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THE EVOLUTION OF PURINERGIC RECEPTORS INVOLVED IN RECOGNITION OF A BLOOD MEAL BY HEMATOPHAGOUS INSECTS

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Many blood feeders use adenine nucleotides as cues for locating blood meal. Structures activity relationship of adenine nucleotides as phagostimulants vary between closely related species of blood feeders. It is suggested that a preexisting diverse pool of nucleotide-binding proteins present in all living cells, serves as a source of receptor proteins for the gustatory receptors involved in blood detection. It is proposed that the selection of any such nucleotide-binding protein is random.

Hematophagy has evolved independently in many taxons of arthropods. The means used for recognition of the blood meal demonstrate convergent evolution. Many unrelated parasites use adenine nucleotides as cues for the presence of blood (Galun, 1987). A careful study of the gorging response of various hematophagous parasites to artificial diets containing adenine nucleotide analogues shows a great diversity of structure-activity relationships (SAR). Whereas in Crustacea phagostimulatory adenine nucleotide analogue SAR are judged on the basis of both behavioral responses and electrophysiological recordings from antennule sensilla, in blood-feeding insects, with one exception, behavioral responses serve as the sole criterion. In the tsetse fly, *Glossina*, the labellar gustatory receptors detect the presence of ATP or its analogues and their electrophysiological responses correspond very well to the behavioral data (Galun & Margalit, 1969; Mitchell, 1976). In other blood feeders, the putative sensilla involved in detection of blood are located inside the food canal. To date, no satisfactory recording has been made from these sensilla. Thus SAR data on hematophagous insects are based on their behavioral responses.

The gorging response of very closely related species, e.g. two aedine mosquitoes, *Aedes aegypti* and *Ae. caspius*, shows a similar SAR – ATP>ADP>AMP – as well as very high potency of the two non-hydrolyzable analogues (AMP-PCP, AMP-PNP) and of 2'3'ddATP (Table I). Two other mosquito species, *Culex pipiens* and *Culiseta inornata*, are also markedly similar to each other (Table II) but quite different from the aedines, although all four belong to the same subfamily, Culicinae. The difference in response between the aedine species and *Culex pipiens* is expressed mainly in the relative importance of the length of the phosphate chain and of the hydroxyl groups on the ribose moiety. The gorging response of *Ae. aegypti* shows the potency sequence ATP>ADP>AMP; removal of each phosphate group reduces the stimulatory activity by approximately one order of magnitude. *Cx. pipiens*, on the other hand, shows the potency sequence ADP>ATP=AMP, and the activity differs very little between the three nucleotide – indicating that the length of the phosphate chain is of minor significance. Removal of one hydroxyl group from the ribose moiety halves the response to ATP for *Ae. aegypti* but reduces it to 3% for *Cx. pipiens*. Removal of the two hydroxyl groups (2'3'ddATP) increases the potency of the molecules 10-fold for *Ae. aegypti* while eliminating all stimulatory effect for *Cx. pipiens* (Table V). A possible explanation for this unexpected increase in potency of ddATP for *Ae. aegypti* is proposed by Galun et al. (1985).

While such marked differences in structural requirements of the nucleotide receptors were observed in the same mosquito subfamily, the same mosquitoes resembled taxonomically distant blood feeders in their gorging response. Thus, the threshold for the feeding response of *Rhodnius prolixus*, the kissing bug, is a function of the length of the phosphate chain as in the mosquito *Ae. aegypti* (Table III) but the blackfly, *Simulium venustum* (Simulidae), like the mosquito *Cu. inornata*, prefers ADP to ATP and its response is far less dependent on the length of the chain (Table IV).

