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COMPARISON OF FOUR DIFFERENT β-ADRENOCEPTOR BLOCKING DRUGS ON LYMPHOCYTE ISOPRENALINE-STIMULATED CYCLIC AMP PRODUCTION

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1 The influence of acebutolol, atenolol, pindolol and timolol on human lymphocyte cyclic AMP (cAMP) and its stimulation by isoprenaline *in vitro* has been studied.

2 Acebutolol and atenolol (10^{-8} – 10^{-6}M) had no significant influence on lymphocyte cAMP levels or on isoprenaline-stimulated increase in cAMP.

3 Pindolol and timolol significantly antagonised the effect of isoprenaline, and pA_2 values were calculated to be 8.12 and 8.04 respectively. This suggests that β_2-adrenoceptors are involved in this phenomenon.

4 Only pindolol produced a significant increase in lymphocyte cAMP, which is consistent with its partial agonist activity.

Introduction

β-adrenoceptor blocking drugs show pharmacological differences in respect of partial agonist activity and degrees of selectivity for β_1 and β_2 receptor sites (Prichard, 1978). Several *in vivo* methods have been developed for comparing the actions of these drugs in man (McDevitt, 1977) but all have limitations, not least of which is the availability of human volunteers for studies which, if not dangerous, are not pleasant and are time-consuming. Furthermore, large numbers of subjects are needed to demonstrate statistically significant differences because of the variation in response which occurs in these test procedures. Because of this, other test procedures have been sought which could more conveniently be applied to the study of β-receptor function in man. Peripheral blood lymphocytes possess an adenylate cyclase system which can be stimulated by β-adrenoceptor agonists such as isoprenaline and salbutamol and blocked by propranolol (Conolly & Greenacre, 1977). We have compared the influence of four β-adrenoceptor blocking drugs, namely atenolol (selective non-agonist), acebutolol (selective, partial agonist), pindolol (non-selective, partial agonist) and timolol (non-selective, non-agonist) on isoprenaline-stimulated cyclic AMP production in human lymphocytes in order to assess its value in the future evaluation of such drugs.

Methods

Cell separation

Eight volunteers (7 male, 1 female) aged 20–49 years, with no allergic histories, each provided 70 ml of blood by venesection. After centrifugation at 250 *g* for 15 min, the cell buffy coat was transferred to an equivalent volume of Hanks Balanced Salt Solution (HBSS). Four ml aliquots were carefully layered into 3 ml Ficoll Hypaque solution and then centrifuged at 1000 *g* for 15 min at 20°C. The interface cells were washed twice in HBSS. Cells were counted in an Improved Neubauer Chamber (Weber and Sons, Sussex). The suspension was diluted to give a final concentration of 1x 10^6 cells/ml. A cell population of 85–95% lymphocytes was obtained, the remaining cells being monocytes and a few granulocytes.

Cell incubation

The incubations were performed in a final volume of 1.5 ml, including cell suspension, theophylline (10^{-2}M), isoprenaline and the β-adrenoceptor blocking drugs. The doses studied ranged from 10^{-8}M to 10^{-4}M of isoprenaline and the concentrations of atenolol, acebutolol, pindolol and timolol used were 10^{-8}M, 10^{-7}M and 10^{-6}M. The incubation time for these

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studies was 12 min at 37°C, and all incubations were performed in duplicate. The tubes were then spun for 15 min at 500 *g* and the supernatant was discarded. Then 1 ml of distilled water was added, the mixture was vortexed and protein was denatured by boiling for 3 min. The tubes were then frozen overnight.

Cyclic AMP extraction

In order to estimate recovery, ^{3}H-cyclic AMP (5000 counts/min, New England Nuclear, USA) was added to all tubes. The tubes were then freeze-thawed five times and tricholoroacetic acid (TCA) 25% was added to all tubes. They were spun at 2000 *g* for 15 min at 4°C for protein removal. The supernatant was transferred to new tubes and the TCA extracted three times with water-saturated ether. The aqueous phase was evaporated, then dissolved in 0.06M sodium acetate buffer (pH 6.2).

