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THE EFFECT OF ALTERED CARBOHYDRATE METABOLISM IN PERTUSSIS-SENSITIZED MICE ON ANAPHYLAXIS

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Abstract—An attenuated reponse to epinephrine-induced hyperglycemia has been demonstrated in rats injected with *Bordetella pertussis* alone and in mice sensitized with horse serum or in combination with *B. pertussis*. This response is not a reflection of reduced liver glycogen. In the mice sensitized with horse serum only, protection against anaphylaxis is afforded by some but not all hyperglycemia-inducing agents tested. Alloxan and epinephrine protect, whereas diazoxide does not. Alloxan was considerably less protective in the horse serum–pertussis-sensitized mouse. Diazoxide, glucose, and epinephrine did not protect. Prednisolone and betamethasone, at doses that protect against lethal anaphylaxis in the horse serum–pertussis-sensitized mouse, also restore epinephrine hyperglycemia in the pertussis rat. Possible mechanisms of protection of these compounds are discussed. No definite link between the carbohydrate abnormality of the pertussis animal and anaphylactic sensitivity has been established.

THE ADMINISTRATION of killed *Bordetella pertussis* organisms to mice enhances the sensitivity of the mice to the lethal effects of histamine, serotonin, and anaphylaxis.[1] Further, Szentivanyi *et al.*[2] demonstrated that pertussis treatment rendered mice hypoglycemic and attenuated epinephrine-induced hyperglycemia. A possible relationship between the hypersensitive state and altered carbohydrate metabolism has received some attention. Several investigators,[3, 4] have reported that alloxan diabetes or glucose loading reduced the anaphylactoid response to histamine liberators and, conversely, that insulin accentuates the anaphylactoid response to these liberators in normal animals. More recently, Kraus[5] demonstrated a similar protective effect of alloxan or glucose against anaphylaxis in ovomucoid-sensitized mice. In contrast to the response seen in pertussis-sensitized mice, epinephrine elicits the usual hyperglycemic response in mice sensitized to antigen (horse serum) alone, and these mice are relatively insensitive to histamine challenge.* In view of these differences, we investigated the role of altered carbohydrate metabolism in both models, in the hope of establishing a possible interrelationship of glycemia and anaphylaxis.

MATERIALS AND METHODS

Carworth Farm CFW male and female mice (20–30 g) and Charles River CD strain male rats (180–200 g) were used for these experiments. The horse serum was obtained from Microbiological Associates, Inc., Baltimore, Md., and kept frozen until used. The *B. pertussis* vaccine was supplied by Eli Lilly & Co. and contained not less than 64×10^9 organisms/ml.

* A. Gulbenkian, A. Yannell Grasso and I. I. A. Tabachnick, unpublished work.

Two methods of sensitization were employed. In the first, undiluted horse serum (HS) was used to sensitize mice. One ml was given i.p. every other day during an 8-day period. These animals were challenged i.v. with 0·2 ml of horse serum 21 days after the initial day of sensitization. Mortality was recorded at 1 hr. In the second method, 0·25 ml of a mixture of horse serum and *B. pertussis* vaccine (1:4, v/v) was administered once i.p. to mice which were challenged i.v. with 0·2 ml horse serum 10 days later. Mortality was recorded at 1 hr. Rats were sensitized by one i.p. administration of 1 ml of *B. pertussis* vaccine and were used 5 days later for glycemic studies.

Epinephrine bitartrate (2 mg/kg, calculated as the free base) was administered subcutaneously 30 min before the animals were bled. Alloxan (100 mg/kg in 0·2 ml saline) was administered i.v. 4 days prior to the use of the animals. Glucose (500 mg/kg) was administered i.v. 5 min before and diazoxide i.p. (160 mg/kg dissolved in 0·1 N NaOH, pH 10·5) 3 hr before the animals were bled for glucose determination or challenged for anaphylaxis.

Prednisolone (50 mg/kg) or betamethasone (12·5 mg/kg) was administered intramuscularly 18 hr before challenge or bleeding to mice and rats and was suspended in 0·2–0·5 ml of 1% gum acacia.

