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The *in vitro* effects of propranolol and atenolol on neutrophil motility and post-phagocytic metabolic activity

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Summary. Propranolol at concentrations of 1×10^{-6} to 1×10^{-4} M consistently increased neutrophil motility as measured in Boyden chambers. The effects were not due solely to stimulation of random migration and chemokinesis but also of directional motility. Propranolol, over a similar concentration range, caused inhibition of post-phagocytic cell metabolic activity (hexose monophosphate shunt, nitro-blue tetrazolium reduction and protein iodination) without any detectable effect on the ingestion rate of *Candida albicans*. Atenolol had no effect on any of these neutrophil functions. Both drugs were without effect on glycolysis and intracellular cyclic AMP levels. Propranolol however, at concentrations which stimulated cell motility, caused increased intracellular cyclic GMP levels. It is suggested that propranolol may stimulate neutrophil motility by promoting increased intracellular cyclic GMP levels or by decreasing neutrophil superoxide production.

INTRODUCTION

Neutrophil function can be modulated by agents which promote fluctuations in the levels of intracellular cyclic AMP (cAMP) and cyclic GMP (cGMP).

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Neutrophil directional motility is inhibited by cAMP-elevating agents (Tse, Phelps & Urban, 1972; Hill, Estensen, Quie, Hogan & Goldberg, 1975; Rivkin, Rosenblatt & Becker, 1975; Anderson, Glover & Rabson, 1977) and stimulated by cGMP-elevating agents (Estensen, Hill, Quie, Hogan & Goldberg, 1973; Anderson, Glover, Koornhof & Rabson, 1976) and leuco-attractants (Anderson *et al.*, 1976; Hatch, Nichols & Hill, 1977). Regulatory effects of the cyclic nucleotides are also evident in the regulation of other phagocyte functions such as random migration (Hill, Estensen, Quie, Hogan & Goldberg, 1975; Anderson *et al.*, 1976; Rivkin & Becker, 1976), candidacidal activity (Bourne, Lehrer, Lichtenstein, Weissmann & Zurier, 1973), lysozomal enzyme release (Zurier, Weissmann, Hoffstein, Kammerman & Tai, 1974; Ignarro, Lint & George, 1974; Ignarro & George, 1974), and post-phagocytic hexose monophosphate shunt activity (Flyer & Finch, 1973; Anderson, Glover & Rabson, 1978). The studies reported here were designed to investigate the effects of the β-receptor blockading agents propranolol (hydrochloride) and atenolol on the functions of human blood neutrophils from healthy adult volunteers. The two β-agonists differ in their pharmacological profiles. Atenolol is cardioselective and possesses no intrinsic activity or membrane stabilizing properties. Propranolol has certain immunomodulatory properties and although the effects on lymphocyte transformation are controversial (Hadden, Hadden & Middleton, 1970; Naka-

zawa, Adolphson, Chaperon, Hobday, Townley & Pauli, 1976) the drug has been reported to increase antibody synthesis in mice (Nakazawa *et al.*, 1976) and rats (Benner, Enta, Lockey, Makino & Reed, 1968). Furthermore Buckley & McGregor (1977) have reported that propranolol increased the number of survivors, decreased the duration of disease and increased the number of peritoneal exudate cells in rats which had been immunosuppressed by ethanol and experimentally infected intraperitoneally with type III pneumococci. We report that atenolol has no effects on any of the neutrophil functions tested. Propranolol, however, caused marked stimulation of neutrophil motility. Stimulation of all three types of neutrophil motility (random migration, chemokinesis and directional motility) was observed. Drug concentrations which stimulated cell motility had an inhibitory effect on post-phagocytic oxidative metabolism with reduction in the levels of hexose monophosphate shunt activity (HMS), nitro-blue tetrazolium reduction and protein iodination. There was no effect on glycolysis or protein synthesis, although a stimulation of intracellular cGMP levels at propranolol concentrations which stimulated cell motility was observed. Intracellular cAMP levels were unaffected.

MATERIALS AND METHODS

Preparation of drugs

Propranolol and atenolol (kindly supplied by I.C.I., South Africa Pharmaceuticals Ltd.) were dissolved in Hanks's balanced salt solution (HBSS) (Grand Island Biological Co., Grand Island, N.Y.) at pH 7·2 giving a concentration range of 10^{-9} to 10^{-3} M. Actinomycin D and puromycin aminonucleoside were obtained from the Sigma Chemical Co. (St. Louis, Mo.), dissolved in HBSS and diluted according to the experimental requirements.