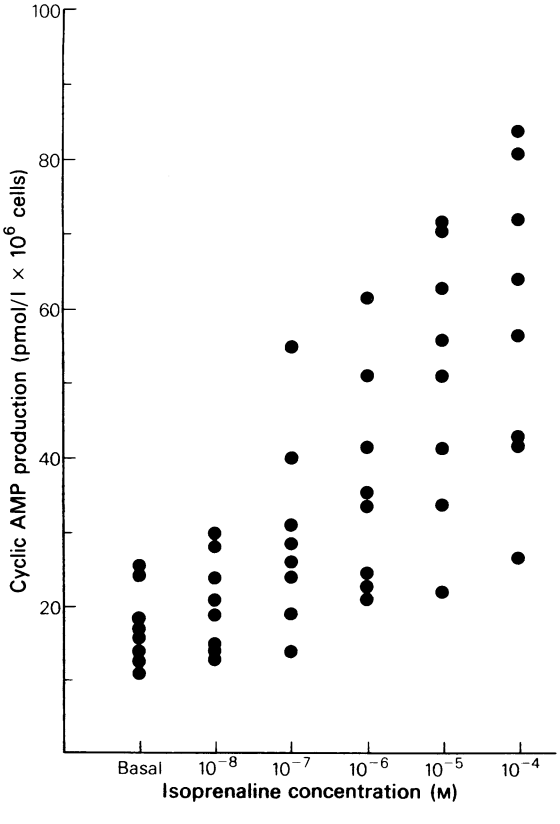

Figure 1 The effect of increasing concentrations of isoprenaline on the production of cyclic cAMP by lymphocytes.

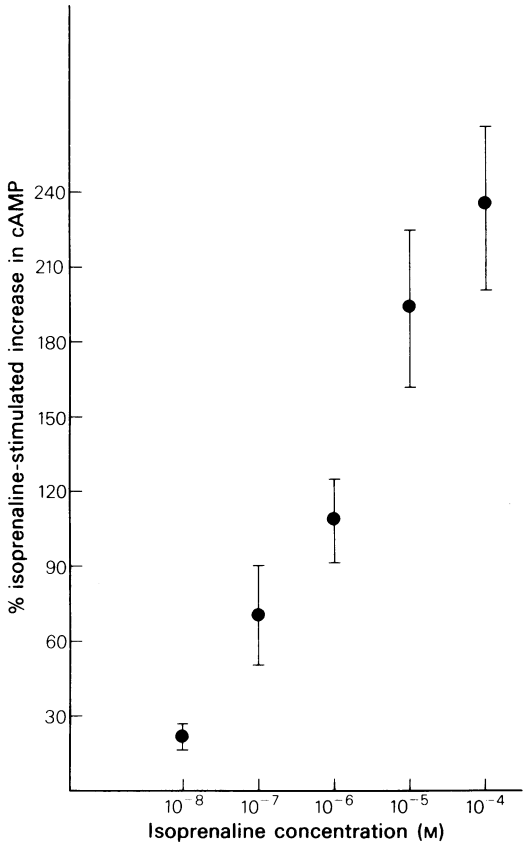

Figure 2 The date from **Figure 1** expressed as a percentage of the isoprenaline-stimulated increase in cyclic cAMP above the baseline level (Mean ± s.e. mean, $n = 8$).

Cyclic AMP assay

Cyclic AMP was assayed by the method of Steiner, Parker & Kipnis (1972) with the use of commercially available reagents for the radioimmunoassay of cyclic AMP (New England Nuclear, Boston, Massachusetts, USA).

pA_2 determinations

pA_2 values for pindolol and timolol were calculated by the method of Schild (1947) as modified by Davis, Conolly & Greenacre (1980). The effect of increasing concentrations of timolol and pindolol on the formation of cAMP by lymphocytes in the presence of a given concentration of isoprenaline (10^{-5}M) was studied. From this dose-response curve and from the

original dose-response curve to isoprenaline in the absence of any antagonist, dose ratios at various isoresponse points were calculated and a Schild plot constructed. pA_2 values and the slope of the Schild plot were derived by linear regression.

Results

Basal levels of lymphocyte cyclic AMP varied between 11.25–25.00 pmol/1 × 10^6 cells, and the increases produced by stimulation with isoprenaline 10^{-8}M – 10^{-4}M varied widely among the eight subjects (Figure 1). Figure 2 shows the same data expressed as percentage of isoprenaline-stimulated increases in cyclic AMP above base-line levels, demonstrating a dose-response effect. A concentration of 10^{-5}M isoprenaline was chosen for the studies of β-adrenoceptor inhibitory drugs as it was submaximal in its stimulating action on cyclic AMP production. Figure 3 shows the percentage inhibition of isoprenaline (10^{-5}M) stimulated cyclic AMP produced by atenolol, acebutolol, pindolol and timolol in concentrations of 10^{-8}M, 10^{-7}M and 10^{-6}M. Atenolol and acebutolol did not produce significant inhibition of cyclic AMP stimulation by isoprenaline. Pindolol and timolol produced significant concentration-dependent inhibition ($P<0.05$) but did not differ from each other in their inhibitory potency or in the slope of their dose-response curves.

The effects of pindolol and timolol at concentration of 10^{-6}M were significantly greater than those of acebutolol and atenolol ($P<0.05$).

pA_2 values for timolol and pindolol were calculated to be 8.04 and 8.12 respectively. The slope of the Schild plot did not differ significantly from unity indicating that both drugs were acting as competitive antagonists (Table 1, Figure 4).

