Hypoglycemia and Its Relationship to Histamine Sensitization in Mice (33800)

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The mechanism by which extracts or whole cell preparations of *Bordetella pertussis* induces histamine hypersensitivity in certain strains of mice is not well understood. Considerable evidence indicates that some sort of blockade against the adrenergic nervous system is produced (1–4), but the mechanism is not clear. Mice treated with *B. pertussis* cells become hypoglycemic (5, 6) and do not become hyperglycemic when treated with catecholamines (7). In addition, it has been reported that mobilization of free fatty acids *in vivo* and *in vitro* is suppressed in tissues of *B. pertussis*-treated mice (4). Whether or not a direct cause-and-effect relationship exists between these metabolic effects and the histamine sensitizing effects of *B. pertussis* is still unanswered. Gözsy and Kátó (8) suggested that the hypoglycemia produced in mice by *B. pertussis* is responsible for their hypersensitivity to histamine and serotonin and for anaphylactoid and anaphylactic shock.

The results reported in this paper do not support the view that hypoglycemia, *per se*, is responsible for histamine sensitivity.

Materials and Methods. Mice. Female CFW mice (obtained from Carworth Farms, New City, N. Y.) were housed in glass jars (5 mice/jar) with beet pulp bedding and were fed (Purina laboratory chow) and watered *ad libitum*.

Drugs and Chemicals. Bovine insulin (B grade, 25.0 units/mg) from Calbiochem and *d*-mannoheptulose (a short duration diabetes inducer) from Mann Research Labs, Inc. were used. Histamine was given as histamine diphosphate (Nutritional Biochemicals Corporation), but all doses of histamine are expressed as the free base. The desired concentrations of drugs were prepared in physiological saline.

Histamine sensitizing factor from B. pertussis. Saline extract (SE) of *B. pertussis* cells was prepared as previously described (9). The SE-treated mice received 20 μg of the lyophilized material intravenously (iv) in 0.2 ml of saline. Control mice received only saline.

Histamine challenge. Sensitivity of mice to histamine was based on number of deaths recorded within 2–3 hr after administering 0.5 mg histamine in 0.2 ml physiological saline intraperitoneally (ip).

Serum glucose tests. All mice were fasted 5 hr before blood was drawn for serum glucose determinations. Blood was obtained from the infra-orbital sinus by means of a Caraway micro tube (Clay-Adams) and allowed to clot for approximately 15 min. Within 30 min after collection, each blood sample was centrifuged in a Clay-Adams microhematocrit centrifuge and 0.1 ml of serum was removed and stored in a 100-μl diSPo capillary pipet (Scientific Products). Serum samples were stored at −15°.

Serum glucose was determined by the glucose oxidase method utilizing Glucostat reagent. Method II, as described in the complete instructions which are included with each order of Glucostat from Worthington Biochemical Corp., Freehold, N. J. was used with the modification, which they describe, of allowing the reaction to go to virtual completion by incubating for 30 min at 37°.

Results. The hypothesis that hypoglycemia might be responsible for histamine sensitivity stimulated an initial experiment to see if SE-treated mice could be protected from histamine shock by exogenous glucose. The SE-treated mice received three injections of 15 mg of *d*-glucose at hourly intervals. The first injection was given ip; the second, subcutaneously (sc) and the third again ip.

Thirty min later the mice were challenged ip with histamine. In a preliminary experiment, some protection by glucose treatment was obtained, but in subsequent more comprehensive experiments there was no evidence of protection even when the sensitizing dose of SE was reduced to a minimal level of 5 μg/mouse.

The relationship between onset and duration of hypoglycemia and of histamine hypersensitivity was next determined. There was a pronounced sensitivity to histamine from 60 min to 21 days after SE injection. In previous work (10), in which we determined histamine LD_{50} values, increased sensitivity could still be detected 84 days after SE administration. Hypoglycemia was not apparent until 120 min after SE treatment (Fig. 1). The serum glucose level remained below normal up to 21 days after SE injection, but in further work (not shown in Fig. 1) the serum glucose level was normal or possibly even above normal at 42 days.