Cell motility studies

These were of two types. The direct effects of propranolol and atenolol on chemotaxis and random migration were assessed by the method of Boyden (1962) whereby polymorphonuclear leucocytes (PMN) which accomplish complete transfilter passage are counted microscopically. To ascertain the exact type of motility influenced by the drugs, the leading front method of Zigmond & Hirsch (1973) was adopted. Neutrophils were obtained from 30 ml of heparinized blood (5 units heparin/ml) donated by healthy male and female laboratory workers. After sedimentation at 37° the buffy layer was centrifuged at 250 *g* for 10 min. The resultant cell pellet was washed three times with HBSS and resuspended to a PMN concentration of 5×10^6/ml.

Leuco-attractants

Three chemo-attractants were used in this study. (a) Fresh serum activated by 500 μg/ml of bacterial lipopolysaccharide (*Escherichia coli* 0127:B8 Difco, Detroit, Mich.) (EAS). The mixture was incubated for 30 min at 37° followed by an eight-fold dilution with HBSS. (b) EAS fractionated by column chromatography to obtain a material which by virtue of its molecular weight and chemotactic activity is probably C5a. EAS (5 ml volumes) was applied to 2×100 cm columns packed with Ultrogel Ac44 (LKB). EAS was eluted with 0·15 M phosphate buffered saline and the eluate collected in 3 ml fractions. Cytochrome c and blue dextran were used as markers. The pre-cytochrome fractions were tested for chemotactic activity at 1/10 dilutions. Fractions containing high activity were pooled. The dilution with optimal leucotactic activity was determined. In experiments using fractionated EAS the HBSS was supplemented with bovine serum albumin (BSA) at a concentration of 2 mg BSA/ml of HBSS. Such protein supplementation was unnecessary for EAS and casein. (c) Denatured casein prepared by alkali treatment of a 5 mg/ml solution in HBSS followed by restoration to pH 7·2. This preparation was stored at −20° and used at a 1:8 dilution for all subsequent experiments.

Leucotaxis assay

PMN chemotaxis was measured with a modified Boyden chamber (Wilkinson, 1971) which utilizes 0·2 ml of drug treated or control cell suspensions in the upper chamber, separated from 0·8 ml of the leuco-attractant by a 5 μm-pore size membrane filter (Millipore Corp., Bedford, Mass.). Chambers were incubated at 37° for 3 h after which filters were removed, fixed in methanol, and stained with haematoxylin. The average number of neutrophils reaching the lower surface of the filter was determined and expressed as an average for triplicate filters. Using this technique the effects of propranolol and atenolol at a final concentration range of 10^{-9} to 10^{-3} M were assessed. Cells were pre-incubated with drugs for 20 min and tested for random motility and chemotactic responsiveness to the three leuco-attractants. The

drugs were present with the cells in the upper chamber throughout the incubation period. In one experiment the effects of 5×10^{-5} M propranolol on the kinetics of PMN chemotactic responsiveness to EAS were investigated (1, 2, 3, 4 and 5 h).

Random migration

This was assessed by measuring the extent of cell migration across the 5 μm pore size filter in the absence of a leucotactic gradient. The HBSS was supplemented with BSA (2 mg/ml). Cells were suspended to 5×10^{-6} PMN/ml in this medium. In random migration studies the drugs were likewise prepared in HBSS/BSA and added in equal concentration to both top and bottom compartments. With these techniques the normal range for chemotaxis with EAS as chemo-attractant was 200–260 cells/HPF; with C5a 80–120 cells/HPF; Casein 120–165 cells/HPF, and in random migration systems 8–16 cells/HPF.

Simultaneous assessment of propranolol effects on different types of neutrophil motility

To ascertain the effects of propranolol and atenolol on specific types of neutrophil motility, ie. random migration, chemokinesis and true chemotaxis, PMN migration was assessed when varying concentrations of EAS (1·5, 3, 6 and 12%) were placed above and below the filter in all possible combinations according to the method of Zigmond & Hirsch (1973). Systems so designed were of two types, without propranolol and with 5×10^{-5} M propranolol above and below the filter. Chambers were incubated at 37° for 40 min, filters were fixed, stained, dehydrated, cleared and mounted top side uppermost. Neutrophil migration was expressed as the distance travelled (/μm) as measured with the optical micrometer on the fine focus of the microscope. Results are expressed in tabular form (checkerboard) and true chemotaxis is taken as the difference between the observed values for positive gradients (above the diagonal lines) and the estimated values based on chemokinesis (in parentheses) according to the formula of Zigmond & Hirsch (1973). The effects on chemotaxis were assessed as previously described (Anderson *et al.*, 1976) by comparing true increments for a given gradient in the presence and absence of propranolol. Percentage stimulation or inhibition is calculated according to the formula:

$$\frac{\text{drug value} - \text{control value}}{\text{control value}} \times 100$$

