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# EFFECT OF ACETYLCHOLINE ON THE CYCLIC GMP LEVEL IN THE RAT HEART AT DIFFERENT AGES

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KEY WORDS: cyclic GMP; acetylcholine; heart; age.

The cholinergic regulation of the heart undergoes changes during aging. Frol'kis and co-workers showed [3, 6] in a series of investigations that the thresholds of the negative chronotropic influences of the vagus nerve on the heart are lower in old animals and that smaller doses of acetylcholine (ACh) cause changes in myocardial contractility. Effects of ACh on function and metabolism of the heart may be realized through activation of guanylate cyclase and elevation of the intracellular level of cyclic guanosine monophosphate (cyclic GMP) [7, 8, 10-12].

The object of this investigation was to study changes in the cyclic GMP level *in vitro* in the myocardium of adult and old rats under the influence of different doses of ACh.

## EXPERIMENTAL METHOD

Experiments were carried out on sections through the heart muscle of adult (6-8 months) and old (24-26 months) Wistar rats. The cyclic GMP content was determined in TCA extracts of sections, previously frozen in liquid nitrogen, neutralized with water-saturated ether, by means of the cyclic GMP kit from the Radiochemical Centre, Amersham (England). ACh was used in concentrations of 0.5, 1, and 2 μM; the sections were incubated with ACh for 1 min.

In the experiments with acetylcholinesterase blockade, neostigmine was used in a concentration of 10 μg/ml incubation medium.

## EXPERIMENTAL RESULTS

No data could be found in the literature on age changes in the cyclic GMP content in heart muscle. The only information available was of a decrease in the intensity of synthesis and breakdown of cyclic GMP with age in the tissues [13] and a decrease in its excretion [9].

Our own observations showed that the cyclic GMP level in the rat myocardium does not change with age: 31.5 ± 2.9 pmoles/g tissue in adults and 18.5 ± 1.5 pmoles/g tissue in old animals. In late ontogeny the basal cyclic GMP level is evidently maintained, so that under resting conditions definite stability of this component of intracellular regulation is ensured.

Different relationships are observed in response to activation of guanylate cyclase by ACh. As Fig. 1 shows, both in adult and in old animals ACh caused a considerable and significant increase in the cyclic GMP concentration. With an increase in the ACh concentration,

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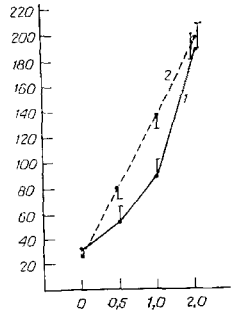


Fig. 1

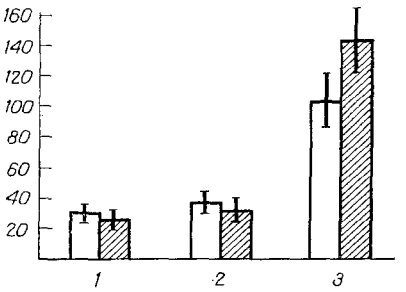


Fig. 2

Fig. 1. Changes in cyclic GMP level under the influence of ACh. 1) Adult rats, 2) old rats. Each point represents mean of 7-8 experiments. Abscissa, ACh concentration (in μM); ordinate, cyclic GMP concentration (in pmoles/g tissue).

Fig. 2. Effect of ACh on cyclic GMP level during acetylcholinesterase blockade. 1) Control, 2) neostigmine, 3) neostigmine + 1 μM ACh. Unshaded columns — adult rats, shaded columns — old rats. Remainder of legend as in Fig. 1.

formation of the cyclic nucleotide also increased. However, the rise in the cyclic GMP level in the myocardium of the old rats was greater than in the adults. For instance, during the action of 0.5 μM ACh the cyclic GMP concentration in the old animals increased by 182%, but in the adults by only 72%; after ACh was given in a dose of 1 μM the increase was 381 and 182%, respectively, so that doubling the ACh concentration raised the cyclic GMP level in the old animals by 2.1 times but in the adults by only 1.4 times. That is why under the influence of these doses of ACh a higher level of cyclic GMP was observed in the old animals. Meanwhile, with a further increase in the dose of ACh (up to 2 μM) the more marked effect was observed in the adult rats. Compared with the action of ACh in a concentration of 1 μM, the cyclic GMP level in the adult rats was increased by 2.7 times but in the old rats by only 1.6 times. As a result of this the cyclic GMP level became equal (differences not significant) in the animals of the two age groups. This, however, is not evidence of the absence of age differences, for the equal level of the cyclic nucleotide was due to different increases, depending on dose, in the adult and old rats.

