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EFFECTS OF THE ATP ANALOG 5′-ADENYLYL METHYLENEDIPHOSPHONATE ON ACID SECRETION IN FROG GASTRIC MUCOSA

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SUMMARY

1. The ATP analog 5′-adenylyl methylenediphosphonate (AMP-PCP) added to the serosal bathing solution of the chambered bullfrog gastric mucosa inhibits acid secretion, raises the transepithelial potential difference, and causes an increase in lag time before resumption of secretion after anoxia. These effects are similar in direction and magnitude to those caused by ATP.

2. While AMP-PCP stimulates the production of inosine in this tissue, it is apparently not itself a precursor for inosine, and seems to act by stimulating catabolism of endogenous purines.

3. AMP-PCP is not a substrate for adenosine deaminase or AMP deaminase, has no marked effects on mitochondrial respiration or phosphorylation, and is not catabolised by gastric mucosa.

4. Washout kinetics of AMP-PCP loaded tissues show this material to be exiting with a single exponential time constant of about 10 min. By contrast, inosine shows 2 washout exponentials of 5 and 30 min. The appropriate analysis suggests that the cells may be more permeable to AMP-PCP than to inosine.

5. It is suggested that the inhibitory action of AMP-PCP (and by extension, ATP and ADP) is due to the possession by these compounds of a nitrogen with an unshared pair of electrons, as has been suggested for other secretory inhibitors.

INTRODUCTION

The ATP analog 5′-adenylyl methylenediphosphonate (AMP-PCP) consists of ATP in which the ester linkage between the β and γ phosphates is replaced with a methylene bridge (see Fig. 1). This compound was first synthesized by Myers *et al.*[1] and is now available commercially. Tested with systems which normally utilize ATP, it has been shown not to interact in many systems, probably because the

Abbreviations: AMP-PCP, 5′-adenylyl methylenediphosphonate; PD, transepithelial potential difference.

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P–C–P bond angles and distances are somewhat different from the parent P–O–P compound[2]. Thus, AMP-PCP is not hydrolysed by meromyosin or heavy meromyosin, and is a poor inhibitor of ATP hydrolysis in these systems[3], is not cleaved by membrane ATPase[2] nor is it able to support Na^+/K^+ transport in erythrocyte ghosts[4]. It is attacked by *Escherichia coli* phosphodiesterase (to give AMP and PCP) but does not react with adenylate cyclase[3], nor does it interact with a number of other ATP-utilizing enzyme systems. However, in size, charge and configuration it closely resembles adenosine nucleotides, and thus should provide a reasonable non-degradable analog for permeability studies in the frog gastric mucosa.

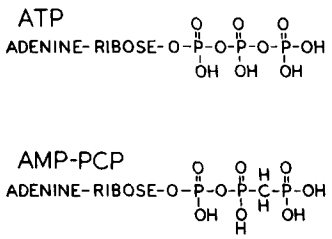

Fig. 1. Formulae of adenosine 5′-triphosphate (ATP) and its α,β-methylene analog, 5′-adenylyl methylenediphosphonate (AMP-PCP).

Our early observations of cytochrome *c* redox changes associated with secretory rate changes in the bullfrog gastric mucosa[5] led to an hypothesis in which the energy for proton transport was derived from the cytochrome system without the intermediation of ATP. The inhibitory effects of ATP added to the bathing solution on acid secretion in this tissue have been explained by a model which involves interactions of ATP with mitochondrial oxidation in the conventional manner[6] and thus would be predicted to be insensitive to AMP-PCP if that model were correct. Thus it was of interest to determine, first, whether AMP-PCP can duplicate the secretory inhibition effects of the natural purine nucleotides, and secondly to determine the washout kinetics of this compound as a model for ATP permeability.

