EFFECT OF AMPIOX AND GUANOSINE TRIPHOSPHATE ON CYCLIC NUCLEOTIDE CONTENT IN MUSCLE TISSUE IN A ZONE OF INFLAMMATION

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In the modern view the state of the cyclic nucleotide system plays an important role in the genesis of inflammation [4, 14]. Accordingly the study of the effect of various biologically active substances and, in particular, of antibiotics used in the treatment of inflammatory conditions, on that system becomes of great importance. Special attention must be paid to the study of the action of antibiotics in conjunction with substances enhancing their effectiveness, for such an approach could help both to elucidate the mechanism of their combined action and to reveal further opportunities for their therapeutic use.

The writers showed previously that guanosine triphosphate (GTP) increased the sensitivity of staphylococcal cells of strain No. 1623 to ampiox (a combined preparation of semisynthetic penicillins – ampicillin and oxacillin – of Soviet manufacture) by 35 times [5]. When a combination of ampiox and GTP was used for the treatment of inflammatory conditions in animals induced by injection of staphylococci, addition of GTP to the ampiox prevented the formation of necrosis and abolished the negative side effects of ampiox on the recipients.

In the investigation described below the content of cyclic nucleotides was studied in muscle tissue in a zone of inflammation in rabbits treated with a combination of ampiox and GTP.

EXPERIMENTAL METHOD

Experiments were carried out on 48 chinchilla rabbits weighing 2.0-2.5 kg. An experimental model of inflammation was obtained by intradermal injection of a bacterial culture [1]: 0.2 ml of a 10^{10} suspension of a staphylococcal culture (strain No. 1623) was injected into rabbits in the dorsal region. The suspension was prepared by washing the agar surface of a 24-h culture with physiological saline.

On the second day after intradermal injection of the culture, when the foci of infiltration were clearly defined, ampiox in a dose of 100,000 units was injected intramuscularly twice a day for 2 days into the rabbits (6 animals) of group 1, GTP was injected in a dose of 10 mg into the rabbits (6 animals) of group 2, and a combination of ampiox with GTP in the above doses was given to the rabbits (6 animals) of group 3. The total dose of ampiox given to each rabbit was 400,000 units and of GTP 40 mg.

The rabbits were killed on the 6th day and cyclic AMP and cyclic GMP were assayed in the muscle tissue of the inflammatory zone by a radioimmunologic method using kits from the Radiochemical Centre, Amersham, England [3]. The results were compared with corresponding data obtained in 24 uninfected rabbits receiving ampiox, GTP, and a combination of both (intact animals – control group).

The results were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

Injection of ampiox into intact animals caused virtually no change in the cyclic AMP content in muscle tissue; injection of GTP and also of a combination of ampiox and GTP caused no significant changes in the cyclic AMP concentration in the muscle (Fig. 1).

KEY WORDS: cyclic nucleotides; muscles; antibiotics; GTP; inflammation.

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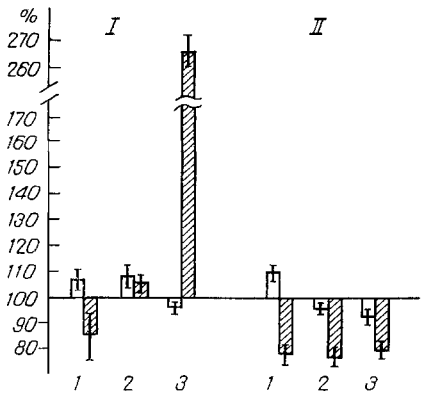

Fig. 1

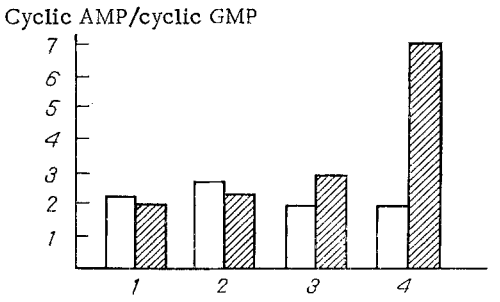

Fig. 2

Fig. 1. Effect of separate and combined injections of ampiox and GTP on content of cyclic AMP (I) and cyclic GMP (II) in muscle tissue (values for animals not having received biologically active substances taken as 100%). Unshaded columns — intact rabbits, shaded columns — infected rabbits. 1) Ampiox; 2) GTP; 3) ampiox + GTP. Ordinate, content of cyclic nucleotides (in percent of control).