Changes in the cyclic GMP concentration in the myocardium thus depend on the dose of ACh and the animal's age. In old rats a larger increase in the cyclic GMP concentration was observed after small doses of ACh. With an increase in concentration of the mediator a more marked effect was found in the adult animals. Consequently, age differences in changes in the cyclic GMP level in the myocardium under the influence of ACh correspond to age differences in its functional effects, expressed as stronger effects of small doses of this mediator in old animals. This suggests that this increase in sensitivity may be due to the changes in the cyclic GMP concentration observed to take place under the influence of ACh.

The mechanism of onset of the age differences in the action of ACh can be attributed to various causes. The most important of them are probably changes in activity of the enzymes of ACh metabolism and in the functional state of acetylcholine receptors. With age, the intensity of ACh synthesis has been shown to fall, and this is accompanied by a decrease in acetylcholinesterase activity in the myocardium [1]. This may lead to changes in the effects of ACh in old animals. Accordingly the effect of ACh in a dose of 1 μM was studied on cyclic GMP level following acetylcholinesterase blockade by neostigmine (Fig. 2). The results indicate that blockade of acetylcholinesterase does not lead to significant changes in the action of ACh on the cyclic GMP level compared with its effect in the absence of neostigmine. Only in adult animals was a rather greater increase in the level of this nucleotide observed than in the first series of experiments, and this was evidently due to the higher acetylcholinesterase activity in the myocardium of the adult rats. Age changes in acetylcholinesterase activity, it may be considered, are not the main cause of activation of the effects of ACh in the myocardium of old animals.

Meanwhile the sensitivity of acetylcholine receptors to ACh is much higher in old animals [4]. Consequently, it can be postulated that the increase in the sensitivity of acetylcholine receptors in old age leads to more marked activation of guanylate cyclase by smaller doses of ACh, and to a more considerable rise in the cyclic GMP level, which was responsible for the enhancement of its effects in the old animals.

It must, however, be emphasized that in the intact organism complex interrelations exist between the effects of mediators on the cyclic nucleotide level, as well as mutual influences of these nucleotides on each other and consequent changes in cardiac activity [2, 5, 8]. Under these conditions the increase in the cyclic GMP concentration, according to some workers [10], is not the only cause of changes in heart function under the influence of ACh.

Age differences in the action of ACh on the cyclic GMP concentration are thus an important, but probably not the only, link in the complex mechanism of age changes in the structure of the cholinergic regulation of the heart.

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## EFFECT OF CYCLIC AMP AND ITS DIBUTYRYL ANALOG ON MACROMOLECULAR BIOSYNTHESIS IN ACTIVELY PROLIFERATING CELLS


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Studies of the role of cyclic adenosine-3,5-monophosphate (cyclic AMP) in cell proliferation and differentiation are of great interest [8, 12-14]. To study the effect of exogenous cyclic AMP on physiological processes in cells both cyclic AMP itself and its dibutyryl analog — $N^6O^2$-dibutyryl-3,5-cyclic AMP — have been widely used. However, exogenous cyclic AMP does not always produce the effects of the cyclic AMP which is synthesized intracellularly and, furthermore, the effects of exogenous cyclic AMP and of dibutyryl-cyclic AMP are not always equivalent [5, 10].

The object of the present investigation was to compare the action of exogenous cyclic AMPs and of dibutyryl-cyclic AMP on macromolecular biosynthesis *in vitro* in cells in a stage of active proliferation. Ehrlich's ascites carcinoma (EAC) cells and thick embryonic carti-


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