Coronary Vasodilator Properties of Purine and Pyrimidine Derivatives

By MARY M. WOLF, B.A. AND ROBERT M. BERNE, M.D.

The vasodilator potency of the adenine nucleotides has been quantitated. Adenosine and AMP were found to be of equal potency and approximately one fourth as effective as ADP and ATP in increasing coronary blood flow. With the exception of UTP, which was also one fourth as potent as ATP, the derivatives of the hypoxanthine, guanine, cytosine and uracil bases lacked vasodilator properties. An increase in myocardial oxygen consumption was observed during infusion of ATP and UTP. However, the elevation in coronary blood flow was greater than that necessary to meet the increased oxygen requirements of the myocardium.

ADENOSINE, and its phosphorylated derivatives AMP, ADP, and ATP are potent vasodilators. However, conflicting reports have appeared on the relative potency of these compounds. The possibility has been entertained that the release of adenine nucleotides may play a physiological role in the adjustment of blood flow to meet changes in the oxygen requirement of muscle. The following quantitative analysis of the vasodilator action of adenine nucleotides in the coronary bed represents part of a program of study of this problem in our laboratory. A wide concentration range was employed in order to ascertain the minimal quantities necessary to bring about maximal flow. Comparisons have been made of the response produced by equal concentrations of all the adenine compounds as well as the related derivatives of the hypoxanthine, guanine, cytosine and uracil bases, with the hope of throwing further light on the molecular constituents necessary for activity. In addition, studies of the cardiac oxygen consumption during infusion of ATP and UTP were made to determine whether the vasodilator action is associated with an increased rate of myocardial metabolism.

METHODS

Experiments were performed on 28 dogs weighing 15-25 Kg. They were anesthetized with intravenously administered sodium pentobarbital (30 mg. per Kg.). The chest was opened in the fourth left intercostal space and artificial respiration was instituted. Following administration of heparin, the circumflex branch of the left coronary artery was cannulated and perfused via the subclavian artery at a constant pressure of 100 mm. Hg. This was accomplished by a pump-perfusion system which permitted regulation of perfusion pressure to any level regardless of the animal's aortic pressure. The coronary blood flow (CBF) was measured by an optically recording rotameter. Records of CBF, perfusion pressure and aortic pressure were taken two minutes after the start of the infusion at which time the response was maximal and constant. A solution of 0.9 per cent NaCl administered at the same rate was found to be without effect on the CBF in each experiment and served as a control. Following each experimental period, the flow was allowed to return to control levels and a record was taken before infusion of the next sample.

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* The following abbreviations are used: adenosine monophosphate, AMP; adenosine diphosphate, ADP; adenosine triphosphate, ATP; inosine monophosphate, IMP; inosine diphosphate, IDP; inosine triphosphate, ITP; guanosine monophosphate, GMP; guanosine diphosphate, GDP; guanosine triphosphate, GTP; cytidine monophosphate, CMP; cytidine diphosphate, CDP; cytidine triphosphate, CTP; uridine monophosphate, UMP; uridine diphosphate, UDP; and uridine triphosphate, UTP.

† Compounds obtained from Sigma Chemical Co. and Pabst Laboratories.

1 Kindly supplied by the Upjohn Co., Kalamazoo, Mich.
In some experiments the effect of ATP and UTP on oxygen consumption of the left ventricle was determined. In this series the common left coronary artery was cannulated with an Eckstein cannula and coronary perfusion pressure was regulated by a pump-perfusion system. A Morowitz type cannula was placed securely in the mouth of the coronary sinus and, between blood sampling, the outflow was directed into the right jugular vein. Measurements of perfusion pressure, aortic pressure and CBF were made as described above. A solution of ATP was infused into the tubing leading to the coronary artery at a rate of 0.1 to 0.3 μM/min. Two minutes after the start of the infusion, at which time the CBF was maximal and constant for the concentration employed, a record was taken and samples of arterial and venous blood were drawn into heparinized syringes containing sodium fluoride. The arterial sample was taken from the tubing just proximal to the coronary cannula and the venous from the tubing distal to the coronary sinus cannula. Immediately following collection of blood samples a second record was taken as a check on the constancy of the flow. Prior to the infusion of nucleotide, control records were taken and coronary arterial and venous bloods were withdrawn for analysis of oxygen content. Five to 10 minute intervals elapsed between collection of samples and control records preceding the infusion of the next higher concentration. The blood samples were kept on ice until oxygen determinations were done in duplicate by the method of Roughton and Scholander. The analyses were completed within 24 hours of collection.