Leucotactic potential of propranolol

To determine if propranolol *per se* possesses chemotactic potential the drug at concentrations of 10^{-6}, 10^{-5}, 5×10^{-5} and 10^{-4} M was placed above and below the filter to yield positive and negative gradients and chemokinesis systems for each drug concentration in the absence of a known leuco-attractant. Using the method of Zigmond & Hirsch (1973) the migratory values for positive propranolol gradients were compared with those obtained in the absence of a positive gradient for each drug concentration.

Pulsing experiments

PMN, prior to assessment of motility, were treated with 5×10^{-5} M propranolol for 60 min after which time they were washed twice, resuspended to 5×10^{6}/ml and placed in the upper chamber. Positive and negative controls consisted of PMN which were identically processed. Negative controls underwent no exposure to propranolol whereas 5×10^{-5} M propranolol was added to positive controls prior to testing of motility and was present throughout the incubation period.

In addition, in another series of experiments PMN were treated with propranolol and EAS (12%) or with EAS alone. After 60 min these cells were washed twice and tested for chemotactic responsiveness to EAS. PMN were also pulsed for 60 min with propranolol + 12% fresh autologous serum or with serum alone, and after washing twice were tested for chemotactic ability to EAS.

Propranolol effects on phagocytosis and post-phagocytic metabolism

Cell preparation. For these studies pure neutrophils were used. Peripheral blood was collected as described above and separated in a Ficoll–Hypaque gradient. Mononuclear cells were discarded and the resultant pellet was resuspended in phosphate buffered saline (PBS, 0·15 M) and sedimented with a 25% volume of 3% gelatin (Difco, Detroit, Michigan) for 45 min. The neutrophil-rich layer was decanted, centrifuged at 250 *g* for 10 min and the resultant cell pellet treated with 0·85% ammonium chloride at 4° for 10 min to lyse residual erythrocytes. The remaining cells which consistently contained more than 90% viable PMN were washed once in PBS and resuspended to an appropriate concentration.

Phagocytosis. The effects of propranolol and atenolol on PMN phagocytosis were assessed at a drug

concentration range of 10^{-9} to 10^{-3} M; 0·5 ml of PMN suspension (6×10^6 PMN/ml) was incubated with 0·1 ml of *Candida albicans* suspension (9×10^7/ml), 0·1 ml fresh autologous serum and 0·2 ml PBS and 0·1 ml of appropriate drug concentration to give a PMN:*C. albicans* ratio of 1:3 in a reaction volume of 1 ml. The mixture was incubated at 37°/20 min on a turntable and the number of extracellular *C. albicans* enumerated microscopically on a haemocytometer. The phagocytic index is expressed as percentage *C. albicans* ingested. In kinetic experiments the effects of propranolol at 10^{-6}, 10^{-5}, 5×10^{-5}, 10^{-4} and 10^{-3} M on the rate of phagocytosis (sampling at 5, 10, 15 and 20 min) was assessed.

Nitro-blue tetrazolium reduction

The semi-quantiative stimulated NBT test was performed according to the method of Sher, Anderson, Rabson & Koornhof (1974). 0·5 ml of heparinized venous blood was incubated with an equal volume of 6% dextran T500 (Pharmacia, Uppsala, Sweden) and incubated at 37° for 1 h. 0·1 ml of this mixture was then added to an equal volume of NBT solution (1 mg/ml, Sigma) and incubated at 37° for 30 min following which a smear was made, air dried, fixed in methanol and stained with 0·1% haemotoxylin. The percentage of NBT (reduced) containing PMN was evaluated microscopically and was consistently greater than 95%.

Hexose monophosphate shunt activity

The extent of HMS activity was measured according to the method of Wood, Katz & Landau (1963) by potassium hydroxide absorption of $^{14}CO_2$ produced from glucose radio-labelled in the C1 position (obtained from New England Nuclear, Boston, Massachusetts as D-glucose 1-[^{14}C]). Pure neutrophil preparations were resuspended in 0·15 M PBS to a concentration of 1×10^7/ml. Each assay was performed in duplicate and utilized 2×10^6 PMN in 0·2 ml and 0·6 ml of radio-labelled glucose containing 0·06 μCi. The remaining 0·3 ml (final reaction volume 1·1 ml) contained 0·1 fresh autologous serum, 0·1 ml of *C. albicans* (6×10^7/ml) and the remaining 0·1 ml accommodated either PBS (controls) or known concentrations of drugs under investigation.