MATERIALS AND METHODS

The experimental preparation is the gastric mucosa of the bullfrog (*Rana catesbiana*) stripped of its external muscle coat and mounted as a flat sheet between two fluid-filled chambers as previously described[5]. The area exposed to each solution was 3.14 cm^2. The solution in contact with the serosal face of the issue is a modified Ringers bicarbonate solution containing (mM): Na^+, 104; K^+, 4; Ca^{2+}, 1; Mg^{2+}, 0.8; Cl^-, 81.8; HCO_3^-, 25; HPO_4^{2-}, 2; and glucose, 20. This solution is gassed with 5% CO_2 in either O_2 (normal) or N_2 (anaerobic conditions). The mucosal bathing solution contained (mM): Na^+, 105; K^+, 4; Ca^{2+}, 1; Mg^{2+}, 0.8; Cl^-, 81.8; and SO_4^{2-}, 14.5. This solution was gassed with pure O_2 or N_2. This choice of solutions was made in an attempt to equalize the concentrations of physiologically active ions across the tissue, while maintaining an unbuffered mucosal solution for secretory measurements. The slight difference in osmolarity between these solutions is not thought to be important.

The chamber employed for experiments in which secretion was to be measured represents an attempt to minimize the volume of the serosal compartment while retaining good fluid circulation and gassing. The serosal compartment has a volume of 5 ml, while the mucosal solution volume is about 15 ml. With this chamber arrangement, recordings of acid secretory rate (by pH-stat) and transmucosal potential difference (from KCl–calomel electrodes) can be made continuously during the experiment.

For experiments with higher concentrations of AMP-PCP, a chamber system previously employed for studies of washout of ATP and ITP and their derivatives[6,7] was used, in order to allow direct comparison of the present results with the previous ones. In this chamber, an even smaller solution volume (3 ml) can be obtained, but acid secretory rate cannot be measured, nor was transepithelial potential difference (PD) measured. In these experiments, therefore, the mucosal side of the chamber was also filled with Ringers bicarbonate and gassed with CO_2-containing gas.

In conducting washout experiments, the serosal compartment was rapidly emptied and refilled with fresh Ringers, and the sample thus obtained was analyzed spectrophotometrically and by the column method previously described[7]. Samples not analyzed immediately were stored frozen until used.

To assess the effects of AMP-PCP on mitochondrial respiration, rat liver mitochondria were prepared by standard methods[8,9] and the respiratory rate determined by the oxygen electrode technique, using a closed cell and a Clark[10] membrane-covered oxygen electrode. The basic test medium was 0.25 M sucrose, 0.01 M phosphate buffer (pH 7.1), to which all additions were made.

Sodium succinate, sodium α-ketoglutarate, ADP, ATP and AMP-PCP were dissolved in water and adjusted to pH 7 before use.

To determine possible cross-reactions with various deaminases, AMP-PCP solutions were checked for activity toward commercial adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4, Sigma Chem. Co. No. A-9626) and AMP deaminase (AMP aminohydrolase, EC 3.5.4.6, Sigma Chem. Co. No. A-8384). The tests were run according to the instructions supplied by the company, monitoring the disappearance of the adenosine compound with a recording spectrophotometer.

Natural purine ribophosphates were purchased from Sigma Chemical Company, St. Louis, Mo., as their purest grade sodium salt. AMP-PCP was purchased from Miles Labs, Kankakee, Illinois, as the sodium salt. Column analysis of the material supplied shows some contamination by a compound which eluted from the column in the position normally occupied by AMP, but has a pronounced absorption peak at 230 mm. Preparative Dowex-1 chromatography was used on one sample to reduce the level of this contaminant, with no effect on the physiological characteristics. The inactivity of AMP-PCP as received toward AMP deaminase and the spectrophotometric data make it unlikely that the contaminant is AMP, but further identification is lacking.

RESULTS

The addition of AMP-PCP at 10 mM to the serosal bathing solution produces effects very similar to those observed with ATP additions. As shown in Fig. 2, AMP-PCP addition under aerobic conditions results in a decrease in the rate of acid

secretion and an increase in potential difference. It is not clear that these changes are complete during the hour of aerobic incubation allowed. A subsequent 40-min anaerobic period reduces the secretory rate to a very low value, as expected, and the recovery from this anaerobic inhibition is only partial and delayed (compare with the pre-AMP-PCP period). Removal of AMP-PCP by washing with Ringers restores acid secretion within 30 min, although complete physiological steady state requires longer, as judged from the potential difference record.