Detailed SAR studies were carried out with only a limited number of blood feeders. Therefore, the analysis does not include many other hematophagous species known to depend on

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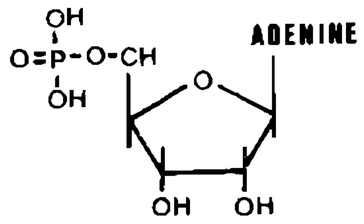
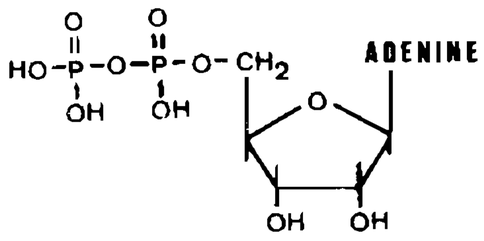
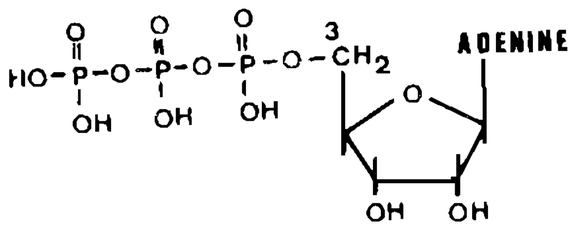
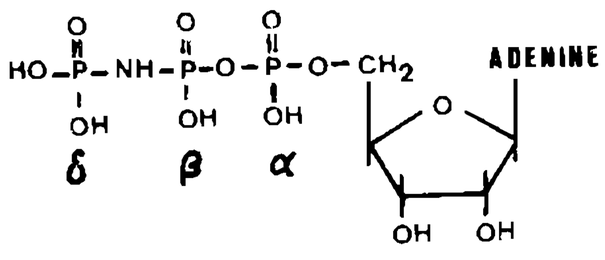
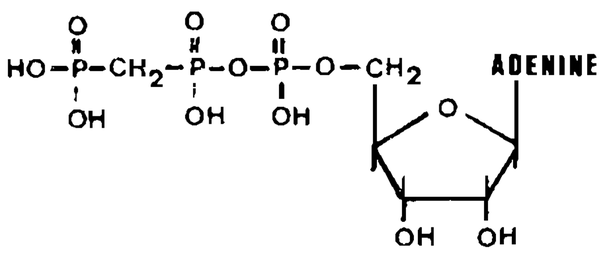
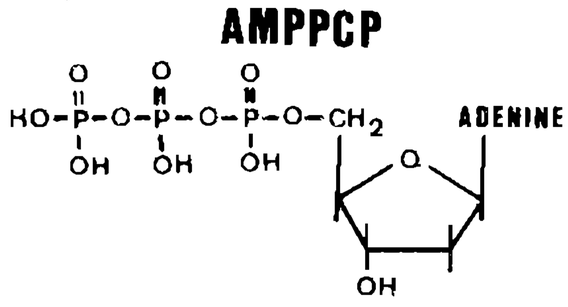
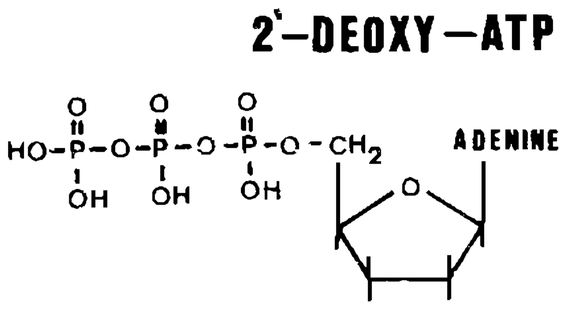
Table I

The gorging response of Aedes aegypti and of Aedes caspius to ATP analogues

	Aedes aegypti *		Aedes caspius **	
	ED_{50} (µM)	Activity index	ED_{50} (µM)	Activity index
 AMP	880	1.4	310	2.6
 ADP	96.2	12.6	150	6.0
 ATP	12.2	100.0	9.1	100.0
 β,IMIDO ATP AMPPNP	2.2	554.0	0.8	1123.0
 β,δ METHYLENE ATP AMPPCP	3.9	312.0	4.9	185.0
 2'-DEOXY-ATP	24.9	49.0	13.0	70.0
 2',3'-DIDEOXY-ATP	1.2	1016.0	2.1	433.0

*) From Galun et al., 1985.