The test apparatus was a 10 ml glass scintillation vial (Packard) which served as the outer chamber stoppered with a tightly fitting perforated McCartney top. Placed inside the outer chamber was a 2 ml autoanalyser cup which served as the inner chamber. The radio-labelled glucose was placed in the outer chamber and 0·6 ml of 1 N KOH in the inner chamber. The outer chamber was stoppered and the apparatus allowed to stand in a 37° water bath for a few minutes. The reaction was initiated by the introduction of the cell suspension to the outer chamber by injection through the cap with a long-needled syringe, and was terminated after varying time intervals by the addition of 2 ml of 2 N HCl. The chambers were allowed to stand for 60 min to permit release of $^{14}CO_2$ and absorption by the KOH, 0·2 ml of which was then transferred to scintillation vials containing 3 ml of acid instagel (Packard), (5·5 ml of 17 N HCl/litre instagel) and activity assessed on a Tri-Carb liquid scintillator for 5 min. Results were expressed as corrected mean counts per minute (c.p.m.). Background controls without neutrophils contained radio-labelled glucose and appropriate additives.

The effects of propranolol and atenolol (10^{-9} to 10^{-3} M) on the HMS activity of resting and stimulated PMN were assessed.

Protein iodination

The iodination of ingested *S. aureus* and *C. albicans* was performed according to the method of Root & Stossel (1974) with minor modifications. To 0·1 ml of a pure neutrophil suspension (1×10^7/ml) in PBS was added 0·1 ml fresh autologous serum, 0·1 ml *C. albicans* (3×10^7/ml) or 0·1 ml *S. aureus* (1×10^9/ml), 0·1 ml of an I^{125} solution in PBS (0·6 μCi/ml) (New England Nuclear, sodium iodate) and 0·1 ml of PBS or known propranolol or atenolol concentration. The reaction volume was made up to 1 ml with 0·6 ml of PBS. Tubes were incubated at 37° for 60 min on a turntable and the reaction terminated by the addition of 2 ml 10% TCA. The resultant protein precipitate was centrifuged and twice washed with TCA. The amount of radioactivity was determined by solid scintillation counting. Controls consisted of tubes with no serum (cells and organisms alone) and tubes with serum alone. The values obtained from these two control systems were summed and deducted from the experimental values to give the true values for phagocytosis-associated protein iodination.

Assay of glycolytic activity

The extent of glycolysis was measured by the amount of lactate production using recognized procedures (Hohorst, 1962). Pure neutrophil suspensions were resuspended to a final concentration of 2×10^7/ml in 0·15 M phosphate buffered saline (PBS) containing 10

mM glucose. Each assay tube (performed in triplicate) utilized 6×10^6 PMN (0·3 ml) in a final volume of 0·5 ml. The remaining 0·2 ml contained either 0·1 ml of fresh autologous serum (5% final concentration) or EAS (5%) and 0·1 ml of PBS in controls and 0·1 ml of propranolol or atenolol in experimental systems. Tubes were incubated at 37° for 60 min and the reaction terminated and the system deproteinized by the addition of 1·0 ml of cold 0·6 N perchloric acid. Tubes were mixed well, centrifuged at 2000 *g* for 10 min and aliquots (0·2 ml) from each tube were assayed for lactate in the presence of NAD and lactate dehydrogenase at 25° for 60 min. The change in optical density at 340 nm (Δ 340 nm) was measured spectrophotometrically. Results were expressed as μg lactate per 6×10^6 PMN per unit time.

Cyclic nucleotide studies

Pure neutrophil preparations were used in these experiments and resuspended to a final concentration of 2×10^7/ml in PBS. To 0·5 ml of cell suspension was added 0·1 ml heat inactivated autologous serum, 0·3 ml PBS and 0·1 ml of PBS alone or PBS containing propranolol to give final concentrations of 10^{-4}, 5×10^{-5} and 10^{-5} M. The tubes were incubated in a water bath at 37°. After a 20 min incubation time the reaction was terminated by the addition of 1 ml cold 0·6 M perchloric acid. PMN in each tube were disrupted in an MSE ultrasonic disintegrator for 1 min (3×20 s bursts) at an amplitude of 10 μm peak to peak. The specimens were then centrifuged at 5000 *g* for 10 min, the pellet was discarded and the supernatant restored to pH 7·2 by the addition of 0·3 M KOH. Specimens were centrifuged for 20 min and the supernatants freeze dried on a Vertis lyophilizer. Specimens were reconstituted in 1 ml of distilled water and aliquots of 20 μl were used for both cAMP and cGMP estimations. Amersham (Amersham England) cAMP and cGMP radio-immunoassay kits and the procedures supplied with each kit were used. Standard curves were run with assay. Duplicate samples were assayed and the final results were expressed as pmol/10^7 PMN.