The influence of the four β-adrenoceptor antagonists on lymphocyte cyclic AMP levels in the absence of isoprenaline is shown in Table 2. Although acebutolol, pindolol and timolol appeared to demonstrate concentration related increases in lymphocyte cyclic AMP content, only in the case of pindolol were the increases significant at concentrations of 10^{-7} and 10^{-6}M ($P<0.05$).

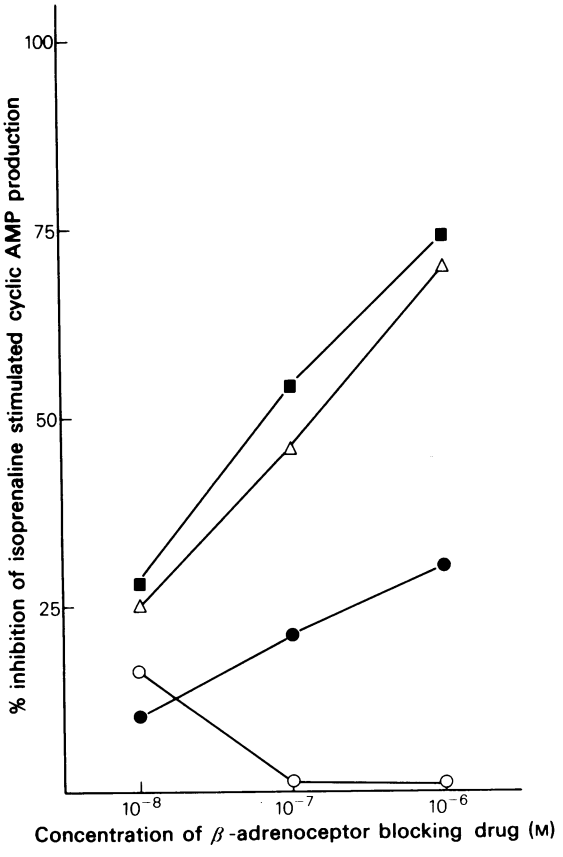

Figure 3 The percentage inhibition of isoprenaline (10^{-5}M) stimulated cyclic AMP production by atenolol (○), acebutolol (●), pindolol (△) and timolol (■).

Discussion

These results confirm those of Conolly & Greenacre (1977) that isoprenaline stimulates cyclic AMP generation in human lymphocytes. They also confirm that such stimulation is inhibited in a concentration-dependent fashion by some β-adrenoceptor antagonists. Conolly & Greenacre (1977) found that propranolol produced a significant inhibition but that practolol did not, suggesting that β_2 receptors are involved in this phenomenon. Our results are in agreement with this, in that the two β_1 selective agents, at atenolol and acebutolol, did not produce significant inhibition at the concentrations used, although the small effects of acebutolol were concentration-related. The non-selective antagonists timolol and pindolol produced a similar degree of inhibition of isoprenaline stimulated cyclic AMP production.

The pA_2 value obtained for pindolol is in agreement with those from animal studies (Moore & O'Donnell, 1970). Despite an extensive search of the literature, no previously published pA_2 values for timolol could be found. However, from experiments in animals (Hall, Robson & Share, 1975) and man (Ulrych, Franciosa & Conway, 1972), timolol was shown to be 5 to 10 times more potent than propranolol. Conolly & Greenacre (1977) calculated

Table 1 The % maximum cyclic AMP response to isoprenaline 10^{-5}M in the presence of increasing concentrations of timolol (n = 6 for each concentration) and pindolol (n = 7 for each concentration) (mean ± s.e. mean)

Concentration of timolol (M)	*% maximum cAMP stimulation in presence of antagonist and 10^{-5}M isoprenaline*	*Concentration of isoprenaline to produce similar response in absence of antagonist* (M)	*Dose-ratio*
10^{-8}	60 ± 9.5	5 × 10^{-6}	2.0
10^{-7}	38 ± 7.8	5 × 10^{-7}	20
10^{-6}	22 ± 4.9	6 × 10^{-8}	166.67
Concentration of pindolol (M)			
10^{-8}	61 ± 9.6	4 × 10^{-6}	2.5
10^{-7}	44 ± 8.4	9 × 10^{-7}	11.1
10^{-6}	25 ± 6.5	7.5 × 10^{-8}	133.33

