Effect of Purine and Pyrimidine Ribosides on an Isolated Frog Ventricle Preparation

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Various purine and pyrimidine ribosides, with the exception of adenosine, have been found to exhibit a positive inotropic effect on an isolated strip of frog ventricle. In a preparation responding maximally to one compound, the addition of a second compound led to a further increase in tension. This relationship was found to apply among the members of a given group (purine or pyrimidine) paired in various combinations, as well as in experiments pairing purine against pyrimidine riboside. Adenosine showed brief stimulation followed by depression, pure depression or no effect.

In the course of an examination of various fractions of heart muscle for cardiotonic activity, inosine was identified as the active component of one highly effective fraction. The subsequent finding, that this compound in low concentration (around $10^{-3}$ M) may cause a significant increase in tension developed by the isolated cat papillary muscle or frog ventricle strip, led to an investigation of other purine and pyrimidine ribosides.

With the exception of adenosine, a positive inotropic response was exhibited by all the compounds tested. The present study was carried out in an effort to determine whether the various active compounds exert their effect independently or by a common mechanism.

Methods

The observations given here were made with a preparation of frog ventricle. Thin strips weighing 5 to 10 mg. were cut from the ventricle of R. pipiens and set up according to Cattell and Gold. Isometric tension was recorded continuously by means of a Statham strain gage (model G7-0, 15-320) and a Sunborn recorder. The tissue bath contained 10 ml. of well oxygenated Ringer's solution, prepared according to Boyle and Conway.

Freshly prepared 0.05 M solutions of the ribosides in isotonic saline or Ringer's solution were added to the bath in the desired volume by capillary pipet. After preliminary experiments, in which the response to the individual ribosides was examined at various concentrations, the manner in which one riboside influenced the effect of another was studied. This was done by adding a given compound to the bath in stepwise fashion until such a concentration was achieved that no further response was obtained. A second riboside was then added in the presence of the saturating concentration of the first. The manipulation of riboside concentration was carried out in this way rather than by rinsing the bath and introducing a new solution, in order that the transient disturbance in contraction amplitude which followed the latter procedure might be avoided.

The initial observations on inosine were made with chromatographically homogeneous material from beef heart. In subsequent experiments commercial preparations of high purity were used. Adenosine, inosine, guanosine, uridine and cytidine were obtained from the Pabst Laboratories (Milwaukee, Wis.), and thymidine from the Nutritional Biochemicals Corporation (Cleveland, Ohio). Melting points and extinction coefficients agreed with reported values and were not altered by recrystallization in the cases in which this was done.

Most of the experiments reported here were carried out in the fall and winter seasons because of the irregular behavior of preparations made from summer frogs.

Results

The spontaneous behavior of the preparation with respect to tension is shown in figure...
1. After a somewhat variable course during the first 2 hours, the general trend was downward. Experimental observations were begun after the third hour.

In connection with 19 preparations, preliminary observations on the effects of the different ribosides acting alone were made at various concentrations. In general, effective stimulating concentrations lay between $3 \times 10^{-5}$ to $10^{-3}$M and appeared to be related to the reactivity of the particular muscle preparation rather than to the riboside. The magnitude of the response was also quite variable, and in some preparations the effect over the entire concentration range was limited to prevention of the slow fall in tension usually seen.

Adenosine at the lower concentrations was without effect. At higher concentrations it produced either a transient increase in tension followed by depression, or an uncomplicated depression. Replacing the adenosine with Ringer’s solution led to a prompt increase in tension, while in the case of the stimulating ribosides return to Ringer’s solution was followed by a prompt decline.

The effect of one riboside, in the presence of saturating concentrations of another, was studied in 29 preparations (cytidine vs. uridine in 7 experiments, thymidine vs. uridine in 4, thymidine vs. cytidine in 2, guanosine vs. inosine in 8, adenosine vs. inosine in 4, and inosine vs. uridine in 4). The same variability noted in the first set of experiments was encountered, but, in general, when one riboside was added to a preparation responding maximally to another riboside a further increase in tension resulted. This was found for the pyrimidine ribosides paired in various combinations, for the purine derivatives and for a pyrimidine and purine riboside (inosine vs. uridine).