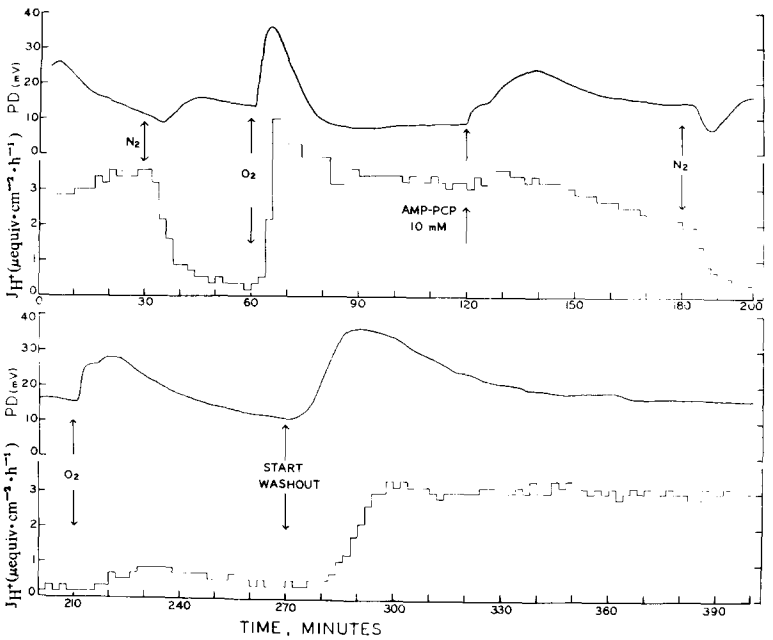

Fig. 2. Effect of AMP-PCP on acid secretory rate and potential difference in the chambered mucosa. After mounting, a preliminary 30-min equilibrium period was followed by a 30-min anaerobic period, with 1 h allowed for recovery. The addition of AMP-PCP at 10 mM to the serosal bathing solution is seen to result in an inhibition of acid secretion and an increase in PD during the following hour of aerobiosis. A subsequent anaerobic period reduces secretory rate to a low level, from which recovery is poor in the following aerobic period. Removal of the AMP-PCP by exhaustive washing restores both secretory rate and PD to near normal levels.

These effects are very similar to those previously reported for ATP[11], and are summarized in Table I. The top entry in this table is a control experiment with ATP, performed in an identical manner to the AMP-PCP experiments. The values obtained are within the range of variation previously observed for ATP under slightly different conditions. The only significant difference between the AMP-PCP experiments and those with ATP is the consistent inhibition with the analog, while the addition of ATP can either stimulate or inhibit the steady-state rate in different stomach preparations.

To analyze for breakdown products, and to determine the washout kinetics, the samples from these runs were collected during the washout period, and analyzed for purine nucleotide composition. While there was no evidence of adenosine nucleotides other than AMP-PCP, there were traces of inosine appearing in the solutions.

TABLE I

SECRETORY RATE, LAG TIME AND PD VALUES OF AMP-PCP

In each case, the compound was added in the indicated concentration to the serosal bathing solution. Figures are given for the average secretory rate (μequiv $H^+ \cdot cm^{-2} \cdot h^{-1}$) during the 30 min previous to addition of the compound (C), for the ratio of the average rate during the second 30-min period following addition to control (I/C), and for the corresponding ratio of secretory rate following recovery from 40 min of N_2 after compound addition to the control rate (R/C). In addition, secretory lag values (min) are given before (C) and after (I) compound addition, as well as the PD just before compound addition and 1 h after addition (C and I). The data in the second line are taken from the experiment shown as Fig. 2. The AMP-PCP sample used in Line 3 has been repurified as described in the text.

Compound	*Concn (mM)*	*Secretion*			*Lag time (min)*		*PD (mV)*	
		C	*I/C*	*R/C*	*C*	*I*	*C*	*I*
ATP	10	3.11	1.17	0.76	4.0	33.0	20.0	30.0
AMP-PCP	10	3.20	0.76	0.17	5.5	14.5	8.0	15.0
	10	3.06	0.61	0.06	8.5	19.0	18.0	23.0
	5	2.08	0.96	0.70	6.5	10.5	11.5	21.0

It was anticipated that the washout kinetics for AMP-PCP should resemble those previously determined for inosine, if the analog, like inosine, cannot be further degraded by the mucosa. One would expect a situation such as shown in Fig. 3, in which there are two exponentials (*plus* the very rapid "chamber compartment", not shown) which can be separated by the technique of subtracting the longer exponential from the data points to obtain the shorter time constant exponential. These two exponentials or quasi-exponentials have previously been identified as the cellular compartment (time constant 30 min) and the serosal connective tissue layer (time constant 5 min). When the same experiment was repeated with AMP-PCP, the kinetics obtained are shown on Fig. 4. A single exponential is obtained, with a time constant of 9.8 min, which seemingly corresponds to neither the serosal connective tissue layer or the cellular compartment. If AMP-PCP did not penetrate the cells, one would have expected a single exponential with a time constant of about 5 min, whereas if its rate of penetration were similar to that for inosine, the two-exponential system would be expected. This point will be discussed later.