Fig. 2. Effect of separate and combined administration of ampiox and GTP on ratio of concentrations of cyclic AMP and cyclic GMP in muscle tissue. 1) Without treatment; 2) ampiox; 3) GTP; 4) ampiox + GTP. Unshaded columns — intact rabbits, shaded columns — infected rabbits.

Injection of ampiox caused no change in the cyclic AMP in the affected muscle of the infected animals, and injection of GTP likewise had no effect on the concentration of this metabolite. Meanwhile combined administration of GTP and ampiox increased the cyclic AMP concentration by more than 2.5 times (by 167%, $P < 0.01$).

The change in the content of cyclic GMP under the influence of ampiox and GTP was a different character. In intact animals ampiox and GTP, whether injected separately or in combination, did not affect the concentration of this metabolite. In the infected animals the action of ampiox and GTP, given separately or in combination, on the cyclic GMP content was in the same direction in both cases, for it reduced the concentration by 20-25% ($P < 0.05$).

These results show that a sharp change takes place in the ratio between the concentrations of cyclic AMP and cyclic GMP in infected muscle tissue in response to combined administration of ampiox and GTP, in favor of cyclic AMP (Fig. 2).

In the early stages of inflammation liberation of primary mediators of inflammation (biogenic amines, catecholamines, and lysosomal enzymes) is observed and, in the course of development of inflammation this leads to activation of the kallikrein–kinin system and to increased formation of prostaglandins [4, 14]. One of them, prostaglandin E_1, can increase the concentration of cyclic AMP which, on the one hand, has an antiinflammatory action, reducing the production of lysosomal enzymes and liberation of histamine and, on the other hand, activates protein kinase, which ultimately is responsible for changes in the biological activity of the cell. The action of cyclic GMP is opposite to that of cyclic AMP with respect to liberation of histamine and lysosomal enzymes [4, 7, 10].

In the present experiments addition of GTP to ampiox in the course of treatment of the infected animals led to a marked rise in the cyclic AMP content in the affected muscle tissue. Meanwhile all the preparations used lowered the cyclic GMP concentration in the muscles of the inflammatory zone. Ultimately the combined use of ampiox and GTP increased the concentration gradients of cyclic AMP and cyclic GMP, and it was evidently this which largely determined the antiflammatory effect of the combined administration of GTP and ampiox. However, considering the fact that injection of ampiox combined with GTP affected the cyclic nucleotide content in the muscle tissue of the zone of inflammation but did not significantly change it in the muscles of the intact animals, one possible mechanism of the action of this combination can be represented as follows.

Ampiox, as an antibiotic of the penicillin series, acts on the principal component of the bacterial membrane — murein [12]. By disturbing the structural integrity of the bacterial mem-

brane, it can give rise to the release of various solubilized enzymes from the cells, including adenylate cyclase, which is present in the bacterial cell in the soluble state [9]. The guanyl nucleotides (including GTP) stimulate the catalytic activity of both membrane-bound [11, 15, 16] and solubilized adenylate cyclase [17]. Inosine monophosphate (IMP) is a common metabolic precursor of the guanyl and adenyl nucleotides.

It can be postulated that under the experimental conditions used injection of ampiox caused inhibition of the enzymic formation of guanyl nucleotides from IMP, for this type of metabolic effect is observed during the action of various antiviral preparations and, in particular, of 2-amino-1,3,4-thiadiazole [6, 8, 13], the composition of which includes structural elements (a five-membered sulfur-containing heterocyclic ring) similar to those of penicillins. Under these conditions addition of GTP to ampiox during treatment of inflammatory conditions in infected animals ought to lead to restoration of the GTP content and of the GTP-activating effect on adenylate cyclase.

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