Ethnic differences in human lymphocytic cyclic AMP production after isoprenaline stimulation and propranolol blockade

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- 1 The stimulatory effects of isoprenaline on human lymphocytic cyclic AMP (cAMP) and blockade by propranolol were studied *in vitro* in healthy Black and White volunteers.
- 2 Basal levels of lymphocytic cAMP were significantly higher in Blacks than in Whites.
- 3 Stimulation with isoprenaline caused a dose-related increase in cAMP, which was in concentrations of 10^{-9} to 10^{-5} M significantly greater in Blacks than in Whites.
- 4 Blockade by 10^{-4} M propranolol did not affect basal cAMP levels significantly, but increases in cAMP levels were significantly smaller in both groups after 10^{-9} to 10^{-2} M isoprenaline, while differences between cAMP levels in Blacks and Whites were still significant at concentrations 10^{-9} to 10^{-3} M.
- 5 The increased cAMP concentration in lymphocytes of Blacks probably reflects a higher degree of β_2 -adrenoceptor activity which could be due to either a greater number and/or greater sensitivity of lymphocytic β_2 -adrenoceptors in Blacks than in Whites.

Keywords lymphocytic cAMP isoprenaline propranolol ethnic differences

Introduction

The incidence of essential hypertension in Black South Africans is significantly higher than in White South Africans (Seedat & Seedat, 1982) and it has furthermore been suggested that β -adrenoceptor blocking agents are less effective anti-hypertensive agents in Blacks than in Whites (Seedat, 1980; Abson *et al.*, 1981).

It is generally accepted that the anti-hypertensive effect of β -adrenergic receptor blockers is related to their antagonism of β -adrenoceptors.

Although some workers (van Zwieten & Timmermans, 1983) have ascribed the anti-hypertensive effect of β -adrenoceptor blocking agents to blockade of β_1 -adrenoceptors, the role of blockade of other β -adrenoceptors cannot be excluded.

It was recently shown that higher doses of penbutolol (Venter & Joubert, 1982) and pro-

pranolol (Venter & Joubert, 1984) are required to produce similar degrees of inhibition of exercise-induced tachycardia in Blacks than in Whites. Analysis of the results suggested either qualitative and/or quantitative ethnic differences in β_1 -adrenoceptors.

These observations prompted us to extend our investigations and to determine whether similar ethnic differences are also exhibited by other β -adrenoceptors. It has been shown by various investigators (Conolly & Greenacre, 1977; Lima *et al.*, 1981) that peripheral blood lymphocytes possess an adenylate cyclase system which is identical to β_2 -adrenoceptors in lungs and other tissues.

Lymphocytic β_2 -adrenoceptors can be stimulated by β -adrenoceptor agonists such as isoprenaline and blocked by antagonists such as propranolol (Coleman & Somerville, 1979). It was therefore decided to use peripheral human

blood lymphocytes to study possible ethnic differences in β_2 -adrenoceptors.

Methods

This study was conducted on lymphocytes obtained from eight matched pairs of healthy Black and White volunteers, which consisted of equal numbers of males and females. Venous blood (10 ml) was collected in a lithium-heparinized silicone coated tube from each volunteer every morning at 08.00 h on six consecutive days (a total volume of 160 ml per day).

After collection each day, the blood was diluted 50:50 with Tris buffered saline and lymphocytes were separated by using Lymphoprep®. Lymphocytes obtained from Black and White volunteers were pooled separately and counted by a Coulter counter. A differential white cell count yielded an average lymphocyte population of 96%.

The lymphocytes were then divided into 2×18 tubes (18 for Blacks and 18 for Whites) with a total of 10^6 cells/tube. The cells were suspended in a final volume of 1 ml phosphate buffered saline (pH 7.2) and theophylline was added to a final concentration of 10^{-3} M, to prevent breakdown of cAMP.

Each set of 18 tubes were divided into two sets of nine. To the first nine tubes propranolol

10^{-4} M was added and to eight of them isoprenaline 10^{-9} to 10^{-2} M. The same was done to the other nine tubes except that propranolol was omitted. This procedure was conducted on 6 consecutive days. Results shown in Figure 1 and Table 1 are the means of the six determinations.

After incubation for 12 min at 37°C , the tubes were centrifuged at 500 g. The supernatant fluid was removed and the cells were lysed with 500 μl lysis buffer. The protein was denatured with 0.5 ml 10% trichloroacetic acid and after centrifugation the supernatants were extracted four times with five volumes of water saturated diethylether to remove the trichloroacetic acid. cAMP determinations were performed using the isotope dilution assay (Boehringer Mannheim Kit). The values were corrected for a recovery of 85% using 8 μCi of $[^3\text{H}]$ -cAMP as tracer.

Statistical analysis comparing basal lymphocytic cAMP levels in Blacks and Whites and also levels obtained after stimulation with various concentrations of isoprenaline in the absence or presence of propranolol was carried out using Student's unpaired *t*-test. A *P* value of 0.05 or less was considered significant.