The spectrophotometric analysis used for obtaining the data in Fig. 4 reveals two components. The first is the adenosine component, and subsequent column analysis shows that this component is all AMP-PCP, with no evidence of other adenosine compounds. The points indicated by the X are an inosine component, and column analysis shows this to be exclusively inosine itself, with no phosphorylated inosine compounds. If AMP-PCP is the source of the recovered inosine, the kinetics of the pathway are different from those previously noted, since the addition of any normal adenosine phosphate leads to the appearance of IMP as the predominant product at shorter washout times. Control experiments have established that there is no inosine recovered from the tissue without the addition of some purine nucleotide.

The possibility that AMP-PCP can be catabolized to inosine prompted a

check for the activity of the two major enzymes implicated in deamination of adenosine to inosine compounds, adenosine deaminase and AMP deaminase. While the commercial sources of both enzymes are mammalian (rabbit muscle and calf intestinal

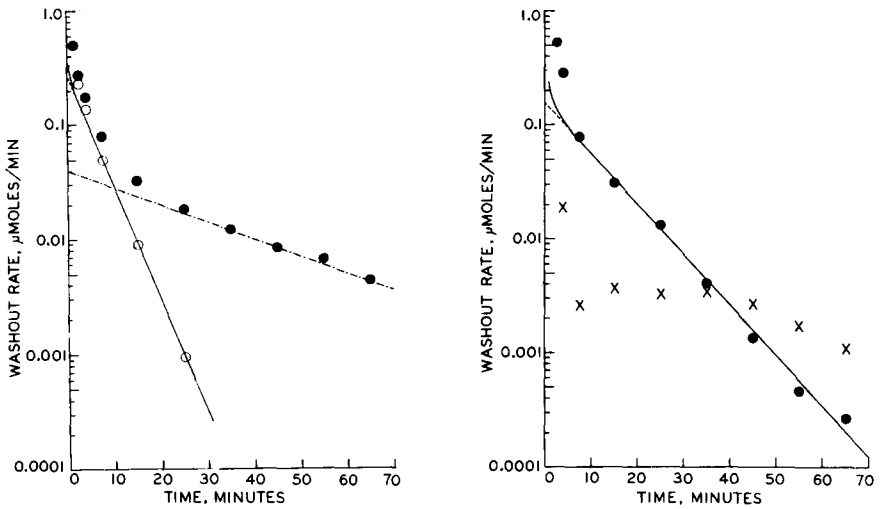

Fig. 3. Washout kinetics of a mucosa loaded with inosine for 3 h, and washed out from the serosal side. Solid points are total inosine washout rates per chamber (3.14 cm^2); the dot-dash line is the exponential drawn through the straight-line portion of these points (and through points from 70–120 min, not shown); the open circles are the result of substracting the dot-dash line from the solid points, and form another quasi-exponential. The solid line is the curve calculated from Eqn 1 with $\alpha = 4.75$ min and $\beta = 0.083 \cdot 10^{-6}$ mole/min for unit area. The dotted line extrapolation was used for the determination of β. These data have been interpreted as showing washout from a cellular compartment (dot-dash line) and from the serosal connective tissue layer (solid line).

Fig. 4. Washout kinetics from a tissue loaded with AMP-PCP for 2 h, final concentration 24 mM in serosal bathing solution. Solid points are the measured adenosine compound washout rate per chamber (3.14 cm^2) while the solid line is the calculated line from Eqn 1 for $\alpha = 9.8$ min, $\beta = 0.050 \cdot 10^{-6}$ mole/min for unit area. As in Fig. 3, the dotted line is the extrapolation used to determine β. Note the lack of any observable long exponential component. The crosses are the rate of appearance of inosine in the bathing solution as a function of time. See text for discussion.

mucosa) it was felt that the preparation of these enzymes from the frog was too time-consuming for the advantages to be obtained. With the enzymes used, there was no disappearance of AMP-PCP in either case, with enzyme concentrations which resulted in reaction rates with the proper substrate which were too fast for accurate measurement. It can be concluded, as expected, that AMP-PCP does not interact with either adenosine or AMP deaminase.