RESULTS

The effect of ATP on coronary vascular resistance was studied over a range of infusion rates of 0.002 to 1.140 μM/min. During the infusion, observations were made of heart rate, aortic pressure, and CBF. In all but one experiment increases in coronary inflow were not accompanied by significant changes in heart rate or aortic pressure. The dose response curve drawn from the data of 48 determinations in 16 animals is presented in figure 1A. The curve is that of a hyperbola and may be expressed by the equation

\[
Y = \frac{X}{A + BX}
\]

where \(X\) is the dose rate expressed as μM of ATP per minute, \(A\) and \(B\) are constants and \(Y\) equals the response expressed as the per cent of maximum CBF obtained with ATP. Maximum levels of CBF with ATP were reached at dose rates of 0.2 to 0.3 μM/minute. Because doses greater than 0.3 μM/minute evoked no further increase in CBF, the responses produced at this dose level were used as a standard for comparison of potency of lower doses of ATP and of the activities of all other compounds included in this series. From figure 1B it can be seen that a rectilinear relationship expressed by the equation

\[
Y = a + bx
\]

exists in the dose rate range from 0.002 to 0.300 μM/min. In this plot \(Y\) equals the re-
In several experiments the comparison was made between the vasodilator activity of ATP and the other members of the adenine series, adenine, adenosine, AMP and ADP. Adenine was consistently found to lack vasodilator properties even when infused at rates of 10 μM/min. Marked elevation in CBF followed infusion of adenosine and its mono- and diphosphates, but quantitative differences were noted between the responses produced by some of these compounds and those of ATP. These data are summarized in figure 2. In this group of experiments, the slope of the ATP log dose line as determined by the method of least squares was 48.38, while that of the entire ATP series as presented in figure 1B was 32.3. Statistical analysis revealed no significant difference in these slopes. Moreover, all of the slopes of the log dose lines for the members of the adenine series belonged to a common universe. Comparisons of the slope of each individual line with that of ATP revealed t values from 0.3 to greater than 0.5.

Determination of potency ratios permitted the division of the adenine nucleotides into two groups. In the first group are ATP and ADP which appear to be approximately equipotent. The ADP/ATP potency ratio is 0.883 with fiducial limits of 0.480 and 1.624 at the 5 per cent level.

AMP and adenosine were found to be roughly equal in potency and approximately one-fourth as active as ATP. Potency ratio of AMP/ATP was 0.280 with fiducial limits of 0.145 and 0.540 at the 5 per cent level, and that of adenosine/ATP was 0.205 with fiducial limits of 0.080 and 0.522 at the 5 per cent level.

Hypoxanthine, inosine, IMP and IDP were found to lack vasoactive properties when compared with ATP in the dose range 0.1 to 0.3 μM/minute. ITP, however, did possess minimal vasodilator properties when administered at 0.3 μM/minute.

Guanine, guanosine, GDP and GTP produced no elevation in CBF either in this dose range, 0.1 to 0.3 μM/minute, or when doses 3 to 4 times greater were infused.

Cytosine and the derivatives of this pyrimidine base, namely, cytidine, CMP, CDP and CTP were also studied. The cytosine compounds had no demonstrable effect on vascular resistance at dose levels four times that of the adenine series. Uracil, uridine, UMP and UDP were similarly inactive but variable results were obtained with UTP. Vasodilator activity was found in 6 of the 8 experiments in which this substance was tested. No significant differences were observed in the CBF responses to the pyrimidines when compared with ATP.
was found by comparing the slope of the UTP log dose line with that of ATP, but the potency ratio of 0.263 indicates that UTP is less active as a vasodilator than is ATP. Fiducial limits in this case were 0.142 and 0.487 at the 5 per cent level. A comparison of the activity of these two compounds can be found in figure 3. 

Ribose and ribose phosphate were without effect on the coronary vessels when infused at the same concentration range as the adenine nucleotides.

ATP and UTP were found to increase myocardial oxygen consumption. However, the elevation in CBF produced by these compounds was greater than that necessary to meet the increased oxygen demand. This is apparent from the elevation in venous oxygen content which occurs during infusion of these nucleotides (table 1).

**DISCUSSION**

The results of these experiments, when viewed in terms of the structural relationship of the substances tested, demonstrate the importance of the six amino group and the ribose moiety attached to the purine base. The conversion of an inactive purine base, adenine, to a potent vasodilator by the addition of the ribose group is an example which supports this general statement. That the ribose alone was not responsible for this effect was shown by the lack of response of the coronary vessels to infusion of ribose or ribose phosphate. Moreover, the fact that addition of ribose to form the nucleotides of the other purines, hypoxanthine and guanine, did not increase the activity of these compounds indicates that some constituent other than ribose exerts an influence on biologic activity. The observation that the deaminated analogues of the adenine compounds, i.e., the hypoxanthine series, lacked vasodilator potency suggests the importance of the six amino group. The inactivity of the guanine series shows that it is not the amino group per se which is responsible, for these compounds possess such a group in the two position.

The enhancement of vasodilator activity with the addition of the second and third phosphate group cannot be explained on the basis of the energy associated with the phosphate linkages. The effectiveness of adenosine and AMP, and the fact that ADP is approximately equal in potency to ATP indicate that high energy phosphate bonds are not essential for activity. Furthermore, it has been shown by Drury, who infused sodium pyrophosphate directly into the coronary vessels, that the high energy phosphate alone has no effect on vascular resistance. Final interpretation of this phenomenon must await uncovering of the mechanism whereby the diminution in vascular resistance under the influence of the adenosine derivatives is brought about.

The results of our studies on myocardial oxygen consumption during infusion of ATP and UTP indicate that the vascular response to the administration of infusion of ATP and UTP on Coronary Blood Flow and Myocardial Oxygen Consumption.

