1974). Alprenolol, in unphysiologically high doses, shares a 'local anaesthetic' property with propranolol and might theoretically influence haemoglobin oxygen affinity by a pharmacological action unrelated to β -adrenergic receptor blockade.

In conclusion, the present study demonstrates a slight, but significant rightward shift of the haemoglobin oxygen equilibrium curve in post-MI patients on long-term β -adrenoceptor blockade with alprenolol. The change, however, is small and hardly of clinical significance. In order to establish a possible *clinical* bearing of this specific pharmacological aspect of β -adrenoceptor blockers further studies on arterial and coronary sinus blood samples are needed.

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