If AMP-PCP were a strong inhibitor of mitochondrial function, by competing with ADP or binding to sites normally involved in ATP turnover, this could explain the inhibitory effects observed. The addition of AMP-PCP to respiring mitochondrial preparations in the oxygen electrode system results in no very striking effects. In the presence of 3.33 mM α-ketoglutarate or succinate, the addition of 0.33 mM ADP gives maximum respiratory rate, with a slight inhibition noted by a subsequent addition of 3.33 mM ATP. This inhibition is also found with 3.33 mM AMP-PCP,

but since it is also found with a similar concentration of ADP it is considered to be an artifact not related to the energetics of the mitochondrial system. These experiments have not been pursued further, since the primary point of interest was to demonstrate that AMP-PCP is not a potent inhibitor of mitochondrial respiration. Neither does it apparently affect oxidative phosphorylation, since the P:O ratios were identical within experimental error in the presence and absence of AMP-PCP. While there may be some second-order effects of the analog on the mitochondrial system, it would not seem that mitochondrial activity is the locus of the inhibition by AMP-PCP.

DISCUSSION

There are several surprising findings as a result of these experiments. The first is that AMP-PCP, although unable to participate in most biological reactions, and apparently being recovered from the tissue unchanged (no evidence of AMP or adenosine, for instance), is able to act as a potent inhibitor of gastric acid secretion. The effects are qualitatively and quantitatively similar to those produced by an equal quantity of ATP (which is degraded) and are completely reversible to removal of the AMP-PCP, as is ATP inhibition. Thus, if AMP-PCP is not a substitute for ATP in reactions involving the energy-storage functions of ATP, the hypothesis previously advanced[6] for the inhibitory action of purine nucleotides must be abandoned.

It is clear, however, that AMP-PCP is not without effect. The appearance of inosine in the washout samples from AMP-PCP-loaded tissues gives a clue to what one of these effects might be. The evidence makes it unlikely that ADPCP was the source of the inosine found. It seems more likely that the entry of AMP-PCP into the cells stimulates the purine catabolic machinery to degrade adenosine compounds, without itself being a substrate for that degradation. Thus the inosine which appears is the result of the activated purine catabolic machinery acting on endogenous adenosine compounds, resulting in the appearance of inosine in the bathing medium.

One problem with this explanation for the increased inosine output after AMP-PCP loading is that the kinetics of inosine washout, both with AMP-PCP loading (Fig. 2) and with ATP or ITP loading[7] are virtually identical, having a long period of constant rate ($0.005\,\mu M \cdot min^{-1} \cdot 3.14\ cm^2$ tissue) followed by an approach to exponential washout. I have previously explained this curve as the result of a saturated IMP phosphorylase which, in the presence of sufficient IMP, produces inosine at constant rate, so that a steady state is set up between inosine production and diffusion from the cell[7]. This explanation requires the presence of IMP, and since IMP seems to be permeable to the cell membranes one would expect to find it in the bathing solutions upon washout. Since IMP does not appear in measurable amounts after AMP-PCP loading, this hypothesis becomes suspect as an explanation for the constant inosine washout rate. I have previously shown that a saturable inosine transport system does not seem to be the explanation, since tissues loaded with inosine show exponential washout kinetics in this range of rates.

The form of the washout of AMP-PCP from the tissues is a bit surprising in light of the previous information from natural compounds. In only one other case is the washout situation uncontaminated by breakdown products of the loaded purine. This is the case of inosine, which, being the excreted end-product of purine

catabolism in this system, is not expected to be further degraded. For comparison with AMP-PCP washout, a washout curve for inosine, taken under essentially identical conditions is reproduced as Fig. 3. We find that this curve can be resolved into 3 separate kinetic "compartments", of 0.5, 5 and 30 min time constant, respectively. These have been identified[6] as the material adhering to the chamber walls, the serosal connective tissue layer (+interstitial space) and the cell cytoplasm, respectively. A cursory examination of Fig. 4 shows that there seem to be only two compartments, the chamber compartment of 0.5 min time constant, and a single exponential of time constant 9.8 min, which is too long for the serosal connective tissue layer and too short for the cells. A detailed examination of the kinetics of such systems is necessary to resolve this apparent paradox.