The blood for glucose determination in mice was withdrawn from the infraorbital sinus. Rats were bled from the dorsal aorta and plasma separated for glucose determination. In both cases, ether was used as the anesthetic. The Technicon Autoanalyzer was used to determine glucose levels.[6] Animals had access to food and water *ad libitum* until the time of drug treatment, with the exception of steroid therapy and alloxan treatment. These animals were allowed food and water until the morning of sacrifice. Liver glycogen were determined by a modification of the methods of Seifter and Roe.[7, 8]

RESULTS

A. Epinephrine-induced hyperglycemia. One half hour after the subcutaneous administration of epinephrine (2 mg/kg) to normal mice, a significant elevation of blood glucose was observed (Table 1). In contrast, mice sensitized 10 days earlier

TABLE 1. EFFECT OF EPINEPHRINE ON BLOOD GLUCOSE IN NORMAL AND HORSE SERUM–PERTUSSIS-SENSITIZED MICE*

Treatment		Experiment I		Experiment II	
Sensitized†	Epinephrine‡	Blood glucose (mg/100 ml)	Statistical analysis	Blood glucose (mg/100 ml)	Statistical analysis
1. No	No	146 (123–167)§	2 > 1, 3, 4 ($P < 0·05$) 1 > 3, 4	141 (94–188)	2 > 1, 3, 4 ($P < 0·05$) 1, 3, 4 NSD
2. No	Yes	296 (273–317)	3, 4 NSD	252 (205–299)	
3. Yes	No	65 (43–87)		94 (47–141)	
4. Yes	Yes	69 (46–90)		127 (80–173)	

* Five mice per treatment group per experiment.
† Sensitized with horse serum–pertussis (as described in Methods).
‡ Subcutaneously, 2 mg/kg 1/2 hr before bleeding.
§ Confidence limits, 95%.

with a combination of horse serum and pertussis vaccine were slightly hypoglycemic and epinephrine did not elevate these levels (Table 1). The subcutaneous administration of epinephrine 2 mg/kg to normal rats induced a marked hyperglycemia which was also attenuated in the pertussis-treated group (Table 2).

The attenuation of the epinephrine hyperglycemia in horse serum–pertussis-sensitized mice was observed as early as day 1 after sensitization and continued through day 14 (Fig. 1). By day 20, a normal glycemic response to epinephrine was

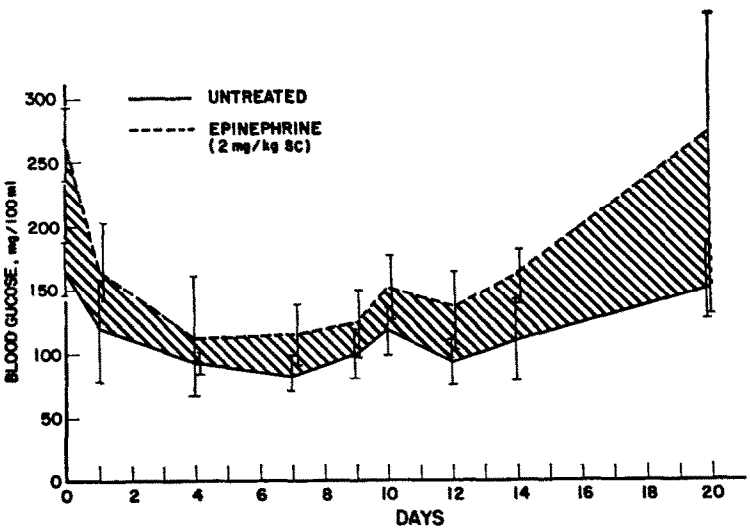

FIG. 1. The cross-hatched area indicates the increase in blood glucose induced by the administration of epinephrine at various times after pertussis sensitization. Vertical bars give standard error of the mean.

observed. The period between days 9 and 11 represented a period of maximal sensitivity to lethal anaphylaxis. Mice were also exceedingly sensitive to exogenous histamine during this period.