Exogenously administered glucose produces an elevation of blood glucose levels in normal mice. In SE-treated mice, exogenous glucose, (0.5 mg/g of body wt) when administered from 30 to 120 min after SE treatment,

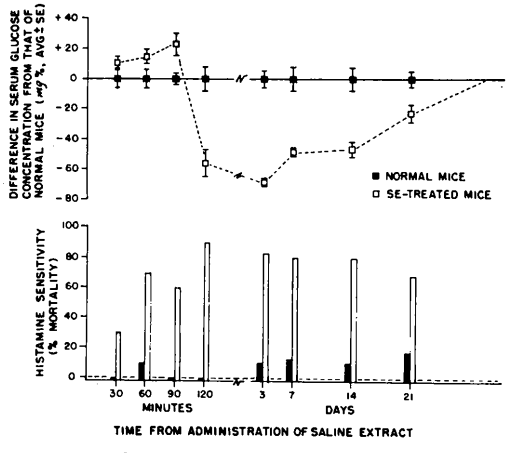

FIG. 1. Relationship between onset and duration of hypoglycemia and histamine hypersensitivity in saline extract (SE) treated mice. The serum glucose values of the experimental mice are compared to those of normal mice set at a "zero" baseline. Normal mice had a serum glucose value of 184 ± 5.8 (av ± standard error) mg/100 ml of serum during the experiment.

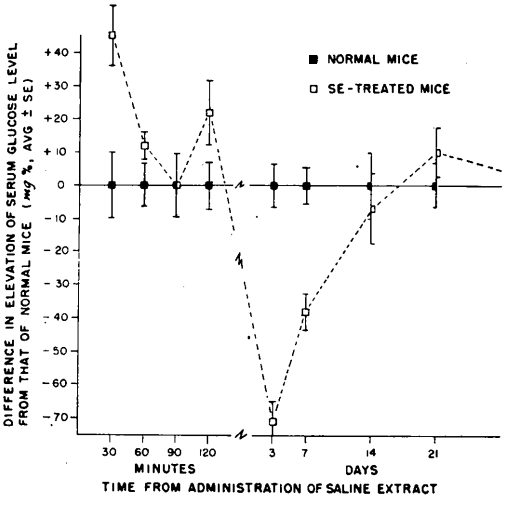

FIG. 2. Comparison of the elevation in serum glucose levels of saline extract (SE) treated mice with that of normal mice after iv administration of exogenous glucose (0.5 mg/g of body wt) at various time intervals after administration of saline extract. The elevation which occurred in normal mice was arbitrarily expressed as zero and the degree of serum glucose rise which occurred in the SE-treated mice was expressed in relation to this base line. The serum sugar rise which occurred in the normal mice after administration of exogenous glucose was 92 ± 3.5 (av ± SE) mg/100 ml of serum.

also produced an elevation of blood glucose. The ability of exogenous glucose to produce hyperglycemia as compared to the response in normal mice was significantly diminished when the glucose was administered 3 or 7 days after the SE treatment. At 14 and 21 days after the SE treatment, the ability of exogenous glucose to produce a rise in serum glucose was essentially restored over its diminished effects at 3 and 7 days (Fig. 2).

The effect of insulin-induced hypoglycemia on histamine sensitivity in mice was compared with that which results from SE treatment. The SE-treated mice received 20 μg of SE iv on the day before histamine challenge or being bled for serum glucose determinations, but only 30 min was allowed to elapse between the ip administration of various doses of insulin and the subsequent blood collection or histamine challenge. Insulin-induced hypoglycemia, equal to or considerably greater than that which resulted from

SE treatment, did not produce histamine sensitivity. Administration of 0.016 units of insulin to mice produced a serum glucose depression 4.1 times greater than that which occurred in SE-treated mice without inducing an increased sensitivity to histamine (Table I). When a profound hypoglycemia was produced by larger doses of insulin (0.04, 0.08, or 0.16 units/mouse), the mice developed hypoglycemic shock and some died before histamine challenge could be given. The surviving mice challenged with histamine died (Table I). These mice apparently were not able to withstand the double insult of these two agents which produce "shock" by separate pathways.