If the washout kinetics observed are to be explained as washout from the serosal connective tissue layer, they should fit the equation governing diffusion in such a system. The appropriate expression for the rate of exit per unit area can be written as[7,12].

$$\frac{dY}{dt} = \beta \sum_{n=0}^{\infty} \exp\left[-(2n+1)^2\, t/\alpha\right] \tag{1}$$

where t is the elapsed time since the start of washout (with zero concentration in the bulk solution) and α and β are derived constants defined below. This expression predicts an infinite rate at $t=0$, a simple exponential with a time constant of α when t is large (since the terms $n>0$ can then be neglected) and a transition region. The exponential can be extrapolated to $t=0$ with an intercept of β. Thus, if a system follows these kinetics, α and β can be measured experimentally.

From the derivation of Eqn 1,

$$\alpha = 4L^2/\pi^2 D \tag{2}$$

and

$$\beta = 2DY_0/L^2 \tag{3}$$

where L is the thickness of the diffusion layer, D is the diffusion coefficient for Y in this layer, and Y_0 is the amount of Y in the layer at $t=0$. A measurement of α will therefore determine D/L^2, as will a measurement of β when Y_0 is known. Neither D nor L can be determined independently unless additional information is available.

If the layer is in equilibrium with the loading solution at $t=0$, and if the layer consists exclusively of components which can dissolve Y to the same extent as the aqueous loading solution, then we can write that

$$Y_0 = [Y_0]L \tag{4}$$

where $[Y_0]$ is the concentration in the layer, and L is its thickness as before (thickness is a measure of volume when the expressions are written for unit area, as here). This $[Y_0]$ will be in equilibrium with the concentration in the loading solution at $t=0$, which is $[Y_s]$. Since $[Y_s]$ can be measured, $[Y_0]$ is determined, and from Eqns 2, 3 and 4 we can write

$$L = \beta\alpha\pi^2/8[Y_0] \tag{5}$$

which determines L, and consequently D is determined from Eqns 2 or 3.

However, if the connective tissue (in the present case) excludes solution such that the volume of trapped free solution is less than L (per unit area) then our calculated value of Y_0 will be too high, our estimate of L too large, and that of D too small. Conversely, if the connective tissue were to bind Y reversibly, we might expect to calculate a value of L which is too small, and correspondingly overestimate D. In the latter case, though, we might expect some modification of the kinetics due to binding itself.

It is instructive to calculate the values from our washout curves and compare them to other determinations. For inosine load and washout, we have previously determined[7] that $\alpha=4.75$ min and $\beta=0.083\cdot10^{-6}$ mole/min (see Fig. 3). We can calculate with the assumption that the barrier is essentially all water that L is 0.022 cm, which gives $D=0.413\cdot10^{-4}$ cm^2/min. This D is only 13% of the free solution value as determined by Bowen and Martin[13]. Such an L, moreover, is smaller than the thickness of the serosal connective tissue layer outside the smooth muscle layer, which would require a permeability barrier to be located within a histologically homogeneous connective tissue layer, which seems unlikely. It seems more reasonable to argue that D may be the same as its free solution value ($3.12\cdot10^{-4}$ cm^2/min) and that L is therefore 0.060 cm, which corresponds to the thickness of the connective tissue barrier measured to the approximate level of the oxyntic cells[7,14]. This argument implies that Y_0 cannot be determined by Eqn 4, but is rather only 36% of that value, consistent with some 64% of the connective tissue being non-accessible to inosine in solution.

A similar argument for AMP-PCP diffusion can be made. Here we find $\alpha=9.8$ min and $\beta=0.050\cdot10^{-6}$ mole/min (Fig. 4). We should expect L to be the same for all compounds of similar size, and accordingly will set $L=0.060$ cm, which gives a computed value of $D=1.49\cdot10^{-4}$ cm^2/min. We cannot compare this with the free solution value for AMP-PCP (which has not been measured), but find that the calculated value is considerably lower than that measured for either ADP ($2.4\cdot10^{-4}$) or ATP ($2.6\cdot10^{-4}$). We also calculate Y_0 to be 45% of the solution value, which allows only 55% of the volume of the layer to be non-aqueous, as compared to the previous determination of 64%.