Estimation of protein synthesis

Protein synthesis in the presence and absence of propranolol (10^{-6}, 10^{-5}, 5×10^{-5} and 10^{-4} M) and serum only (10%) or C5a or EAS (10%) was measured according to the extent of uptake of radio-labelled amino acids (New England Nuclear, amino acid mixture, tritium labelled). Pure neutrophils, 5×10^6 in 0·5 ml, were mixed with 0·2 ml of 50% fresh autologous serum or EAS, or C5a (0·2 ml of pure column preparations) and 0·1 ml of PBS or appropriate propranolol cencentrations. The volume was made up to 1 ml with 0·3 ml PBS. Experiments were duplicated substituting tissue culture medium 199 (TC199) for PBS. Incubation was terminated at varying time intervals (0, 1, 3, 5, 10, 20, 40 and 60 min) by the addition of 10% TCA. The precipitate was washed twice, dissolved in 1 ml 1 N KOH and 0·2 ml transferred to counting vials containing acidified insta-gel. The incorporation was measured and expressed as total counts per 5×10^6 PMN.

Effects of actinomycin D and puromycin aminonucleoside

These drugs were dissolved in HBSS to give a concentration range of 10^{-6} to 10^{-3} M for puromycin and 10, 25 and 50 μg/ml for actinomycin. The effects of these concentrations *per se* on neutrophil motility were determined. PMN were pre-treated with the same drug concentrations for 30 min at 37° prior to the addition of 5×10^{-5} M propranolol and then tested for chemotactic responsiveness to EAS.

RESULTS

Studies of neutrophil motility

The effects of varying concentrations of propranolol on chemotaxis to EAS and casein are shown in Fig. 1a and on chemotaxis to C5a and in the random migration system in Fig. 1b. At propranolol concentrations of between 10^{-6} and 10^{-4} M, there was a consistent increase in chemotaxis to all three leucoattractants and in random migration. Statistically significant stimulation of chemotaxis and random migration was evident at propranolol concentrations of 10^{-5}, 5×10^{-5} and 10^{-4} M. The stimulation was consistently observed with these drug concentrations and was maximal at 5×10^{-5} M. Inhibition of locomotion was observed at 10^{-3} M propranolol. Propranolol *per se*, at the concentrations which caused stimulation of cell motility, possessed no leucotactic activity.

The results of the experiments utilizing different combinations of EAS above and below the filter in the presence and absence of 5×10^{-5} M propranolol, this being the concentration which exerted maximal effects in Fig. 1a and b, are shown in Table 1. It is evident that stimulation of cell motility by propranolol occurred in the presence of a gradient, ie. above the diagonal

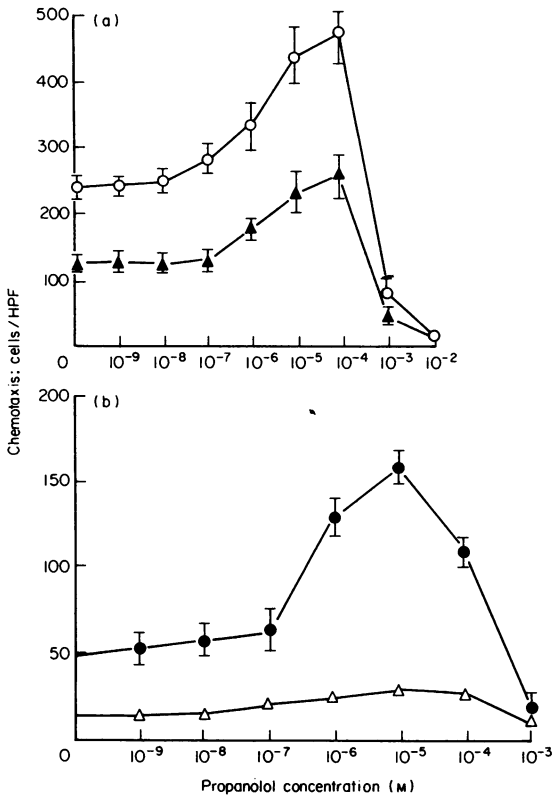

Figure 1 (a) The effects of varying propranolol concentrations on neutrophil chemotaxis to EAS (o) and casein (▲). Results are expressed as mean and standard error. (b) The effects of varying propranolol concentrations on neutrophil chemotaxis to C5a (●) and on random migration (△). Results are expressed as mean and standard error.