**) Galun, umpublished data

ED_{50} the dose inducing 50% of the test insects to gorge, was calculated from dose response results, using SAS computerized probit procedure.

Activity index : $\frac{ED_{50} \text{ of ATP}}{ED_{50} \text{ of analogues}} \times 100$

TABLE II

The gorging response of *Culex pipiens* & *Culiseta inornata* to ATP analogues

Analogue	*Culex pipiens**		*Culiseta inornata**	
	ED_{50} (μM)	Activity index	ED_{50} (μM)	Activity index
ATP	24.5	100.0	199.0	100.0
ADP	11.8	207.0	90.5	219.0
AMP	27.1	90.0	906.0	22.0
AMP-PCP	48.0	51.0	338.0	59.0
AMP-PNP	20.0	120.0	168.0	118.0

*From Galun et al., 1987.

TABLE III

The gorging response of *Aedes aegypti* & *Rhodnius prolixus* to ATP analogues

Analogue	*Aedes aegypti**		*Rhodnius prolixus***	
	ED_{50} (μM)	Activity index	ED_{50} (μM)	Activity index
A tetra P	15.8	77.2	3.2	118.0
ATP	12.2	100.0	3.8	100.0
ADP	96.5	12.6	45.0	8.4
AMP	880.0	1.4	630.0	0.6

*From Galun et al., 1985.
**From Friend & Smith, 1982.

TABLE IV

The gorging response of *Culiseta inornata* & *Simulium venustum* to ATP analogues

Analogue	*Culiseta inornata**		*Stimulim venustum***	
	ED_{50} (μM)	Activity index	ED_{50} (μM)	Activity index
ATP	199	100	12	100
ADP	90	219	5	240
AMP	906	22	100	12
AMP-PCP	338	59	highly effective	

*From Galun et al., 1985.
**From Smith & Friend, 1982.

ATP as a phagostimulant. However, even the small number of examples given here indicates quite clearly that the gustatory adenine nucleotide receptors (purinergic receptors) do not show a single evolutionary trend and must have evolved anew many times. The question arises: why do so many unrelated taxons focus on adenine nucleotides as the cue for the presence of blood, and once having selected them, why do they exhibit such diversity in the structural requirements of their gustatory chemoreceptors?

In should like to suggest an answer to two parts of the question: ATP is a very effective 'metabolic symbol' (terminology of Tomkins, 1975). Tomkins refers to environmental reference chemicals as symbols - meaning chemicals that accumulate when the cell is exposed to a particular environment. The metabolic symbols do not necessarily bear a structural relationship to the environment they represent. Thus, ATP is not a chemical analogue of hemoglobin. Yet a high concentration of ATP encountered by a questing blood feeder indicates a high concentration of an easily digestible and nutritions protein – as ATP is confined to

TABLE V

The gorging response of *Aedes aegypti* & *Culex pipiens* to ATP analogues

Analogue	*Aedes aegypti**		*Culex pipiens***	
	ED_{50} (μM)	Activity index	ED_{50} (μM)	Activity index
A tetra P	15.8	77.2	very low	
ATP	12.2	100.0	24.5	100
ADP	96.5	12.6	11.8	207
AMP	880.0	1.4	27.1	90
AMP-PCP	3.9	312.0	48.0	51
AMP-PNP	2.2	554.0	20.4	120
dATP	24.9	49.0	833.0	2.9
dADP	248.5	4.9	524.0	4.6
ddATP	1.2	1016.0	0	0

*From Galun et al., 1982.
**From Galun et al., 1987.

the cellular fraction of the blood which contains the major portion of the blood protein. In shed blood, ATP is quickly degraded by deamination and dephosphorylation into non-stimulatory components such as xanthine. Thus, prior to its deterioration, shed blood is often not imbibed by many blood feeders unless exogenous ATP or ADP is added.