TABLE I. Comparison of Histamine Sensitivity in Mice Which Have Been Made Hypoglycemic with SE or Various Doses of Insulin.[a]

Mice	Insulin treatment (units/mouse)	Serum glucose depression below normal mouse levels (mg/100 ml of serum)[b]	Histamine sensitivity (D/T)[c]
Normal	None	0	0/20
SE treated	None	—19	16/19
Normal	0.004	—22	0/10
Normal	0.008	—34	0/10
Normal	0.016	—78	0/10
Normal	0.04	—112	7/10
Normal	0.08	—119	7/9
Normal	0.16	—128	5/5

[a] SE was given iv 1 day before, and insulin doses ip, 30 min before obtaining blood samples or giving histamine challenge.

[b] Differences between mean serum glucose values of various groups as indicated and "normal" control mice. The control group and SE-treated group contained 20 mice each and the insulin-treated groups had 10 mice each.

[c] D/T = deaths/number of mice tested.

The fact that mice can be sensitive to histamine even when their serum glucose level is considerably elevated was demonstrated with the short-term diabetogenic agent, *d*-mannoheptulose. Mice were sensitized with 20 μg of SE given iv and the following day 40 mg of *d*-mannoheptulose/-mouse was given subcutaneously 2 hr before histamine challenge or obtaining blood samples. The *d*-mannoheptulose given to SE-treated mice produced a serum glucose level considerably above that of normal mice without interfering with the histamine sensitivity produced by SE (Table II).

Discussion. Hypoglycemia in mice after treatment with whole cell preparations of *B. pertussis* is a well-documented phenomenon (5, 6, 11). Our results reported here show that semipurified extracts of *B. pertussis* cells also depress serum glucose levels. The significance of this hypoglycemia with respect to the histamine sensitivity which develops in mice after *B. pertussis* treatment is not clear. Ganley (12) found that alloxan diabetes protected *B. pertussis*-treated mice against histamine challenge; suggesting that histamine sensitivity might be directly linked to depressed blood glucose levels. This was apparently verified when he demonstrated that the protection induced by alloxan could be reversed by insulin (12). Gözsy and Kátó

TABLE II. Persistence of Histamine Sensitivity in SE-Treated Mice Made Hyperglycemic with *d*-Mannoheptulose.[a]

SE treatment (μg/mouse, iv)	*d*-Mannoheptulose (mg/mouse, sc)	Serum glucose, compared to normal mice (mg/100 ml of serum)[b]	Histamine sensitivity (D/T)[c]
20	none	—19	9/10
20	40	+58	9/10

[a] SE was given iv 1 day before, and *d*-mannoheptulose, subcutaneously, 2 hr before obtaining blood samples or giving histamine challenge.

[b] Differences between mean serum glucose values of the two groups of 10 mice each as indicated and "normal" control mice.

[c] D/T = deaths/number of mice tested.

(8) also proposed that the hypoglycemia which occurs after *B. pertussis* treatment of mice was sufficient to explain the increased sensitivity to histamine and serotonin.

On the contrary, there are experimental observations described in the literature, as well as those presented in this paper, which argue against hypoglycemia, *per se,* as being responsible for histamine sensitivity in mice. Stronk and Pittman (6) noted that a toxic pertussis vaccine produced a greater hypoglycemia than did a detoxified vaccine, but its histamine sensitizing ability was not greater. Fishel and Szentivanyi (7) were unable to protect *B. pertussis*-treated mice against histamine challenge by giving 45 mg of glucose to mice during a 2.5-hr interval preceding histamine challenge. We confirmed their results and were unable to detect any degree of protection by administration of 45 mg of glucose even when minimal amounts of SE were used.

Some parallelism exists in the temporal relationship between hypoglycemia and histamine sensitivity, but histamine sensitivity appears earlier and persists longer than hypoglycemia. The hypothesis that histamine sensitivity is a result of hypoglycemia, *per se,* was weakened by the observation that an insulin induced hypoglycemia of equal or somewhat greater severity to that produced by SE treatment did not result in histamine sensitivity. Histamine challenge given after insulin doses which produced profound hypoglycemic shock did increase the mortality. However, this was probably due to an additive effect of these two "shock-inducing" substances rather than to an augmented histamine sensitivity.