This variation in Y_0 percentage between inosine and AMP-PCP could, of course, be a variation in the individual tissues employed for the measurements, although such a variation seems a little large. The value of D for AMP-PCP, however, is only 60% of what one would assume its true value must be, based on the determinations of Bowen and Martin[13] for a variety of nucleotides and -sides, none of which have diffusion coefficients lower than $2.25\cdot10^{-4}$ cm^2/min.

If we assume that the diffusion coefficient for AMP-PCP is $2.5\cdot10^{-4}$ cm^2/min (an average of ADP and ATP values determined under conditions approximating ours) a very interesting result is obtained. From the measured value of α and this assumed value of D, L is calculated to be 0.078 cm, which is quite close to the value measured in section[14] of 0.08 cm for the total thickness of the tissue, cells and all. This raises the possibility that the AMP-PCP is distributed across a space which includes the interior of some cellular compartment and that the diffusion of material from this compartment is rapid enough to merge with the connective tissue washout. In this view, the failure to observe the cellular washout compartment with AMP-PCP loading is not because the material never enters the cells, but because it enters and

leaves the cells too rapidly to be distinguishable from the connective tissue washout. Thus, the cellular cytoplasm is acting merely as an extension of the unstirred layer.

This is an interesting possibility, but should not be taken too definitively, since the number of experiments is restricted, and the free solution diffusion coefficient has been assumed in these calculations, since measurements are not available.

The only other explanation for the failure to find a diffusion time-constant of about 30 min would be to maintain that the AMP-PCP did not penetrate the cells at all. This requires, as we have seen, rather gross changes in the assumed value for the diffusion coefficient, changes which seem restricted to the analog and not applicable to any other purine nucleotide. This seems unlikely.

A direct answer to this question could be obtained by the use of radioactive analog, following the location of the label by autoradiography. Technical considerations, chiefly the lack of availability of such labeled analog, have prevented this attempt.

Finally, the chemical basis for inhibitory effects of AMP-PCP and the natural purine phosphates remain to be understood. One common feature of all of these compounds is their possession of a nitrogen with an unshared pair of electrons, in conformity with the hypothesis proposed by LeFevre *et al.*[15] for gastric secretory inhibitors. In the case of purines, the nitrogen in question is part of the purine ring structure, and is not a completely sufficient requirement, since adenosine and inosine, which likewise have this structure, are rather inefficient inhibitors compared to ATP, ITP and AMP-PCP[6]. The hypothesis previously advanced[6] which imputed the inhibitory effects of ATP and ITP to interactions with the mitochondrial respiratory chain must apparently be abandoned, since AMP-PCP does not seem to affect mitochondrial respiration. It would seem that the inhibitory effects are not a consequence of the "natural" roles of the adenosine nucleotides in these cells, but rather represent a "side effect" due to other properties of the molecules. Further work with nitrogen-containing analogs will be necessary to clarify this point.

CONCLUSIONS

In conclusion, it has been demonstrated that the ATP analog AMP-PCP is an inhibitor of gastric acid secretion in this preparation, with inhibitory effects closely resembling ATP. Although its addition stimulates a small release of inosine, apparently by stimulating catabolysis of endogenous purines, the analog itself is not broken down, which makes it a good model compound for studies of the kinetics of washout. As expected, the analog is inactive toward adenosine deaminase and AMP deaminase, and is inactive as an inhibitor of mitochondrial respiration. The kinetics of its washout are perhaps to be understood as indicating a permeability of the cell plasma membrane to AMP-PCP which is greater than that for inosine, which is the only other purine compound for which no catabolism is observed. If this is true, it is perhaps not surprising that the effects of other adenosine phosphates can be best explained by assuming cellular permeability, and it reinforces the conclusion that the lack of stimulatory effect of ATP added under anaerobic conditions to this system is not due to a failure of ATP to penetrate, but rather due to the acid secretory mechanism being insensitive to cytoplasmic ATP levels. The inhibitory

effects of the analog, however, force the abandonment of an hypothesis previously advanced which connected the inhibitory effects of ATP with an action on the respiratory rate of the gastric mucosal mitochondria.

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