B. Liver glycogen. Since the attention of hyperglycemia seen in the pertussis-sensitized (PS) animals could be due to a deficiency of liver glycogen possibly resulting from reduced food intake, daily weight changes and food consumption over a 5-day period were measured in ten normal and ten PS rats. On the fifth day, five of the normal rats and five PS rats were given epinephrine (2 mg/kg s.c.) and half an hour later all the rats were bled and their glucose levels determined. After the blood was drawn, the rat was sacrificed, and a portion of its liver was removed for glycogen analysis. Table 3 reveals that the epinephrine-treated PS rats have typically attenuated hyperglycemic glucose levels, whereas the normal rats develop significant hyperglycemia in response to epinephrine. The daily weight gain of the normal rats is greater (9·2 g vs. 5·7 g) than that of the PS rats. Also normal rats consume slightly more food than PS rats. However, there is no apparent effect on glycogen levels.

C. The effects of hyperglycemic agents on anaphylaxis. The results of treatment with hyperglycemic agents in mice sensitized with either horse serum alone or with the

TABLE 2. COMPARISON OF EPINEPHRINE (EPI)-INDUCED* HYPERGLYCEMIA IN NORMAL, PERTUSSIS-TREATED† (P) AND STEROID-TREATED* PERTUSSIS RATS

Exp. no.	1. Normal	2. Normal + EPI	3. P	4. P + EPI	5. Beta-methasone + P	6. Beta-methasone + P + EPI	7. Pred-nisolone + P	8. Pred-nisolone + P + EPI	Statistical significance ($P < 0{\cdot}05$)
				(Plasma glucose mg/100 ml)					
I‡	179 (143–216)§	431 (395–468)	168 (131–205)	254 (217–290)					2 > 1, 3, 4 4 > 1, 3 1, 3 NSD
II‡	193 (124–256)	434 (365–497)	223 (154–286)	296 (227–359)	279 (210–342)	538 (469–601)			6 > 5, 4, 3, 2, 1 2 > 5, 4, 3, 1 5 > 1, 3 4 > 1, 3
III‡	183 (117–249)	442 (376–508)	172 (106–238)	268 (202–334)			230 (164–296)	396 (330–462)	2, 8 NSD 2, 8 > 1, 3, 4, 7 4 > 1, 3 1, 3, 7 NSD

* Epinephrine, 2 mg/kg, s.c. was administered 1/2 hr prior to sacrifice.
Prednisolone, 50 mg/kg, i.m. was administered 18 hr prior to sacrifice.
Betamethasone, 12·5 mg/kg, i.m. was administered 18 hr prior to sacrifice.
† Rats were treated i.p. with 1 ml of pertussis vaccine 5 days before use.
‡ Treatment group, 20–40 rats.
§ Confidence limits, 95%.

combination of horse serum and pertussis are shown in Table 4. Mean control blood glucose values for HS animals ranged from 120 to 135 mg/100 ml, and protection from lethal anaphylaxis in these control animals from 0 to 30 per cent. In contrast mean blood glucose values of control PS mice ranged from 47 to 102 mg/100 ml, but anaphylactic mortality was similar to that of HS mice.

TABLE 3. EFFECT OF PERTUSSIS SENSITIZATION (PS*) IN THE RAT ON EPINEPHRINE-INDUCED HYPERGLYCEMIA, LIVER GLYCOGEN, DAILY WEIGHT CHANGE, AND DAILY FOOD CONSUMPTION

Treatment		Plasma glucose (mg/100 ml)	Liver glycogen (mg/100 ml)	Δ Weight (g/day/rat)	Food consumed (g/day/rat)
None†	1.	191 (176–206)‡	6054 (4872–7240)	9·2 (7·8–10·6)	20·9 (15·2–26·6)
PS†	2.	173 (157–189)	5042 (4092–5992)	5·7 (4·6–6·8)	16·6 (15·3–17·9)
Epinephrine† (2 mg/kg s.c.)	3.	601 (574–628)	5309 (4274–6344)		
PS + epinephrine† (2 mg/kg s.c.)	4.	210 (182–220)	4652 (3945–5359)		
Statistical analysis	5.	3 > 1, 2, 4 P < 0·01 4 > 2 P < 0·05	NSD	1 > 2 P < 0·01	1 > 2 P < 0·01

* Sensiized with pertussis only (as described in Methods).

† Five rats per group.

‡ Confidence limits, 95%.

When *alloxan* was given to the HS mice, significantly elevated blood glucose levels were consistently seen, and an improved protection against lethality was afforded. In one of these three experiments, the degree of protection, though large, was statistically insignificant. Alloxan treatment of PS mice also resulted in hyperglycemia, but the degree of protection in these mice was less than that observed in the HS mice (Table 4, column 2).