The hypothesis that alloxan treatment protects *B. pertussis* mice from histamine challenge by its diabetogenic action and subsequent elevation of blood glucose levels is weakened by the results reported here for the short-term diabetogenic agent, *d*-mannoheptulose. This agent produced a definite rise in serum glucose levels of SE-treated mice, without inducing protection against the lethal effects of histamine. We do not have an explanation for the disparity in the results obtained with the two diabetogenic agents, but it seems that one must look beyond an explanation based only on differences in blood sugar levels to explain increased histamine sensitivity. Substances in *B. pertussis* apparently induce changes in cells or tissues which modify their metabolism or functional reactivity and these changes are not quickly rectified by restoring or elevating blood sugar levels. It seems that histamine sensitivity and hypoglycemia are two separate, coexistent phenomena which occur in the mouse after treatment with fractions or whole cell preparations of *B. pertussis* without having a "cause-and-effect" relationship. Substances from *B. pertussis* do indeed block part of the adrenergic nervous system and produce striking changes in carbohydrate and fat metabolism (1, 2, 4). Physiological manifestations of these changes are probably exhibited in numerous ways, thus making it very difficult to determine whether *B. pertussis* substances produce these effects by a single mechanism or by a number of different pathways.

Summary. Hypoglycemia, *per se,* does not seem to explain the histamine sensitivity which occurs in mice after treatment with fractions or whole cell preparations of *Bordetella pertussis.* Onset and duration of histamine sensitivity were earlier and longer lasting, respectively, than those of the hypoglycemia which resulted from treatment with an alkaline saline extract (SE) of *B. pertussis.* Insulin-induced hypoglycemia which was equal to or even 4.1 times greater than that resulting from SE treatment did not sensitize mice to histamine. The SE-treated mice made hyperglycemic with *d*-mannoheptulose, a short-term diabetogenic agent, were not protected from the lethal effects of histamine challenge.

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Enhancement of Net Sodium Transport in Isolated Bullfrog Intestine by Sugars and Amino Acids (33801)

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(Introduced by E. E. Selkurt)

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Many examples of coupling between the transport of sodium and that of other solutes, including sugars and amino acids, by mammalian small intestine have been reported (1–3). As yet, however, the nature of the mechanisms involved in these coupling phenomena remains largely obscure (3). Recent studies have suggested that the isolated small intestine of the bullfrog is a particularly useful model system for the *in vitro* investigation of coupled transfer processes. Levin (4) has shown that this preparation maintains a remarkably stable transmural potential difference (pd) for several hours *in vitro*, both in chloride and in sulfate media, that the potentials developed under these conditions are increased by actively transported sugars and amino acids, and that the effects of sugars and amino acids on the transmural pd are additive. These results have been confirmed and extended (5). In addition, it was shown that the effects of sugars and amino acids on transmural pd are paralleled by equivalent increases in short circuit current (i.e., these substances do not cause any significant change in tissue resistance). In sulfate media, short circuit current was found to correspond to net sodium transport (5, 6) so that, under these conditions, measurements of short circuit current can be used to estimate the net rate of sodium transfer and the effects on it of added solutes. The present paper reports the results of a preliminary survey of the effects of a number of sugars and amino acids on the short circuit current across the isolated small intestine of the bullfrog, under conditions where this current corresponds to net sodium movement.

Materials and Methods. Adult bullfrogs were used in this investigation. The animals were stunned by a blow on the head, the abdomen was opened, and a segment of intestine either immediately proximal or distal to the hepato-pancreatic duct was removed rapidly and transferred to oxygenated Ringer's solution. The segment was opened lengthwise and mounted between the two halves of a conventional Ussing chamber (7) having a circular aperture with an area of 0.33 cm^2 between them. Both sides of the chamber were filled with equal volumes of identical Ringer's solutions. The chamber contents were maintained at 26 $\pm$ 0.2° and continuous circulation and oxygenation of the solutions in both halves of the chamber during the experiment were effected as described by Ussing and Zerahn (7).

The bathing solutions were phosphate Ringer's of the type described by Adrian (8)

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