SODIUM FLUORIDE ACTIVATION OF HUMAN LYMPHOCYTE ADENYLATE CYCLASE IS REDUCED IN OLD AGE

Sympathetic nervous system responsiveness in man decreases with advancing age (Vestal *et al.*, 1979; Bertel *et al.*, 1980). We have previously shown, using the human lymphocyte model, that the isoprenaline or prostaglandin E_1 stimulated cyclic AMP response is markedly reduced in the elderly (Dillon *et al.*, 1980). The observed reduction in cyclic AMP response is not, however, associated with a decrease in cellular β -adrenoceptor density (Doyle *et al.*, 1981a).

The stimulation of adenylate cyclase through either the β -adrenoceptor or prostaglandin receptor requires a functioning GTP regulatory unit. Alterations in the GTP regulatory unit would influence translation of the drug-receptor interaction into a biochemical event and thus effect the response. As sodium fluoride also stimulates cyclic AMP production by a process involving the GTP regulatory unit (Rimon *et al.*, 1980; Downs *et al.*, 1980) we used this non-receptor mediated event as a means to study the mechanism of altered adenylate cyclase responsiveness in the elderly.

Samples of venous blood were obtained from 9 healthy young people (6 F, 3 M) aged between 20 and 29 years with a mean age of 24 years and from 9 healthy elderly people (6 F, 3 M) aged between 67 and 88 years with a mean age of 76 years. All subjects were drug free. The elderly subjects were ambulant and community living. Blood was diluted 1:1 with phosphate buffered saline and lymphocytes isolated by density gradient centrifugation (Boyum, 1967). Aliquots of cells (2×10^6) were incubated with 1 mM theophylline and 10 mM sodium fluoride at room temperature in a final volume of 0.8 ml. This concentration of sodium fluoride produces maximum stimulation of adenylate cyclase (Martin *et al.*, 1980). The cells were incubated for periods ranging from 0 to 30 min. The reaction was terminated by placing the tubes in a boiling water bath for 5 min. Aliquots were assayed in duplicate for cyclic AMP content by a competitive protein binding assay (Radiochemical Centre, Amersham). The concentration of sodium fluoride used in these experiments did not interfere with the cyclic AMP assay.

Figure 1 shows the cyclic AMP production by lymphocytes from young and elderly people in the presence of 10 mM sodium fluoride. Baseline lymphocyte cyclic AMP concentrations (in the absence of sodium fluoride) in the elderly were significantly lower than in the young (4.8 ± 0.8 and 12.4 ± 1.4 pmol/ 10^6 cells respectively: mean $\pm$ s.e. mean, $P < 0.01$). The time required for measurable adenylate cyclase activation by sodium fluoride was greater in lymphocytes from elderly people than from young people. In lymphocytes from elderly subjects there

Figure 1 Cyclic AMP production in the presence of 10 mM NaF by lymphocytes from young (●) and elderly (○) people.

was no response observed before 16 min. In lymphocytes from young people there was an almost instantaneous response becoming significantly different from baseline values at 16 min ($P < 0.05$). Peak concentrations were observed at 24 min for the elderly group and 26 min for the young group with significantly different peak values. Mean maximum cyclic AMP production by lymphocytes from young people was 38.7 ± 9.5 pmol/ 10^6 cells and 8.7 ± 1.2 pmol/ 10^6 cells from the elderly ($P < 0.001$).

The baseline values obtained for cyclic AMP production agree with our previous results (Doyle *et al.*, 1981b). The present data show a decreased cyclic AMP response to stimulation by sodium fluoride, a process which does not involve cell membrane receptors. It therefore appears that a post receptor mechanism is responsible for the age related decrease in lymphocyte cyclic AMP production following various stimuli. This could involve a decreased activity or responsiveness in adenylate cyclase due to regulatory mechanisms or membrane structural changes associated with the ageing process.

The time lag in sodium fluoride activation of adenylate cyclase in the elderly could be due to alterations in the rate of entry of the fluoride ion into the cell, perhaps secondary, to age related alterations in membrane permeability. In conclusion we speculate that the observed decreased production of cyclic AMP in response to various stimuli, isoprenaline,

prostaglandin E₁ and sodium fluoride, are due to age associated alterations in cell membrane structure.

This work was supported by the Medical Research Council of Ireland.

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Received November 27, 1981

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TOCAINIDE PROTEIN BINDING IN NORMAL VOLUNTEERS AND TRAUMA PATIENTS

It is a general principle of pharmacology that plasma concentration of free drug reflects concentration at receptor sites better than does plasma concentration of total drug (i.e., pharmacological and toxicological effects are better correlated with free concentration). Further, substantial experimental evidence confirms the reliability of this principle. For example, in volunteers the reversal of isoprenaline-induced tachycardia by propranolol is much better correlated with free than with total plasma concentration (McDevitt *et al.*, 1976). Similarly, in epileptics phenytoin toxicity is more closely correlated with free concentration of this drug (Booker & Darcy, 1973).

Drugs such as quinidine, lignocaine, propranolol and disopyramide are highly bound and their free fractions are strongly correlated with the plasma concentration of α_1 -acid glycoprotein (α_1 -AGP), an acute phase reactant (Piafsky, 1980; Routledge *et al.*, 1980). The concentration of this glycoprotein has been shown to vary as much as five-fold in typical populations and effective use of the above drugs as

well as interpretation of their serum concentrations may require knowledge of free fractions. Thus, it is appropriate to establish the protein binding characteristics of new compounds which share structural features with any of these drugs.

Tocainide is an orally effective lignocaine-like antiarrhythmic drug reported in a preliminary study to exhibit a high serum free fraction (Lalka *et al.*, 1976). These data are suspect because blood was collected into Vacutainers® which probably contained tris (butoxyethyl) phosphate (TBEP), a plasticizer that has been shown to be a potent inhibitor of the binding of several drugs to α_1 -AGP (Borga *et al.*, 1977; Piafsky, 1980).

The present study was designed to improve our understanding of tocinide binding by using well defined blood collection methods, studying many more subjects, monitoring α_1 -AGP concentrations, and examining serum from trauma patients who exhibited extraordinarily high concentrations of this protein (Piafsky, 1980; Edwards *et al.*, 1982). Blood