Bordetella pertussis Does Not Induce β-Adrenergic Blockade

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Bordetella pertussis organisms induce histamine sensitivity and diminish the normal hyperglycemic response to epinephrine in experimental animals. These effects have been attributed to β-adrenergic blockade. However, under conditions in which the decrease in epinephrine-induced hyperglycemia after B. pertussis administration was demonstrable, there was no change in rat reticuloocyte β-adrenergic receptor number or affinity measured by iodohydroxybenzylpindolol binding or in isoproterenol-stimulated adenylate cyclase activity. Therefore, there was no generalized β-adrenergic blockade induced by B. pertussis. The observed effects can be explained by the hypersecretion of insulin resulting from B. pertussis administration.

Administration of Bordetella pertussis organisms, or a protein from the supernatant culture medium, to rats or mice causes a decrease in the hyperglycemic response to epinephrine and an increase in sensitivity to the lethal effects of histamine (9, 11, 15, 16, 21). Because both of these effects are mimicked by β-adrenergic antagonists, it has been postulated that “the pertussis organism possesses, or causes the host to elaborate a specific substance with a steric configuration complementary to the β-adrenergic receptors,” resulting in a generalized and sustained β-adrenergic blockade (20). Both experimental administration of B. pertussis and clinical whooping cough are reported to cause β-adrenergic blockade (2, 10).

Since the development of high-affinity specific ligands such as iodohydroxybenzylpindolol (IHYP) (1) and dihydroalprenolol (12), it has become possible to evaluate “β blockade” directly by measurement of β-adrenergic receptor number and affinity. In the present study, IHYP binding and catecholamine-responsive adenylate cyclase were measured in reticuloocyte membranes from rats given B. pertussis vaccine. The data show that under conditions in which the characteristic decrease in epinephrine-induced hyperglycemia is demonstrable, there is no evidence for blockade of the rat reticuloocyte β receptor.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Taconic Farms, Inc., Germantown, N.Y.), weighing 250 ± 40 g, were treated according to the protocol in Table 1. B. pertussis organisms were given in the form of whole-cell B. pertussis vaccine (Bureau of Biologics lot 7b) which was detoxified by mild heating and contained thimerosal (1:10,000). Each animal received a single intraperitoneal injection of 0.5 ml containing approximately 2.4 × 10¹⁰ organisms at the time indicated. Soluble material from B. pertussis vaccine was prepared by sonic disruption of Lot 7b vaccine (30-s pulse × 6 at an output setting of 50, with a Sonifer Cell Disruptor, model W140D, Heat Systems-Ultrasonics, Inc., Plainview, N.Y.). Disrupted cell fragments were removed by centrifugation at 100,000 × g for 40 min. The supernatant material was evaluated for: (i) ability to reproduce untreated vaccine effects on hyperglycemic response to epinephrine; and (ii) effect of IHYP binding to reticuloocyte membranes in vitro.

Hemolysis was induced in groups III, IV, and V by intramuscular injection of 1-aceetyl-2-phenylhydrazine (APH; Sigma Chemical Co.) on days 2, 3, and 4. This treatment resulted in reticuloysis of >90% in each group. Each treatment group (Table 1) was subdivided before sacrifice. Half of each group (five to seven animals) was used to obtain reticuloocytes and determine basal serum glucose (hexokinase method, Statzyme reagents, Worthington Diagnostics, Freehold, N.J.). The other half was challenged with 0.5 ml of 1-epinephrine bitartrate (Sigma) subcutaneously 30 min before sacrifice to determine the response of serum glucose to epinephrine (Table 2). Preparation of reticuloocyte membranes, measurement of specific IHYP binding, and assay of adenylate cyclase were performed as previously described (3). The specificity and reversibility of IHYP binding and the effects of β-adrenergic agonists and antagonists were established for the rat reticuloocyte system in that study (3).