Epinephrine caused significant blood glucose elevations in HS mice, and in all experiments, anaphylactic deaths were reduced. In PS mice, blood glucose levels were not significantly elevated nor was any protection elicited by epinephrine pretreatment (Table 4, column 3).

Glucose administration did not effect significant elevation of blood glucose levels in HS mice and gave no protection against anaphylactic mortality. Similar results were obtained in PS mice (Table 4, column 4).

Diazoxide, an antidiuretic, benzothiadiazine, and a potent hyperglycemic agent,[9] effected significant elevations in blood glucose levels in HS mice, but did not protect significantly against lethal anaphylaxis. Diazoxide was consistently less hyperglycemic in PS mice than in HS mice and did not reduce anaphylactic mortality (Table 4, column 5).

The lack of epinephrine response in horse serum–pertussis-sensitized mice strongly resembled the diminished reactivity of adrenalectomized mice to epinephrine.[3] It was therefore considered that we were observing relative adrenal insufficiency due to reduction of ACTH availability or even a direct effect on the adrenal cortex. Massive doses of ACTH (400 μg/kg) given intramuscularly to the pertussis-sensitized mouse

TABLE 4. EFFECT OF VARIOUS HYPERGLYCEMIC AGENTS ON BLOOD GLUCOSE LEVELS AND ANAPHYLAXIS IN SENSITIZED MICE

Exp. no.	Control 1.		Alloxan† 2.		Epinephrine† 3.		Glucose† 4.		Diazoxide† 5.		Statistical significance ($P < 0.05$	
	Glucose*	% Prot	Glucose	% Prot	Glucose	% Prot	Glucose	% Prot	Glucose	% Prot	Glucose	% Protection‡
					Horse serum-sensitized mice							
I	133 (112–154)§	0	471 (337–606)	90	296 (221–371)	44	182 (127–237)	20	376 (217–535)	0	2, 3, 5 > 1 1, 4 NSD	2, 3 > 1 1, 4, 5 NSD
II	120 (85–154)	12	445 (353–536)	50	288 (214–362)	60	90 (76–104)		574 (514–632)	10	2, 3, 5 > 1, 4	2, 3 > 1 1, 5 NSD
III	135 (111–158)	30	328 (186–470)	60	399 (340–478)	78	181 (118–244)	70	615 (503–727)	30	2, 3, 4 > 1 1, 4 NSD	3 > 1 1, 2, 4, 5 NSD
					Pertussis–horse serum-sensitized mice							
I	102 (87–117)	0	398 (143–652)	40	119 (109–129)	0	126 (69–184)	0	283 (197–368)	0	2, 5 > 1 1, 3, 4 NSD	1, 2, 3, 4, 5 NSD
II	58 (45–70)	0	543 (470–616)	40					410 (239–581)	0	2, 5 > 1	1, 2, 5 NSD
III	47 (29–65)	20	489 (398–580)	10	102 (75–104)	10	90 (77–104)	0	397 (342–451)	22	2, 5 > 1, 3, 4 1, 3, 4 NSD	1, 2, 3, 4, 5 NSD

* Mice per group, 9–10; % Prot indicates % protection; glucose expressed as mg/100 ml whole blood.
† Alloxan, 100 mg/kg i.v. 4 days prior to challenge or bleeding.
Glucose, 500 mg/kg i.v. 5 min prior to challenge or bleeding.
Diazoxide, 160 mg/kg i.p. 3 hr prior to challenge or bleeding.
Epinephrine, 2 mg/kg s.c. 1/2 hr prior to challenge or bleeding.
‡ The tests of significance were carried out via Fishers exact probability test.
α, The probability of the null hypothesis being false when it is indeed true, was set at 0·05.
§ Confidence limits, 95%.