Table 1. Effects of propranolol on PMN chemokinesis and true chemotaxis using EAS as chemo-attractant

	EAS (%)				
EAS above filter (%)	0	1·5	3	6	12
(a) No propranolol					
0	30				
1·5		40	53(40)	57(41)	65(43)
3		42(42)	42	55(42)	66(44)
6		41(44)	42(45)	45	69(45)
12		41(47)	43(47)	41(50)	50
(b) 5×10^{-5} M proranolol on both sides of the filter					
0	34				
1·5		66	112(71)	113(77)	117(81)
3		72(76)	81	109(81)	120(82)
6		75(81)	80(81)	81	115(83)
12		71(83)	70(83)	72(83)	85

The effect of varying concentration gradients and absolute concentration of EAS on the motility of human neutrophils in the presence and absence of 5×10^{-5} M propranolol. Figures along the diagonal from upper left to lower right show the distance migrated (μm) in increasing concentrations of the leuco-attractant in the absence of a concentration gradient. Above the diagonal, the cells are moving in a positive gradient. The figures in parentheses are estimates of what migration would have been in each of the tests, assuming that the cells detected the absolute concentration of the chemo-attractant but not the gradient.

(positive gradients, ie. chemotaxis) and below the diagonal (negative gradients) and in the absence of a gradient (chemokinesis, within the diagonals). These results indicate that propranolol produced both stimulation of random migration (13%) and chemokinesis (65, 95, 80 and 70% for 1·5, 3, 6 and 12% EAS above and below the filter respectively).

To assess the effects of propranolol on directional motility the true increments in chemotaxis between control and drug treated cells were compared. Results of these calculations made from the data contained in Table 1 are summarized in Table 2. It can be seen that propranolol caused a marked stimulation of true chemotaxis for all positive gradients tested ($P < 0{\cdot}001$). Atenolol was without effect in this system (results not shown).

Table 2. Analysis of the effects of propranolol on true chemotaxis. Data recorded in Table 1

Positive gradients		True chemotactic increments (μm)		Stimulation of true chemotaxis (%)
EAS (%)	EAS (%)	Control	5×10^{-5} M propranolol	
1·5	3	13	41	215
1·5	6	16	36	125
1·5	12	22	36	63
3	6	13	28	115
3	12	22	38	73
6	12	24	32	33

Table 3. Results of a series of six experiments showing the effect on PMN chemotactic responsiveness to EAS after pre-treating cells with 5×10^{-5} M propranolol and EAS (12%) or serum (12%)

Upper chamber	Cells per HPF Mean	Range	*P* value
PMN + HBSS	152	116–201	
PMN + propranolol (PROP)	289	219–315	<0·001
PMN pulsed with PROP (60 min)	163	109–199	NS
PMN pulsed with EAS (60 min)	55	32–74	<0·001
PMN pulsed with EAS + PROP (60 min)	232	189–261	<0·001
PMN pulsed with serum (60 min)	141	103–181	NS
PMN pulsed with serum + PROP (60 min)	305	226–341	<0·001

In one experiment the kinetics of normal neutrophil chemotactic responsiveness to EAS at 1, 2, 3, 4 and 5 h in the presence and absence of 5×10^{-5} M propranolol was evaluated. Results are shown graphically in Fig. 2. Propranolol caused increased chemotaxis at all time intervals tested.

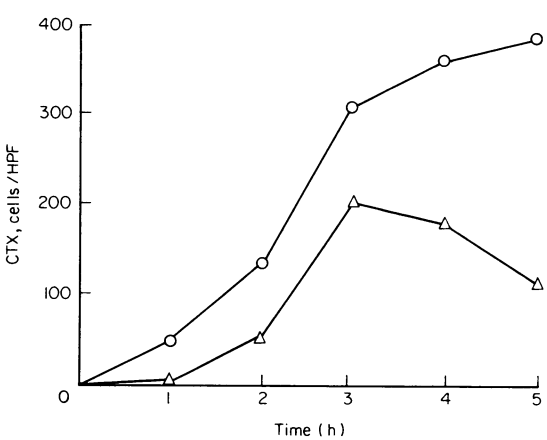

Figure 2. Chemotactic responsiveness of normal neutrophils to EAS at varying time intervals in the presence (o) and absence (Δ) of 5×10^{-5} M propranolol.

