Histamine Hypersensitivity of Mice Induced by 5′-AMP* (32903)

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To date, four interventions which affect the histamine sensitivity of certain strains of mice have been described: (i) the injection of *Bordetella pertussis* organisms (1,2); (ii) adrenalectomy (1,2); (iii) the administration of endotoxin (3,4), and; (iv) the establishment of beta-adrenergic blockade (5). In addition, these procedures appear to share the ability to alter the responsiveness of the autonomic nervous system which is particularly manifested in the first and fourth groups by the failure of epinephrine to elicit the normal increase in blood glucose (6).

The failure of epinephrine action in the histamine hypersensitive mouse stimulated interest in attempting to offset the latter state with cyclic 3′,5′-adenosine phosphate (3′,5′-AMP) and 5′-adenosine phosphate (5′-AMP). The cyclic nucleotide, whose intracellular concentration is increased following activation of adenyl cyclase, is believed to mediate the metabolic effects of catecholamines, whereas 5′-AMP represents the phosphodiesterase-catalyzed breakdown of 3′,5′-AMP (7,8). During the course of these investigations, it became clear that 5′-AMP not only elicited hyperglycemia in normal mice of CFW strain but also sensitized them to histamine. These observations are presented in this report.

Materials and Methods. Female mice of the CFW and CFI strains obtained from Carworth Farms were employed in these studies. They were housed in groups not exceeding 50 in number and were allowed pellets and water *ad libitum*. At the time of the experiments, the animals weighed between 18 and 20 gm. The chemical compounds were purchased from commercial sources: histamine diphosphate and adenosine 3′,5′-cyclic phosphate (Nutritional Biochemicals Corp. Cleveland, Ohio), and adenosine 5′-monophosphate (Schwarz BioResearch, Inc., Orangeburg, N. Y.). The drugs were dissolved and diluted in sterile, nonpyrogenic, physiologic saline immediately before each experiment; dosages of histamine represent amine base. Blood glucose measurements, using the glucostat method (Worthington Biochemical Corp. Freehold, N. J.) were made 30 min following the intraperitoneal injection of saline or nucleotide. The effect of 3′,5′-AMP and 5′-AMP on the histamine resistance of normal mice was tested by mixing appropriate concentrations of the amine and nucleotide and injecting intraperitoneally 0.2 ml of the mixture. Deaths were recorded for a 2-hour period.

Results. Table I shows that both 3′,5′-AMP and 5′-AMP elicited hyperglycemia in the CFW strain of mice. In this strain, 5′-AMP markedly reduced the histamine resistance whereas the cyclic nucleotide lacked this activity. In terms of hyperglycemia, the CFI mouse was susceptible to the action of 3′,5′-AMP but not 5′-AMP; neither nucleotide appeared to influence the histamine sensitivity of this strain.

Discussion. Since most of the metabolic events influenced by exogenously administered or endogenously released catecholamines (and other hormones), are believed to involve the action of cyclic 3′,5′-AMP as shown in the cat, rat, dog, and the human individual (7), it was not surprising to find in the present studies that the cyclic nucleotide induced hyperglycemia in both strains of mice. It seems, therefore, not unreasonable to assume that the events mediated by 3′,5′-AMP including induction of hepatic and skeletal muscle glycogenolysis, lipolysis in adipose tissue, etc. (9), in the mouse resemble those described for other species.

On the other hand, the hyperglycemic response to exogenous 5′-AMP is not a common event and even appears to be confined to individual strains within species (Table I). In attempting to interpret these data, emphasis will be placed on skeletal muscle gly-

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TABLE I. The Influence of 3′,5′-AMP and 5′-AMP on the Blood Glucose and Histamine Sensitivity of CFW and CFI Strains of Mice.

Strain and nucleotide	Dose	Blood glucose[a] (mg/100 ml ± SE)	Histamine toxicity[b] (mg/kg) 125	62.5	31.3	15.7	7.8
CFW							
Saline	0.2 ml	160 ± 3.3	0/10	0/10	1/10	0/10	0/10
5′-AMP	25 mg/kg	180 ± 4.3					
	50 mg/kg	201 ± 6.8	8/10	5/10	6/10	4/10	1/10
	100 mg/kg	212 ± 5.6					
3′,5′-AMP	25 mg/kg	178 ± 7.2					
	50 mg/kg	191 ± 8.6	0/10	1/10	0/10	0/10	0/10
	100 mg/kg	219 ± 9.6					
CFI							
Saline	0.2 ml	158 ± 6.4					
5′-AMP	50 mg/kg	168 ± 7.2	0/10	0/13	0/5	0/5	—
	100 mg/kg	164 ± 3.4					
3′,5′-AMP	50 mg/kg	196 ± 5.9	0/10	0/5	1/10	0/8	—
	100 mg/kg	264 ± 12.8					

[a] Glucose concentration 30 min after intraperitoneal injection of saline or nucleotide, 8 mice per group.