18 hr before epinephrine seemed to have no effect on the inhibition of epinephrine hyperglycemia. Studies described in Table 2 showed that, in the normal rat, plasma glucose levels ranged from 179 to 193 mg/100 ml and were elevated by epinephrine administration to 431–442 mg/100 ml. Plasma glucose values for rats which received only pertussis 5 days earlier ranged between 168 and 223 mg/100 ml, and after epinephrine they ranged from 254 to 296 mg/100 ml. This system therefore seemed appropriate to test the possible relationships between adrenocorticoid activity and epinephrine sensitivity. Prednisolone itself did not cause significant hyperglycemia in the pertussis-treated rat, but when epinephrine was administered to prednisolone-treated rats, a hyperglycemic response was elicited which was of the same magnitude as that seen in normal rats given epinephrine alone (Table 2). Betamethasone caused a similar restoration of epinephrine-induced hyperglycemia in the pertussis-treated rat and, in addition, betamethasone alone elicited a significant but moderate hyperglycemia in the pertussis rat (Table 2). A somewhat similar trend is seen with both corticoids in horse serum–pertussis-sensitized mice (Table 5), but considerable inter-experiment variability was encountered. It should be noted that these doses of prednisolone and betamethasone consistently afforded almost complete protection against lethal anaphylaxis in the horse serum–pertussis-sensitized mouse.

DISCUSSION

In order to gain some insight into the mechanisms involved in anaphylaxis and to determine a possible relationship between carbohydrate metabolism and anaphylaxis, two types of sensitized mice have been treated with various hyperglycemic agents and challenged to induce anaphylactic shock.

In horse serum-sensitized mice, alloxan, epinephrine, and diazoxide elicited significant hyperglycemia, but of these three, only alloxan and epinephrine afforded protection against lethal anaphylaxis. Görög and Szporny[10] have indicated that the protective action of alloxan is a result of diminished glucose oxidation, thereby decreasing the amount of energy available for the liberation of the anaphylactic-inducing substances. Alternatively, the severe diabetic stress caused by alloxan elicits a hypersecretion from the adrenal cortex,[11] and the glucocorticoids so produced protect against lethal anaphylaxis in this system.[12]

The protective effect of epinephrine in horse serum-sensitized mice may be a reflection of carbohydrate intolerance induced by this amine (inhibition of glucose uptake or indirectly by catecholamine inhibition of insulin secretion[13]) or could be due to the prevention of the excessive dilation of blood vessels observed in anaphylaxis, thereby preventing circulatory collapse.

In contrast, diazoxide did not protect horse serum-sensitized mice from anaphylactic shock, although it causes considerable hyperglycemia. The mechanism of action of diazoxide hyperglycemia is not clear, but we have demonstrated that diazoxide potentiates the metabolic effects of the catecholamines and is blocked by inhibitors of the metabolic effects of catecholamines,[9] Further, Rubin *et al.*[14] have shown that diazoxide is hypotensive and blocks the vasoconstrictor action of a number of substances including catecholamines. Consequently, the hyperglycemic response to diazoxide, although reflecting a potentiation of normal levels of circulating catecholamines, could not exert a protective action against lethal anaphylaxis, because the vascular actions of diazoxide (hypotension by arteriole dilation and prevention of

TABLE 5. EFFECT OF STEROIDS ON EPINEPHRINE-INDUCED HYPERGLYCEMIA IN HORSE SERUM–PERTUSSIS-SENSITIZED (PS) MICE*

Exp. no.	1. PS†	2. PS + Epinephrine (EPI) (2 mg/kg)‡	3. PS + Betamethasone (12·5 mg/kg)‡	4. PS + EPI + Betamethasone	5. PS + Prednisolone (50 mg/kg)‡	6. PS + EPI + Prednisolone	Statistical analysis ($P < 0{\cdot}05$)
			(Blood glucose mg/100 ml)				
I	94 (52–136)§	146 (104–188)	133 (91–175)	225 (183–267)			4 > 3, 2, 1
II	69 (27–111)	136 (94–178)	90 (48–132)	190 (149–233)			2, 4 > 1, 4 > 3
III	65 (23–107)	69 (26–110)	91 (48–132)	147 (105–189)			4 > 1, 2, 3
IV	109 (67–151)	138 (95–179)	103 (61–145)	290 (248–332)			4 > 1, 2, 3
V	87 (45–129)	116 (74–158)			115 (73–157)	176 (134–218)	6 > 1, 6, 5, 2 NSD
VI	103 (61–145)	175 (133–277)			115 (73–157)	291 (249–333)	6, 2 > 1, 5
VII	87 (45–129)	143 (101–185)			114 (72–156)	310 (267–351)	6 > 1, 2, 5
VIII	86 (44–128)	116 (74–158)			90 (48–132)	178 (136–220)	6 > 1, 5, 2
IX	65 (23–107)	69 (27–111)			65 (23–107)	118 (76–160)	1, 2, 5, 6 NSD