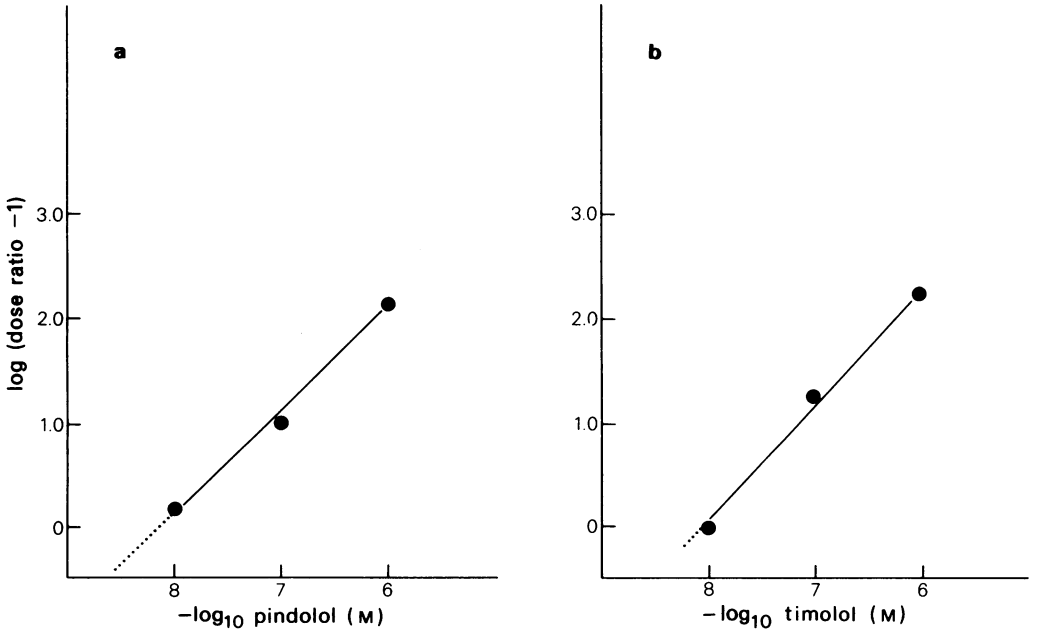

Figure 4 a) Schild plot for pindolol from the mean of seven experiments. pA_2 value for pindolol against isoprenaline is 8.12, the slope of the line is 1.02 and r = 0.996.
b) Schild plot for timolol from the mean of six experiments. pA_2 value for timolol against isoprenaline is 8.04, the slope of the line is 0.90 and r = 0.996.

Table 2 Lymphocyte cyclic AMP (pmol/1 × 10^6 cells) (mean ± s.e. mean) after incubation with acebutolol, atenolol, pindolol and timolol compared with control value (n = 8)

	Control	*Drug concentration* (M)		
		10^{-8}	10^{-7}	10^{-6}
Acebutolol	17.4 ± 1.8	16.2 ± 2.2	18.9 ± 3.1	21.2 ± 3.3
Atenolol		11.5 ± 1.7	16.7 ± 2.8	14.3 ± 1.4
Pindolol		16.8 ± 12.8	23.2 ± 2.0*	24.4 ± 2.8*
Timolol		15.6 ± 2.1	19.4 ± 2.6	21.8 ± 2.7

*Significantly different from control (P<0.05)

a pA_2 value of 8.34 for propranolol against isoprenaline stimulation of cAMP formation in the human lymphocyte. This suggests that timolol is of similar potency to propranolol in blocking the β-adrenoceptor in the human lymphocyte.

There are several limitations to this modified method of determining pA_2 values among which are firstly the fact that it is not known whether there is a parallel displacement of the agonist dose-response curve by the antagonist, so justifying the calculation of a pA_2 value, and secondly it has been reported by Coleman & Somerville (1977) that the use of a single fixed dose of agonist can give rise to considerable error depending on the agonist dose chosen. However, the volume of blood required from a single person to obtain sufficient lymphocytes to determine a pA_2 by the conventional method of dose-response curves to isoprenaline in the absence and presence of increasing concentrations of antagonist is unacceptably large (more than 300 ml) and also the pH of the isoprenaline solution above 10^{-2}M is such that it inhibits the formation of cAMP by the lymphocytes.

Pindolol possesses a marked degree and acebutolol a lower degree of partial agonist activity (Prichard, 1978), and it had been predicted that this might have influenced their effect on stimulation of lymphocyte cyclic AMP by isoprenaline. However, in the concentrations of agonist and antagonist used and with the relatively small numbers of samples it was not possible to demonstrate a difference in these effects of pindolol and timolol on one hand, and acebutolol and atenolol on the other. However, when the four drugs were compared in respect of their ability themselves to stimulate the generation of lymphocyte cyclic AMP, pindolol demonstrated a significant agonist effect at concentrations of 10^{-7}M and 10^{-6}M. Acebutolol and timolol also appeared to produce dose-dependent increases, but these were not statistically significant. Although acebutolol possesses partial agonist activity this has not been claimed for timolol. Further studies are therefore required to assess the value of such an increase in lymphocyte cyclic AMP as a screening procedure to detect partial agonist activity.

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