Pulsing experiments

Results of pulsing experiments are shown in Table 3. Induction of increased cell motility is dependent upon the presence of serum or EAS during the pre-incubation period. Propranolol stimulation is more efficient in the presence of serum than EAS, presumably because the latter promotes 'deactivation'. The decreased normal responsiveness is probably due to prolonged washing and centrifugation.

Table 4. Effects of propranolol on PMN glycolysis and NBT reduction

Propranolol concentration (M)	EAS associated glycolytic activity mean lactate production as μg/6 × 10 PMN/60 min with standard deviation	Percentage PMN containing reduced NBT expressed as mean and standard deviation
0	47 ± 5·1	95 ± 3·4
10^{-3}	42 ± 6·2	11 ± 3·1
5×10^{-4}	45 ± 5·8	33 ± 5·0
10^{-4}	48 ± 5·3	37 ± 9·0
5×10^{-5}	49 ± 4·9	50 ± 6·0
10^{-5}	47 ± 5·3	66 ± 8·0
5×10^{-6}	48 ± 6·1	81 ± 7·0
10^{-6}	48 ± 5·2	91 ± 4
10^{-7}	48 ± 5·5	96 ± 6

Effects of actinomycin D and puromycin

Both of these drugs at the concentrations tested had no effects on neutrophil motility. Likewise they had no effect on the propranolol (5×10^{-5} M) mediated stimulation of cell motility (results not shown).

Phagocytosis and post-phagocytic metabolism

Neither propranolol nor atenolol at varying concentrations and at varying time intervals had any effect on the ingestion of *C. albicans*. Propranolol, but not atenolol, casued marked inhibition of HMS activity and protein iodination (Fig. 3). Although the inhibition of both functions was apparent at almost all drug concentrations tested (10^{-5} to 10^{-3} M) statistical significance was achieved only at concentrations of 10^{-4} M upwards ($P < 0{\cdot}001$).

Inhibition of both activities was progressive and dose dependent. Inhibition of NBT reduction by propranolol paralleled the inhibition of the HMS and protein iodination (Table 4). Glycolysis was unaffected by propranolol (Table 4).

Cyclic nucleotide studies. Propranolol caused increased levels of intracellular cGMP at the three concentrations tested. There was no apparent effect on the

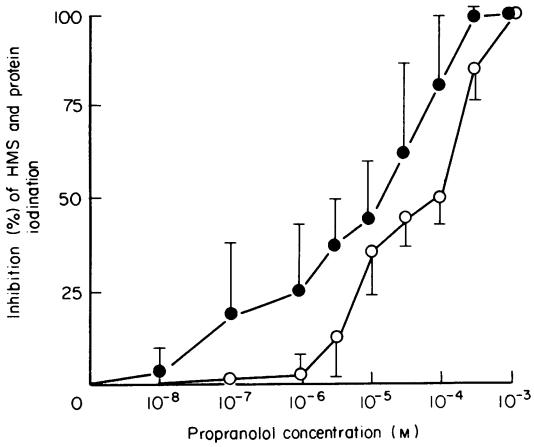

Figure 3. Effects of varying concentrations of propranolol on neutrophil post-phagocytic HMS (o) activity and protein iodination (•). Results are expressed as mean inhibition (%) and standard deviation of normal post-phagocytic activity.

Table 5. Effects of propranolol at concentrations which stimulate PMN motility on intracellular cyclic nucleotide levels

Propranolol concentration (M)	Cyclic nucleotide concentration expressed as pmoles/10^7 PMN/20 min mean and standard deviation of four experiments	
	cAMP	cGMP
0	40·3 ± 12·5	4·8 ± 1·1
10^{-4}	37·7 ± 10·3	12·2 ± 5·4
5×10^{-5}	30·1 ± 11·3	10·8 ± 4·2
10^{-5}	42·2 ± 11·1	10·5 ± 5·1

levels of intracellular cAMP. Results are shown in Table 5.

Propranolol effects on protein synthesis

In control systems no increased protein synthesis in the presence of EAS or C5a was detectable using both PBS and TC 199 as the cell suspending medium. Likewise propranolol *per se* at the four concentrations tested and in the presence of either EAS or C5a had no effect on neutrophil protein synthesis (results not shown).

DISCUSSION

There is some evidence to suggest that propranolol may affect immune reactivity. Beta-blockers such as propranolol and butoxamine have been reported to enhance *in vivo* antibody synthesis to heterologous protein in rats (Benner *et al.*, 1968). Propranolol has also been reported to increase antibody production and lymphocyte transformation in mice (Nakazawa *et al.*, 1976), although Hadden *et al.* (1970) were unable to detect any effect of propranolol on the PHA induced transformation of human lymphocytes. Furthermore, Buckley & McGregor (1977) have reported that propranolol significantly increased the number of survivors, the number of peritoneal exudate cells and decreased the mean duration time of disease in ethanol treated mice experimentally infected with type III pneumococci.