* Five mice per treatment group per experiment.
† Sensitized with horse serum and pertussis (as described in Methods).
‡ Consult text for routes and times of administration.
§ Confidence limits, 95%.

vasoconstriction by various agonists including catecholamines) would enhance and not antagonize the challenge-induced vascular collapse.

In contrast, the influence of hyperglycemia on anaphylactic mortality in pertussis-sensitized mice was less pronounced than that seen in the mice sensitized with horse serum alone. Epinephrine did not protect these mice from the lethal effects of systemic anaphylaxis and was not hyperglycemic. Although both diazoxide and alloxan produced significant hyperglycemia, diazoxide did not protect and alloxan only mildly protected the PS mice against lethal anaphylaxis.

The reduction of protection observed in the PS mice compared with the HS mice may reflect increased cellular permeability resulting from pertussis sensitization.[15, 16] Indeed, the administration of pertussis vaccine as an adjuvant leads to marked alterations in tissue permeability and facilitates subsequent vascular collapse upon challenge with the appropriate antigen or amine (histamine or serotonin).[1] The lack of efficacy of epinephrine in the PS mice was not surprising. Bergman and Munoz[17] have shown that it is difficult to protect mice with epinephrine against the circulatory failure induced by histamine when these mice have been treated with the "histamine-sensitizing factor" of *Bordetella pertussis*.

Our liver glycogen studies, which confirm the earlier work of Fishel and Szentivanyi,[18] seem to exclude lack of liver glycogen as a factor in the attenuation of epinephrine-induced hyperglycemia in the PS animal.

The ability of prednisolone and betamethasone to restore epinephrine-induced hyperglycemia in this animal, therefore, does not reflect the need for increased glycogen deposits but rather supports the concept that pertussis sensitization results in a relative adrenal insufficiency,[1] by either decreasing secretion or increasing tissue requirements for steroid in the animal.[19] Ramey and Goldstein[20] have suggested that catecholamine efficacy is directly related to the presence of corticoids, and in their absence the receptor threshold for catecholamines increases. Since Bergman and Munoz[21] have suggested that the primary cause of death in mouse anaphylaxis is circulatory failure, it is also conceivable that the actions of these steroids on survival might result from their effects on the microcirculatory system[22] by counteracting the vasodilatation induced in anaphylaxis.

Fishel and Szentivanyi[18] have demonstrated that pertussis animals resemble mice which have been treated with a β-adrenergic receptor blocking agent in that they are sensitive to histamine and show attenuated epinephrine hyperglycemia. However, diazoxide potentiates the hyperglycemic response to exogenous epinephrine,* but yields no significant protection against anaphylactic challenge. The apparent lack of correlation between hyperglycemia and protection in diazoxide-treated mice may reflect (as discussed above) a synergizing or summing of the hypotensive and anti-vasoconstrictor properties of diazoxide with the vascular collpase seen in anaphylactic shock. Consequently, although hyperglycemia is being elicited, the concomitant but possibly independent pharmacological actions of diazoxide exacerbate rather than prevent the circulatory failure.

While no clear relationship has yet been established between the carbohydrate abnormality of the pertussis animal and anaphylactic sensitivity, two factors are apparent which tend to correlate the two events: both conditions prevail simultaneously and those steroids that protect against anaphylaxis restore epinephrine-induced

* I. I. A. Tabachnick, A. Gulbenkian and A. Yannell Grasso, unpublished work.

hyperglycemia. Not to be overlooked, however, is the possibility that a state of hyperinsulinism exists in the pertussis-treated animal. Insulin has been shown to exacerbate sensitivity to anaphylactic challenge in sensitized mice[5] and to increase cellular permeability.[23] In addition, pertussis mice have low blood glucose levels and depressed response to glucose loading as well as their lack of epinephrine-induced hyperglycemia. This provocative aspect is currently being explored.

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