SHORT COMMUNICATION

The effect of aging on β-adrenoceptor-stimulated cyclic AMP formation in human lymphocytes

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Summary

1. Responsiveness of the β-adrenoceptor adenylate cyclase system was measured in lymphocytes from healthy young and old subjects by incubating the cells with isoprenaline in the presence of a phosphodiesterase inhibitor and by measuring production of adenosine 3':5'-cyclic monophosphate (cyclic AMP) with a competitive binding assay.

2. The two groups did not differ significantly in the levels of cyclic AMP produced or in the concentration of isoprenaline required to give half-maximal stimulation of the cells (ED₅₀).

Key words: β-adrenoceptors, aging, cyclic AMP, lymphocytes.

Abbreviations: cyclic AMP, adenosine 3':5'-cyclic monophosphate; cyclic GMP, guanosine 3':5'-cyclic monophosphate; EDTA, ethylenediaminetetraacetate.

Introduction

There is conflicting evidence on whether the responsiveness of the β-adrenoceptor adenylate cyclase system is altered in the elderly.

A study in man in vitro showed that the physiological response to both isoprenaline and propranolol was reduced in healthy elderly subjects (Vestal, Wood & Shand, 1979). A fall in β-adrenoceptor numbers with aging has also been reported (Schocken & Roth, 1977).

Some work in vitro has shown a decreased response to β-agonists with aging (Berger, Preiss, Hesse-Wortmann & Gries, 1971; Ericsson, 1974; Cooper & Gregerman, 1976; Chung, Dillon, Kelly & O’Malley, 1979) whereas other studies have demonstrated the converse (Kranz & Wollenberger, 1976; Kalish, Katz, Pineyro & Gregerman, 1977).

In the present study we have examined the stimulation of adenosine 3':5'-cyclic monophosphate (cyclic AMP) formation in lymphocytes from healthy old and young human subjects as a measure of the primary receptor response to β-agonists.

Materials and methods

Subjects

Ten old and 10 young subjects were studied (for details see Table 1). All were healthy volunteer subjects; none had any history of atopy, smoked or took regular medication.

Blood samples

Each subject was tested on two separate occasions. All subjects were free from any medication for 3 days before blood sampling and abstained from alcohol and caffeine for 12 h. Between 09.00 and 10.00 hours, 30 or 40 ml of venous blood was drawn into ethylenediaminetetraacetate (EDTA).

Preparation and incubation of lymphocytes

Lymphocytes were prepared by a modification of the technique of Boyum (1968). Portions of blood were layered over an equal...
volume of lymphocyte separation medium (Flow Laboratories Ltd, Irvine, Ayrshire, Scotland, U.K.) and centrifuged at 400 g for 30 min. The lymphocyte layer was taken off; the cells were washed in medium 199 containing Hanks salts, Hepes buffer (25 mmol/I) and L-glutamine (100mg/l) (Gibco Europe Ltd, Paisley, Scotland, U.K.), and resuspended to a final concentration of 1–3 x 10^6 cells/ml (above 95% of these cells being viable).

The incubation procedure was a modification of the method of Makino, Ikemori, Kashima & Fukuda (1977). Aliquots of cell suspension were preincubated at 37°C for 10 min. Medium 199 (as above) was added containing 3-isobutyl-1-methylxanthine (to give a final concentration of 1 mmol/l) and (±)-isoprenaline (Suscardia, Pharmax Ltd, Bexley, Kent, U.K.). After a further 15 min incubation the cells were sedimented by centrifugation, the supernatants discarded and cell pellets frozen in solid carbon dioxide/acetone and thawed in distilled water. Protein was precipitated by boiling and sedimented by centrifugation. The supernatants were stored at -20°C. Cyclic AMP was assayed by a competitive protein-binding method with a commercial kit (cyclic AMP assay kit, The Radiochemical Centre, Amersham, Bucks., U.K.). This assay is highly specific for cyclic AMP; a 200-fold excess of guanidine (3':5'-cyclic monophosphate (cyclic GMP) is required to give 50% inhibition of binding. Absence of interference by other substances was confirmed by obtaining binding curves in the presence of cell extracts previously treated with phosphodiesterase.