The present studies indicate that propranolol but not atenolol causes stimulation of neutrophil motility and that propranolol *per se* is non-chemotactic. Application of the method of Zigmond & Hirsch (1973) which enables an analytical dissociation of the locomotory response reveals that the stimulation observed was not only of random migration and chemokinesis but also of true directional motility. Propranolol concentrations which stimulated cell motility also caused elevation of intracellular cGMP levels. Haddock, Patel, Alston & Kerr (1975) have reported stimulation of lymphocyte cGMP levels via α-adrenergic stimulation with noradrenaline plus propranolol. It has been previously reported that agents which promote elevated cGMP levels also stimulate cell locomotion (Estensen *et al.*, 1973; Hill *et al.*, 1975; Anderson *et al.*, 1976). It has also been reported, however, that certain agents which promote elevated neutrophil cGMP levels have no stimulatory effect on cell motility (Anderson *et al.*, 1976; Wilkinson, 1976), so that the situation with regard to cGMP stimulation of cell motility is not entirely clear.

An inverse relationship between stimulation of neutrophil motility and inhibition of post-phagocytic HMS activity, protein iodination and nitro-blue tetrazolium reduction was evident. Certain leuco-attractants have been shown to stimulate neutrophil HMS activity (Goetzl & Austin, 1974; Goldstein, Feit & Wassermann, 1975) and ascorbic acid (Goetzl, Wassermann, Gigli & Austen, 1974) and levamisole (Wright, Kirkpatrick & Gallin, 1977) which stimulate motility also cause stimulation of HMS activity *in vitro*. This would indicate a possible role of the HMS in cell locomotion. We have found, however, that levamisole and propranolol (Anderson *et al.*, 1978) cause inhibition of resting HMS activity and HMS

activity stimulated by EAS and phagocytosis. Likewise levamisole (unpublished observations), propranolol and ascorbate (McCall, de Chatelet, Cooper & Ashburn, 1971) (confirmed in this laboratory, unpublished) inhibit protein iodination indicating that all three may inhibit the production of superoxide and hydrogen peroxide. It is possible that although superoxide is necessary for the anti-microbial activity of the phagocytic cell its formation may be toxic to the neutrophil and thereby interfere with certain cell processes essential for a normal locomotory response. Agents which may inhibit pathways involved in the production of superoxide and hydrogen peroxide such as levamisole, propranolol (Anderson *et al.*, 1978) and possibly ascorbate (McCall *et al.*, 1971) could therefore be expected to potentiate chemotaxis. There are arguments against this theory such as the controversial effects of levamisole on the HMS and the fact that dibutyryl cAMP and cAMP elevating agents which inhibit HMS (Anderson *et al.*, 1978) activity also inhibit chemotaxis. These agents however cause inhibition of other cell processes such as adherence (Lomnitzer, Rabson & Koornhof, 1976) and their effects are therefore complex.

In the series of pulsing experiments a serum (or protein) requirement for propranolol stimulation of motility was observed. Serum or EAS and propranolol induced stimulation of motility and eliminated deactivation of chemotactic responsiveness to EAS. In one kinetic experiment using EAS as leuco-attractant propranolol caused consistent stimulation of chemotactic responsiveness at all time intervals tested and also maintained a high level of responsiveness at later time intervals.

No effects of propranolol on neutrophil glycolysis were observed, therefore it is unlikely that the stimulatory effects on motility are due to increased ATP production. Likewise no effects on protein synthesis, as measured by uptake of radio-labelled amino acids were found. In fact no protein synthesis above control level was found in the presence of EAS or C5a alone or in the presence of propranolol indicating no detectable protein synthesis during chemotactic stimulation and the lack of any effect of propranolol on neutrophil amino acid uptake. This was further confirmed by the absence of any effect of puromycin aminonucleoside or actinomycin D on the propranolol induced stimulation of neutrophil motility.

The effects of propranolol on neutrophil function would appear to be a specific property of this drug and not of beta-blockers in general since atenolol, a cardioselective agent, was without effect. Since this class of compounds is fairly large, however, this may not be an accurate conclusion and the effect of other beta-blockers on leucocyte function should be investigated. In our hands the profile of effects of propranolol on human blood neutrophil function is similar to that of levamisole. Propranolol may be of value in the treatment of conditions associated with defective leucocyte motility.

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