Diphosphoglycerate rather than ATP as a physiological phagostimulant factor for *Rhodnius prolixus*[1]

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Summary. Diphosphoglycerate in μM concentrations stimulates feeding in *Rhodnius prolixus*. Since diphosphoglycerate is present at a high concentration in red blood cells, and is resistant to apyrase hydrolysis, it is suggested that it is a physiological phagostimulant for this blood-sucking insect.

ATP is one of the nucleotides which has been suggested to be a physiological phagostimulant for *Culex pipiens*[2]. After testing several phosphate-rich compounds, Friend[3] classified them according to their potency for *Rhodnius prolixus* in inducing gorging. With gorging activity for these insects expressed as the median effective dose (ED_{50}), ATP was found to be the most effective nucleotide[4]; this activity decreased significantly in the presence of Ca^{++}[3]. Since ATP is present in high concentration in blood it has been considered to be a natural physiological phagostimulant for *R. prolixus* as well as for other haematophagous insects.

Recently, the existence in the salivary gland of *R. prolixus* of a typical and potent apyrase able to hydrolyze both ATP and ADP to AMP was described[5,6]. It was shown[6] that the enzyme is excreted in the insect's saliva, and is continuously injected into the blood meal, where it could efficiently hydrolyze the ATP present; this suggests that ATP may not be as important a factor in gorging as was previously supposed.

In the present work it is shown that 2,3-diphosphoglycerate, which occurs in a concentration 3-fold higher than ATP in erythrocytes, has an ED_{50} lower than ATP for *R. prolixus*, which suggests that it may in fact be a better physiological phagostimulant for these bugs.

Materials and methods. The insects were obtained from a colony of *R. prolixus* reared and maintained in the laboratory at a relative humidity of 50-60% at 25±2 °C, and fed on human citrated blood every 25-35 days. Following the last ecdysis the 3rd instar larvae were starved for 40 to 45 days and were then fed through a specially designed feeding apparatus[7] on NaCl 0.15 M, pH 7.0, containing or not containing 2,3 diphosphoglycerate. Intake of saline was estimated by the difference in body weight before and after feeding. Groups of 40 insects were used. The gorging effect was expressed as the ED_{50} estimated by the method of Litchfield and Wilcoxon[8], using the number of fully fed insects as percent of the total. Hydrolysis of ATP and 2,3 diphosphoglycerate (DPG) with the salivary secretion from *R. prolixus* was carried out as described for measurement of apyrase activity[6]. The reagents ATP and DPG were from Sigma Chemical Co. (USA). Other reagents were of analytical grade.

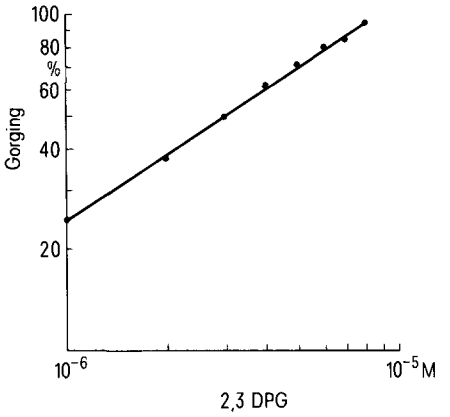
Results and discussion. The figure expresses the phagostimulant activity of DPG for *R. prolixus*. An ED_{50} of 3.0×10^{-6} M (confidence limits 2,4-3,7) was then estimated. This value is slightly lower than that reported for ATP under the same conditions by Smith and Friend[9]. Since the concentration of DPG inside red blood cells, where it binds to hemoglobin on an equimolar basis[10,11] is 3-fold higher than that of ATP, the data suggest that DPG could be an important stimulator of blood feeding for these insects. This possibility was reinforced by the finding (table) that ATP, but not DPG, was rapidly hydrolyzed by the insect's salivary apyrase. Since this apyrase is a calcium-dependent enzyme[5,6] these observations could explain the previous finding[3] that Ca^{++} drastically decreases the phagostimulant activity of ATP.

Besides its direct effect on platelet aggregation, ATP is also important for vasoconstriction, perhaps inducing contraction of myofibrils in the muscles along the small vessels[12]. Plasma ATPase[13] would also favor the produciton of ADP, an important platelet aggregating factor which in turn initiates blood clotting. Although ATP and ADP can induce phagostimulation by themselves under experimental conditions, their physiological actions on the blood circulation are probably not important for the insect's gorging.

It has been shown that DPG as well as ATP, ADP and AMP are allosteric effectors of hemoglobin[10]. Interestingly, there is a great similarity between the phagostimulant action and the allosteric inhibition of hemoglobin-oxygen interaction by these substances. For the latter effect there is an order of magnitude difference for the affinity among them: DPG > ATP > ADP > AMP. It was also shown that the binding occurs inside the central cavity situated between the 2 β chains of hemoglobin, where the α-amino as well as other basic groups are located. The affinity shown by the less efficient allosteric effectors is dependent on the progressive shortening of the distances between terminal phosphate groups[10]. The phagostimulant activity of these substances shows a very similar order of effect as represented by their ED_{50}: DPG, 3.0×10^{-6} M (this work) and ATP, 6.0×10^{-6} M; ADP, 66.0×10^{-6} M and AMP, 1000×10^{-6} M[4]. These results indicate that a functional similarity may exist between the central cavity of the hemoglobin molecule and that of the insect chemoreceptor (Macarini, in preparation). Furthermore, since the phagostimulants are necessary in the 1st 2 min of gorging[14], it is suggested that they may function in a saturating manner. During the process of feeding it is necessary for some hemolysis to occur to permit the release of DPG. It can be said, however, that the hemolysis of as few as 1% of the red blood cells is enough to produce enough DPG to induce 100% gorging[15].