Selection of ATP or ADP as a phagostimulant by so many taxons of blood feeders may also have resulted from widespread availability of ATP-ADP binding proteins participating in the energy metabolism of every living cell. Thus, directions for synthesizing a nucleotide binding site are already present in the genome of every cell, and do not have to be created de novo. This pool of proteins, with its marked affinity for adenine nucleotides, could have been the source of the vertebrate purinergic receptors present in the nervous and vascular systems and in the smooth muscle of many internal organs. There, adenosine and its non-cyclic nucleotides function as neurotransmitters or modulators of physiological activities (see review of purinergic receptors of vertebrates, Burnstock & Brown, 1981). Carr et al. (1987) suggest a diferent evolutionary pathway for internal neuroactive agents — e.g., origin from external chemoreceptors of primitive aquatic organisms. I suggest that nucleotide-binding sites on proteins existed at the earliest stages of evolution, and could serve as a source of specific binding sites for internal and external chemoreceptors.

Some events in the evolution of the various blood feeders led to the unmasking of the genetic information for the nucleotide binding site in the genome of the gustatory neurons on the mouthparts — and to the incorporation of this receptor protein into the neural dendrite. This may have occurred late in evolution and therefore some genera of the same family lack the property, while other genera possess it (i.e. culicines detect ATP while anophelines do not need them in order to recognize their blood meal) (Galun et al., 1985). A similar proposal was made by Kitteredge & Takahashi (1972) for crustacean pheromone receptors evolving from internal hormone receptors.

Because information for many different nucleotide binding sites exists in the cell genome, the unmasking of such information may expose a diversity of binding sites – which may in turn explain the diversity of the structural requirements shown by the purinergic gustatory receptors even in different genera of the same family.

REFERENCES

BURNSTOCK, G. & BROWN, C.M., 1981. *An introduction to purinergic receptors.* p. 1-45. In: Burnstock, G. Ed., Purinergic Receptors. Chapman & Hall, London.

CARR, W.E.S.; ACHE, B.W. & GLEESON, B.A., 1987. Chemoreceptors of crustacea: Similarities to receptors for neuroactive substances in internal tissues. *Environ. Hlth. Perspectives, 71* :31-46.

FRIEND, W.G. & SMITH, J.J.B., 1982. ATP analogues and other phosphate compounds as gorging stimulants for *Rhodnius prolixus J. Insect. Physiol., 28* :371-376.

GALUN, R. Regulation of blood gorging. *Insect. Sci. Appl., 8* :in press.

GALUN, R.; FRIEND, W.G. & NUDELMAN, S., 1987. Purinoreceptors involved in recognition of blood by culicine mosquitoes (in preparation).

GALUN, R.; KOONTZ, L.C. & GWADZ, B.W., 1985. Engorgement response of anopheline mosquitoes to blood fractions and artificial solutions. *Physiol. Ent., 10* :145-149.

GALUN, R.; KOONTZ, L.C.; GWADZ, B.W. & RIBEIRO, J.M.C., 1985. Effect of ATP analogues on the gorging response of *Aedes aegypti. Physiol. Ent., 10* :275-281.

GALUN, R. & MARGALIT, J., 1969. Adenine nucleotides as feeding stimulants of the tsetse fly *Glossina austeni. Nature, 222* :583-584.

KITTEREDGE, J.S. & TAKAHASHI, F.T., 1972. The evolution of sex pheronome communication in Arthropoda. *J. Theor. Biol., 35* :467-471.

MITCHELL, B.K., 1976. Physiology of an ATP receptor in labellar sensilla of the tsetse fly *Glossina morsitans. J. Exp. Biol., 65* :259-271.

SMITH, J.J.B. & FRIEND, W.G., 1982. Feeding behavior in response to blood fraction and chemical phagostimulants in the blackfly *Simulium venustum. Physiol. Ent., 7* :219-226.

TOMKINS, G.M., 1975. The metabolic code. *Science, 189* :60-76.