[b] No. dead/no. injected following intraperitoneal administration of a mixture of nucleotide and histamine.

cogenolysis for it is at this level a function of 5′-AMP has been proposed (8). This event is believed to be mediated by: (i) conversion of inactive phosphorylase *b* to the active form-phosphorylase *a*—with the reaction catalyzed by a 3′,5′-AMP–sensitive phosphorylase *b* kinase; (ii) conversion of phorylase *b* to phosphorylase *a* by Ca^{2+} and a kinase activating factor (10,11), and; (iii) activation of phosphorylase *b* by 5′-AMP without conversion to the *a* form (8). The latter was of interest to us since an epinephrine-induced glycogen breakdown in the skeletal muscle of certain strains of mice has been shown to occur in the presence of only negligible quantities of phosphorylase *a* (12). Thus the responsiveness of the CFW strain may rest upon the functioning of 5′-AMP in activating phosphorylase *b* whereas this system may be deficient in the CFI strain.

The histamine-sensitizing activity of 5′-AMP in the CFW strain and the absence of this effect in the CFI cannot be explained on the basis of present data. Certain comments, however, seem appropriate. Firstly, there seems to be a strain relationship in the sensitizing effect of 5′-AMP and pertussis organisms with the CFW susceptible and the CFI unresponsive to both procedures (Table I) (2). Secondly, the sensitizing effect of 5′-AMP and the possible site of its hyperglycemic action (skeletal muscle) serve to emphasize a suggestion made some time ago relative to the impairment of peripheral glucose uptake, normally influenced by muscle glycogenolysis, in the pertussis-sensitized and/or beta-adrenergically blocked mice. The impairment may result in accumulation of 5′-AMP with the development of a histamine hypersensitivity. Lastly, the pertussis-sensitized mouse has been employed as an experimental model of atopic disease (14). The effectiveness of theophylline in the therapy of these diseases is believed to be, at least in part, due to its action on phosphodiesterase suppressing the conversion of 3′,5′-AMP to 5′-AMP (9) thus prolonging the action of the cyclic nucleotide. The present results suggest an additional factor to be considered; the suppression of phosphodiesterase may be effective due to decreased "production" of the sensitizing 5′-AMP.

It is obvious that more extensive studies will be required to define the role of 5′-AMP

in the histamine-phenomenon. Studies in progress are emphasizing phosphorylase *b* as a possible site of adrenergic blockade.

Summary. The injection of 3′,5′-AMP elicited an increase in blood glucose concentration of both the CFW and CFI strains of mice and had no effect on the histamine sensitivity of either strain. Administration of 5′-AMP was followed by hyperglycemia and histamine hypersensitivity in the CFW mice; the CFI animal was unresponsive to 5′-AMP in terms of both phenomena.

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Pyelonephritis in the Mouse. III. Therapeutic Experiments (32904)

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As part of our study of pyelonephritis in mice we have investigated the therapeutic effects of agents used to treat the disease in man and have explored the possibilities of serotherapy.

Methods. The strains of *Proteus mirabilis* and *Pseudomonas aeruginosa* were those used previously as were the techniques for infection and kidney culture (1). The following therapeutic agents were tested: streptomycin, neomycin, kanamycin sulfate, nalidixic acid, 3,4-dimethyl-5-sulfanilamidoisoxazole (Gantrisin), 2-sulfanilamidopyrimidine (sulfadiazine), Ampicillin, penicillin G, polymyxin, oxytetracycline (Terramycin), D-4-amino-3-isoxazolidone (Oxamycin), and chloramphenicol (Chloromycetin). Antibiotics were given by the intraperitoneal (i.p.) or subcutaneous (s.c.) route, chemical agents by the i.p. or oral (p.o.) route. Many different schedules were tried. In some experiments chemotherapeutic treatments were begun from 1 to 5 days after i.v. infection and given for 5 or 7 days, in 2 doses on weekdays, one half daily dose on Saturdays and Sundays. In other tests a one dose i.v. treatment was administered 1 hour after challenge. Mice were sacrificed from 1 to 7 days after discontinuance of therapy. The sources of materials used in serotherapy tests were: (i) pooled serum from mice that had survived i.v. challenge with *P. mirabilis* or *Ps. aeruginosa* (MS); (ii) serum from rabbits that had received 3 i.v. doses of formalin killed organisms (RS); and (iii) commercial human gamma globulin (GG). For therapy, immune serum and GG were given by various routes in 1 or more 0.25 ml doses before or soon after i.v. challenge. Kidney cultures were done a week after infection.

Results. Chemotherapy experiments. In 67 chemotherapy tests in which duplicate sets of 10 controls were employed, the percentage of positive kidney cultures was identical 32 times, differed by 10% 25 times, and by 20%