### Table 1

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**NUCLEOTIDES AND CORONARY BLOOD FLOW**

**DISCUSSION**

The results of these experiments, when viewed in terms of the structural relationship of the substances tested, demonstrate the importance of the six amino group and the ribose moiety attached to the purine base. The conversion of an inactive purine base, adenine, to a potent vasodilator by the addition of the ribose group is an example which supports this general statement. That the ribose alone was not responsible for this effect was shown by the lack of response of the coronary vessels to infusion of ribose or ribose phosphate. Moreover, the fact that addition of ribose to form the nucleotides of the other purines, hypoxanthine and guanine, did not increase the activity of these compounds indicates that some constituent other than ribose exerts an influence on biologic activity. The observation that the deaminated analogues of the adenine compounds, i.e., the hypoxanthine series, lacked vasodilator potency suggests the importance of the six amino group. The inactivity of the guanine series shows that it is not the amino group per se which is responsible, for these compounds possess such a group in the two position.

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The results of our studies on myocardial oxygen consumption during infusion of ATP and UTP indicate that the vascular response...
cannot be explained solely on the basis of increased metabolic rate. Although some increase in oxygen consumption was found, it was considerably less than the corresponding elevation in CBF. This is unlike the results obtained with epinephrine and dinitrophenol (unpublished observations), both of which produce a parallel increase in oxygen consumption and CBF. However, the metabolic activity of ATP and UTP is not to be discounted, for these compounds do not behave like acetylcholine (unpublished observations) or nitroglycerine which produce an elevation in CBF without a significant change in oxygen consumption. In view of our findings, therefore, it is probable that the action of the nucleotides on coronary vascular resistance is a complex one. While the metabolic effect undoubtedly contributes to the phenomenon, the major action appears to be a direct one on the smooth muscle of the vessels.

The vasodilator potency of UTP, in view of the inactivity of the lower members of the uracil series, is difficult to explain at this time. The fact that the addition of the third phosphate group to the inactive uridine diphosphate endows this substance with vasoactive properties suggests that it is not the parent compound per se which is responsible for this activity, but rather that it acts as an intermediate in a scheme resulting in production of an active component. Enzyme systems catalyzing the transphosphorylation of ADP by UTP forming ATP have been described. However, it is not likely that such a reaction could explain our observations for it would not provide for a net increase in vasodilator material, but merely for a shift from one potent compound to another within a stabilized system. Furthermore, if transphosphorylation to the adenosine compounds were the mechanism behind this phenomenon, one would expect similar results with the triphosphate derivatives of the other purine and pyrimidine bases. This was not found to be the case.

**Summary**

Maximal vasodilator effects on the dog's coronary arteries can be obtained with infusions of ATP at rates of 0.2 to 0.3 μM/minute. Comparison of the potency of this compound with the other members of adenine series of nucleotides have revealed ADP to be approximately of equal potency while AMP and adenosine are about one-fourth as effective in diminishing vascular resistance. Adenine was found to lack vasodilator properties as did all the members of the hypoxanthine series with the exception of ITP which produced a small increase in CBF. The derivatives of the purine base, guanine, were similarly inactive as were the derivatives of the pyrimidine base, cytosine. UTP was the only member of the uracil derivatives which evoked an increase in CBF and was found to be approximately one-fourth as potent as ATP.

Although an increase in myocardial oxygen consumption was observed during infusion of ATP and UTP, the elevation in CBF was greater than that necessary to meet the increased oxygen demand. The action of these compounds is believed, therefore, to be primarily on the vessels and not secondarily due to an increased metabolic rate.

**SUMMARIO IN INTERLINGUA**

Maximal effectos vasodilatori in le arterias coronari de canes es obtenibile per medio de triphosphato adenosinic infundite a proratas de 0,2 a 0,3 μM per minutum. Le comparation del potentia de iste composito con le potentias del altere membros de series adeninic del nucleotidos ha revelate que diphosphato adenosinic ha approximativemente le mesme potentia como triphosphato adenosinic, durante que monophosphato adenosinic e adenosina reduce le resistentia vascular con solmente circa un quarto de ille efficacia. Il esseva trovate que adenina ha nulle potentia vasodilatori. Le mesmo vale pro omne membros del series hypoxanthinic con le exception de triphosphato inosinic que produceva un parve augmento del fluxo coronari de sanguine. Le derivatos del base purinic, guanina, esseva similemente in-active e etiam le derivatos del base pyrimidinic, cytosina. Triphosphato uridinic esseva le sol membro del series de derivatos uracilic que evocava un augmento del fluxo coronari de sanguine. Su efficacia esseva circa un quarto del efficacia de triphosphato adenosinic.

Ben que un augmento del consumption myocardial de oxygeno esseva observate duringle
infusion del triphosphatos adenosinic e uridinic, le elevation del fluxo coronari de sanguine exceedeva le grado de elevation requirite pro satisfacer le augmentate demanda de oxygeno. Per consequisite nos crede que le action de iste compositos es un action primari super le vasos e non un action secundari a un metabolismo accelerate.

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