Results of typical experiments are presented in figures 2 to 8.

**Discussion**

The simplest explanation, for the finding that one riboside will exert its effect in the presence of a saturating concentration of another riboside, is that the compounds act, at least in part, through independent mechanisms. The various nucleotides (phosphorylated ribosides) participate as co-factors in a variety of enzymatic reactions, a circumstance which adds weight to such an explanation. A plausible view of the results may be postulated on this basis. Within the cell, the nucleotide level is the resultant of various synthetic and degradative reactions. In the present preparation, loss from the cell of one or more of the more readily permeable precursors by diffusion into the bath will lead to depletion of the corresponding co-factor. When its level is reduced to the point at which the reaction (or reactions) in which it participates cannot be maintained at a rate required by the cell for normal activity, tension development may decline. By the addition of an appropriate precursor (riboside) to the bath, nucleotide level and cell function are restored. There is no further increase in tension once optimal co-factor concentration has been achieved.

The foregoing view may provide insight into the apparently anomalous behavior of adenosine. Adenosine triphosphate (ATP) is quantitatively the dominant nucleotide in the cell, and its resynthesis is actively promoted at various steps in the metabolism of the cell. The failure of adenosine to increase tension may only mean that ATP levels had not declined below the optimal value by the time adenosine was added. Since a number of pathways are available in the cell for resynthesis of ATP, the decline in tension some-
times caused by adenosine may be due to competition for available high-energy phosphate by the adenylate system, which may lead secondarily to a further depression in levels of other, less abundant nucleotide cofactors. Earlier reports are in general agreement with the present work concerning the effect (or lack of one) of adenosine on cardiac contractility. It may be suggested that the effects of the adenine nucleotides, about which there is some disagreement, cannot properly be compared with those of the nucleoside, since there is, in all likelihood, a great impediment to permeability conferred by the addition of the phosphate groups.

The presence of active deaminases in the heart, that rapidly convert adenylie to inosinic derivatives, may cast doubt on the validity of the foregoing thesis. It may be expected that in the presence of adequate ATP levels the concentration of inosine co-factors would be maintained and that inosine would be without effect. However, the significance of the interconversion of the adenylie and inosinic systems in intact myocardium remains undefined, making it impossible to assess the importance of the question raised.

It is of interest to note that riboside effects are not unique in heart. Uridine and cytidine have been reported to aid in the

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**Fig. 2 Top left.** At arrows 1, 2, 3 and 4, cytidine was added to the bath to give concentrations of $10^{-3}$, $2 \times 10^{-3}$, $3 \times 10^{-3}$, and $4 \times 10^{-3}$ M, respectively. At 5 and 6, uridine was added to give concentrations of $10^{-3}$ and $2 \times 10^{-3}$ M, respectively. (The values given for this and subsequent figures represent the final concentration prevailing in the bath after each addition.)

**Fig. 3 Bottom left.** At arrows 1, 2 and 3, thymidine was added to the bath to give concentrations of $10^{-3}$, $2 \times 10^{-3}$ and $3 \times 10^{-3}$ M, respectively. At 4 and 5, uridine was added to give concentrations of $10^{-3}$ and $2 \times 10^{-3}$ M, respectively.

**Fig. 4 Top right.** At arrows 1, 2 and 3, thymidine was added to the bath to give concentrations of $10^{-3}$, $2 \times 10^{-3}$ and $3 \times 10^{-3}$ M, respectively. At 4 and 5, cytidine was added to give concentrations of $10^{-3}$, and $2 \times 10^{-3}$ M, respectively.

**Fig. 5 Bottom right.** At arrows 1, 2 and 3, guanosine was added to the bath to give concentrations of $10^{-3}$, $5 \times 10^{-3}$ and $10^{-3}$ M, respectively. At 4, 5 and 6, inosine was added to give concentrations of $10^{-3}$, $2 \times 10^{-3}$ and $3 \times 10^{-3}$ M, respectively.
Fig. 6 Top. At arrows 1 and 2, adenosine was added to the bath to give concentrations of $10^{-4}$ and $5 \times 10^{-4}$ M., respectively. At 3, the adenosine solution was removed, and the chamber rinsed and refilled with fresh buffer.