Substrate	Salivary protein	Percent hydrolyzed in 10 min
ATP	5 μg	38
DPG	5 μg	0

Apyrase activity of *Rhodnius prolixus* salivary secretion on 1 mM ATP and DPG.



The gorging response of 3rd instar larvae of *Rhodnius prolixus* to 2,3 diphosphoglycerate in 0.15 M NaCl, pH 7.0

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Metabolic rates in excised tissues of carp

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Summary. Oxygen consumption in vitro of carp tissues ranged from 7.60 $\mu l \cdot min^{-1} \cdot g^{-1}$ for brain to undetectable for fat in the following order: brain, kidney, intestine, dark muscle, hepatopancreas, ventricle, gill filaments, testis, ovary, erythrocytes, vertebrae, ordinary muscle, blood, and fat. Summated oxygen consumption in vitro of the tissues approximately coincided with oxygen consumption in vivo of intact animal.

Oxygen consumption in vitro (Q_{O_2}) of tissue is a basic parameter of metabolic activity of the tissue. The values of Q_{O_2} have been determined in several fishes, but they were limited to only a few tissues for any single species. Brain, liver and muscle from menhaden, toadfish and scup[1]; brain, liver and gills from goldfish[2]; kidney and gills from cutthroat trout[3]; muscles from 2 tunas[4]; gills from 2 sunfishes[5]; rectal gland, spleen and kidney from dogfish[6]; and gills from Atlantic cod[7] have been examined. This paper presents data on Q_{O_2} for 14 tissues covering most of the main organs of carp. The relationship between these figures and the oxygen consumption of the intact animals is discussed.

Material and methods. The experiments were carried out on 88 carps *Cyprinus carpio* of 213±82 g ($\bar{X} \pm SD$) kept for 2 weeks or more. They were fed with pellets made from fish meal (38%), wheat flour (29%), soybean cake (15%), vitamins and minerals, except 1 or 2 days before experimentation. A fish was instantly killed by a spinal abscission and the tissues immediately excised. Excised tissues were prepared for Q_{O_2} determination by the following methods, which have been found to give the most reliable results for various tissues (Oikawa and Itazawa, in preparation). Most of the tissues were prepared by chopping with scissors about 400 times, until the tissue became pasty, in a chilled weighing bottle. Gill filaments, erythrocytes, blood and fat were examined with intact tissue, and vertebral centra were prepared by grinding them by a file. Oxygen consumption of the tissue preparation was determined by Warburg's manometric method at 20 °C using Cortland saline[8] containing 5.6 mM glucose as the substrate solution. A substrate solution containing Na-pyruvate, Na-fumarate and Na-L-glutamate besides glucose gave almost the same results for various tissues as the solution containing glucose only (Oikawa and Itazawa, in preparation).

Results and discussion. The highest value of Q_{O_2} was obtained with the brain which showed 7.60 $\mu l \cdot min^{-1} \cdot g$ (wet wt)$^{-1}$, while the lowest one was with fat, for which the value did not differ significantly from zero. The 14 tissues examined were arranged in the following order mainly based on statistically significant differences ($p < 0.05$): brain, kidney > intestine > dark muscle, hepatopancreas > ventricle, gill filaments > testis > ovary, erythrocytes, vertebral centra, ordinary muscle > blood > fat (table 1). Summation of tissue respiration in vitro was attempted to compare with the respiration in vivo of the intact animal using the same individuals. Oxygen consumption in vivo of a carp of 110–140 g was determined by a constant flow method at 20 °C making 6 measurements each time. The oxygen consumption was 1.67±0.10 $\mu l \cdot min^{-1} \cdot g$ (wet wt)$^{-1}$ ($\bar{X} \pm SD$). After the experiments, the fish were sacrificed to weigh the various tissues composing the body. Oxygen consumption in vitro of a whole animal was estimated by dividing the summated oxygen consumption of various tissues by the summated weight of the tissues.

Table 1. Oxygen consumption in vitro of carp tissues

Tissue	Preparation	Q_{O_2} ($\mu l \cdot min^{-1} \cdot g^{-1}$)		
		N (fish)	$\bar{X}$	SD
Brain	Chopped[c]	10	7.60	±0.83
Kidney	Chopped	10	6.91	±1.15
Intestine	Chopped	10	5.35	±0.92
Dark muscle[a]	Chopped	10	4.26	±0.95
Hepatopancreas	Chopped	11	4.22	±1.03
Ventricle	Chopped	10	3.33	±0.80
Gill filaments	Intact	13	3.10	±0.72
Testis	Chopped	10	2.46	±0.57
Ovary	Chopped	10	1.87	±0.51
Erythrocytes	Intact	8	1.53	±0.71
Vertebral centra	Ground	7	1.08	±0.40
Ordinary muscle	Chopped	10	1.04	±0.52
Blood	Intact	8	0.42	±0.13
Fat[b]	Intact	8	0.25	±0.65

[a] Reddish muscle of the lateral regions of the body. [b] Fat collected from the intracranial region. [c] Chopping was carried out with scissors.