**Statistical analysis**

The basal and stimulated cyclic AMP levels and the concentrations of isoprenaline required to give half-maximal cyclic AMP production (ED_{50}) in the two groups were compared by Wilcoxon's test for unpaired data.

**Results**

There were no significant differences between the two age groups in basal cyclic AMP levels, production of cyclic AMP in response to isoprenaline stimulation or in the ED_{50} for isoprenaline (Table 1).

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Cyclic AMP (pmol/10^6 cells)</th>
<th>ED_{50} of (±) isoprenaline (mol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>24.5 ± 6.76 ± 1.01</td>
<td>32.97 ± 5.02</td>
</tr>
<tr>
<td>(18–30)</td>
<td></td>
<td>6.8 ± 1.2 x 10^-6</td>
</tr>
<tr>
<td>Old</td>
<td>73.5 ± 6.68 ± 0.56</td>
<td>34.21 ± 4.42</td>
</tr>
<tr>
<td>(68–79)</td>
<td></td>
<td>6.4 ± 1.2</td>
</tr>
</tbody>
</table>

There was some variation in cyclic AMP levels in a single subject measured on several occasions (mean coefficient of variation 36%) and also among the subjects in each group (mean coefficients of variation of 42 and 37% in the young and old groups respectively). The reproducibility of measurements on replicate samples was very high.

**Discussion**

The results of the present study do not agree with those of several earlier studies in man, which have suggested that β-adrenoceptor sensitivity is reduced with aging. Vestal et al. (1979) found that the effect of isoprenaline on the resting heart and the ability of a given free concentration of propranolol to antagonize this effect were decreased in older healthy subjects. Such results may indicate reduced β-adrenoceptor sensitivity with aging, but considerable cardiovascular system adjustment also takes place in such situations in vivo and thus heart rate is only an indirect measure of β-adrenergic response. Schocken & Roth (1977) reported a decrease in β-adrenoceptor density on human lymphocytes with advancing age, and Chung et al. (1979) found both a reduction in the ED_{50} and maximal response to isoprenaline of cyclic AMP formation in such cells. However, these latter workers compared healthy young subjects with geriatric patients and it is well established that alterations in drug pharmacokinetics and dynamics occur with disease (see Smith & Rawlins, 1973; Crooks & Stevenson, 1979). Furthermore Schocken & Roth's (1977) evaluation of the specific binding of the lipid soluble β-antagonist [3H]dihydroalprenolol to lymphocyte membranes probably leads to an overestimate of receptor numbers (see Nahorski & Richardson, 1979). The reported change in receptor numbers with aging could therefore be attributable in part to a change in the extent of lipid partitioning of [3H]dihydroalprenolol. Since there is a close association between β-adrenoceptor numbers and cyclic AMP production (Levitzki, 1976) the present
results suggest that lymphocyte \( \beta \)-adrenoceptor numbers did not alter with age.

Some of the variation within and between subjects may reflect differences in the relative densities of lymphocyte subpopulations. Pochet, Delespesse, Gausset & Collet (1979) showed that \( \beta \)-adrenoceptors are not equally distributed in the different subpopulations of purified human T and B lymphocytes. Apparent changes in \( \beta \)-adrenoceptor sensitivity can also result from altered levels of circulating catecholamines and exogenous \( \beta \)-agonists (Greenacre & Conolly, 1978; Mackenzie, Popkin, Sheppard, Stillner, Davis & Fenimore, 1980). Since there is a linear rise in plasma adrenaline and noradrenaline concentrations with aging (Lake, Ziegler, and B lymphocytes. Apparent changes in \( \beta \)-adrenoreceptor adenylate cyclase system in exogenous \( \beta \)-agonists (Greenacre

In conclusion, we have found no evidence of an age-related change in the responsiveness of the \( \beta \)-adrenoceptor adenylate cyclase system in healthy human subjects.

Acknowledgments

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References


Nahorski, S.R. & Richardson, A. (1979) Pitfalls in the assessment of the specific binding of \(-\)\( \text{H} \)dihydroalprenolol to \( \beta \)-adrenoreceptors. British Journal of Pharmacology, 66, 469–470W.