Fig. 7 Bottom. At arrows 1, 2 and 3, adenosine was added to the bath to give concentrations of $10^{-4}$, $5 \times 10^{-4}$ and $10^{-4}$ M., respectively. At 4, inosine was added to a concentration of $10^{-4}$ M.

Maintenance of function of the isolated, perfused cat brain, presumably by virtue of their roles in galactoside and phospholipid synthesis respectively. The changes that were not corrected by the pyrimidines were prevented by the inclusion of the liver in the perfusion system.

The significance of these observations, derived from highly artificial in vitro systems, is a matter for speculation. A heart subjected to hypertensive or valvular disease may reach a point at which the resynthesis of one or more of the less abundant nucleotides cannot keep pace with the rate of metabolic breakdown (or a point at which normal levels become inadequate), thus creating a reversible biochemical "lesion" which results in a loss of contractility and manifestations of frank failure. If the liver contributes to nucleotide homeostasis of peripheral tissues, as suggested by the brain perfusion experiments, impairment of liver function secondary to cardiac failure may intensify the biochemical defect. The possible role of the various purine and pyrimidine nucleotides in the spontaneous failure that occurs in the isolated heart and heart-lung deserves some consideration. These preparations have been used extensively as models in the study of various aspects of cardiac failure, but the nature of the defect (or defects) responsible for the deterioration in work capacity remains obscure.

While attention in the field of muscle function has been focused primarily on ATP, the present results suggest that, in the effort to define the determinants of myocardial contractility, the functional role of the quantitatively lesser nucleotides merits further study.

**Summary**

Various purine and pyrimidine ribosides, adenosine excepted, have been found to exhibit a positive inotropic effect on the frog ventricle. In a preparation responding maximally to one compound, a further increase in tension followed the addition of a second compound. This relationship was found to apply among the members of a given group (purine or pyrimidine) paired in various combinations, as well as in experiments pairing purine vs. pyrimidine riboside; it indicated that the active ribosides manifest their musculotropic effect through independent mechanisms. It
has been suggested that the mechanical failure of the isolated ventricle strip may result in part from depletion of the various quantitatively lesser nucleotide co-factors, and that the ribosides act by promoting resynthesis of the corresponding ribotides. The failure of adenosine to stimulate has been supposed due to the presence of adequate levels of ATP (by far the most abundant of the various nucleotides), while the depression frequently produced by this riboside may result from further depletion of the other nucleotides by competition for available high-energy phosphate by the adenylate system.

**SUMMARIO IN INTERLINGUA**

Esseva constatate que varie ribosidos purinic e pyrimidinic—con le exception de adenosina—exerce un positive effecto inotropic super le isolate ventriculo del rana. Quando un preparato de ventriculo ranin habeva respondite maximalemente a un del compositos, un augmento additional de tension sequave le addition de un secunde composito. Iste relation se manifestava non solmente inter le membros del un e del altere gruppo—i.e. inter purinas e inter pyrimidinas—quando illos esseva appareate in varie combinationes sed etiam quando un purina esseva appareate con un pyrimidina. Per consequente il poter esser stipulate que le ribosidos manifesta lor effectos musculotropicae per varie mechanismos que es mutualmente independent. Il ha esseva suggerite que le disfallimento mechanic de isolato preparatos de ventriculo resulta in parte ab le exhaustion del varie, quantitative minus prominent co-factores nucleotidic, et que le ribosides age a promover le resynthese del correspondent ribotides. Le facto que adenosina non exerce un effecto stimulatori ha esseva interpretate come resultante possibilmente del presentia de adequate concentrationes de triphosphato adenosinic (que es, per multo, le plus abundante del varie nucleotidos), durante que le depression que es frequentemente producete per iste ribosido es possibilmente le resultato de un depletion additional del altere nucleotidos in consequentia del rivalitate del systema adenylatic relative al disponibile phosphato a alte energia.

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