Results

Results expressed as pmol cAMP per 1×10^6 lymphocytes, are listed in Table 1, and illus-

Figure 1 The effects of increasing concentrations of isoprenaline on the production of lymphocytic cAMP in the presence (closed symbols) or absence (open symbols) of 10^{-4} M propranolol in Blacks (○, ●) and Whites (△, ▲).

Table 1 Lymphocytic cAMP production (pmol/ 1×10^6 cells; mean $\pm$ s.d.) after isoprenaline stimulation and/or propranolol (10^{-4} M) blockade.

Volunteers	Basal levels	Isoprenaline (M)						
		10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-2}
Blacks (n = 8)	25.8 $\pm$ 15.3 **	34.3 $\pm$ 6.9 *	50.0 $\pm$ 7.6 **	68.8 $\pm$ 6.8 **	90.6 $\pm$ 9.6 **	137.1 $\pm$ 19.7 **	134.4 $\pm$ 18.0	140.5 $\pm$ 15.8
Whites (n = 8)	13.8 $\pm$ 3.2	25.8 $\pm$ 2.8	33.5 $\pm$ 3.0	45.1 $\pm$ 2.9	63.9 $\pm$ 4.4	103.3 $\pm$ 12.7	137.7 $\pm$ 14.8	144.5 $\pm$ 12.6
Isoprenaline (M) + 10^{-4} M propranolol								
Blacks (n = 8)	27.2 $\pm$ 6.0	28.5 $\pm$ 5.1 **	30.5 $\pm$ 5.4 **	38.8 $\pm$ 4.1 **	44.2 $\pm$ 5.2 **	62.5 $\pm$ 8.1 **	100.8 $\pm$ 11.4 **	121.2 $\pm$ 7.8 **
Whites (n = 8)	12.0 $\pm$ 1.8	12.0 $\pm$ 2.7	11.9 $\pm$ 3.2	15.6 $\pm$ 3.4	19.0 $\pm$ 5.1	28.8 $\pm$ 5.9	47.6 $\pm$ 6.3	112.6 $\pm$ 13.8

Significant difference between groups: * $P < 0.05$, ** $P < 0.01$

trated in Figure 1. Basal levels of lymphocytic cAMP are significantly ($P < 0.01$) higher in Blacks than in Whites.

Stimulation of lymphocytes with isoprenaline caused a dose-related increase of lymphocytic cAMP in both groups, but levels of cAMP were significantly higher in Blacks than in Whites at isoprenaline concentrations of 10^{-9} M ($P < 0.05$) and 10^{-8} to 10^{-5} M ($P < 0.01$).

After blockade with 10^{-4} M propranolol, basal cAMP levels in Blacks remained significantly ($P < 0.01$) higher than in Whites and did not differ significantly from pre-blockade values in both instances.

Stimulation with 10^{-9} M and 10^{-8} M isoprenaline did not cause any significant changes in cAMP levels of either Blacks or Whites, but isoprenaline 10^{-7} to 10^{-3} M caused dose-related increases in both groups. At 10^{-2} M isoprenaline however, there was no significant difference between cAMP concentrations of the two groups.

Discussion

Our results confirm those of Conolly & Greenacre (1977), and Lima *et al.* (1981), namely that isoprenaline stimulates cAMP generation in lymphocytes. This generation can be antagonised competitively by β -adrenoceptor blockers such as propranolol. The increased cAMP concentration in lymphocytes of Blacks probably reflects a higher degree of β_2 -adrenoceptor activity, both at basal level as well as after stimulation with isoprenaline. This could be explained by either a greater number and/or greater sensitivity of lymphocytic β_2 -adrenoceptors in Blacks than in Whites.

The fact however that the same maximal cAMP concentration was attainable in the two groups would perhaps suggest a higher sensitivity of β_2 -adrenoceptors rather than higher numbers in Blacks. Formal radioligand studies are required to distinguish between these possibilities. Although blockade by propranolol did not affect basal levels of cAMP in either Blacks or Whites, it did reduce cAMP production with resultant depression of dose-response curves after stimulation with isoprenaline (Figure 1). It therefore appears that Blacks have higher β_2 -adrenoceptor activity in lymphocytes than Whites. If this also applies to vascular β_2 -adrenoceptors, it may have important clinical implications. Peripheral vascular resistance which plays a major role in the establishment of arterial blood pressure is largely maintained by a balance between arteriolar constriction of cutaneous vessels

where α_1 -adrenoceptors are present, and arteriolar dilatation of skeletal vessels where an abundance of β_2 -adrenoceptors are present.

A balance between vasoconstriction and dilation is therefore responsible for the net peripheral resistance. Blockade of β_2 -adrenoceptors by agents such as propranolol inhibits dilatation of muscle arterioli and will therefore be inclined to increase peripheral resistance. Whilst Blacks appear to be equipped with a

relatively more active β_2 -adrenoceptor system, β_2 -adrenoceptor blockade in Blacks might result in a greater increase in peripheral resistance than in Whites, hence the resistance of Black hypertensives to β -adrenoceptor blockade.

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