RESULTS AND DISCUSSION

Control animals (group I) responded normally to epinephrine, with an 81% increase in serum glucose (Table 2). The APH-treated, unvaccinated animals (group III) also showed a normal
response (98% increase). Vaccination alone caused the expected blunting of hyperglycemia after epinephrine (35% increase; different from control, \( P < 0.005 \)). When vaccine was given either before (group IV) or after (group V) \( \beta \)-adrenergic blockade, the glycemic response to epinephrine was completely eliminated. The reason for increased vaccine effect in the hemolyzed animals is not known, but it is apparent from the response of group III that \( \beta \)-adrenergic receptor alone has no effect on epinephrine responsiveness.

Because the hemolysis did not interfere with the expected effects of pertussis administration and because the development of reticulocytosis was not affected by pertussis treatment, this model system was considered a reasonable one in which to examine pertussis effects on the \( \beta \) receptor and adenylate cyclase. Also, the rat reticulocyte \( \beta \)-adrenergic receptor has the characteristics of a \( \beta_2 \) receptor (3), which is the same as that in liver, bronchi, and lymphocytes, the targets of the apparent blocking effects of pertussis in vitro (10) and in clinical whooping cough (2). The generation of a massive reticulocytosis after \( \beta \)-adrenergic blockade allowed the study of the effect of pertussis vaccination on the \( \beta \) receptor under two different conditions. In group IV, the pertussis vaccine was administered just before hemolysis, and thus before the production, differentiation, and release of the reticulocytes into the circulation. Group V animals received the pertussis dose after hemolysis, near the peak of reticulocytosis. Because kinetic data indicate that reticulocytes remain in the circulation for 3 days during a massive response such as this (6), the cells from group V animals which were circulating at the time of vaccination were essentially the same cells as those collected for membrane preparation and assay. By studying reticulocytes generated both before (group V) and after (group IV) vaccination, we would detect any potential \( \beta \)-blocking effects of \( B. pertussis \).

As shown in Fig. 1, there was no difference in the ability of isoproterenol to displace specifically bound (3) \( ^{125}\)I-HYP from membranes of control versus vaccinated animals. There was also no reduction in specific \( ^{125}\)I-HYP binding sites in the membranes from the two groups of vaccinated animals as compared with unvaccinated (group IV, 49 fmol/mg of protein; group V, 44 fmol/mg of protein versus group III, 33 fmol/mg of protein). Similarly, the activity of adenylate cyclase and the response to isoproterenol were the same for reticulocyte membranes from control and pertussis-treated animals (Fig. 2). Thus, both the affinity and number of reticulocyte \( \beta \)-adrenergic receptors as measured by direct binding studies and the adenylate cyclase response to isoproterenol are unaltered in rats receiving \( B. pertussis \).

To evaluate the direct effect of a soluble extract of pertussis vaccine on I-HYP binding, the whole-cell vaccine was disrupted by sonic treatment, and the supernatant (100,000 \( \times \) g) material was used for study. The extract from 2.4 \( \times \) 10\(^9\) organisms produced the same effect on the glucose response to epinephrine as did the whole vaccine (Table 3). The vaccine extract was then assayed for its ability to alter I-HYP binding. As shown in Table 4, pertussis vaccine extract had no effect on I-HYP binding in vitro in either the absence or presence of isoproterenol or excess unlabelled HYP.

These findings, that \( B. pertussis \) does not cause generalized \( \beta \)-adrenergic blockade, in vivo or in vitro, make it imperative that we reexamine the evidence upon which that hypothesis was based. One of the arguments for \( \beta \)-blockade was based on the observation that the response of adenylate cyclase activity or cyclic AMP accumulation to isoproterenol is diminished in cer-

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood glucose (mg/dl ( \pm ) SEM)</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Basal 126 ( \pm ) 6</td>
<td>228 ( \pm ) 15</td>
</tr>
<tr>
<td>II</td>
<td>119 ( \pm ) 5</td>
<td>161 ( \pm ) 14</td>
</tr>
<tr>
<td>III</td>
<td>117 ( \pm ) 5</td>
<td>232 ( \pm ) 11</td>
</tr>
<tr>
<td>IV</td>
<td>120 ( \pm ) 4</td>
<td>108 ( \pm ) 10</td>
</tr>
<tr>
<td>V</td>
<td>122 ( \pm ) 8</td>
<td>115 ( \pm ) 30</td>
</tr>
</tbody>
</table>

* Sprague-Dawley male rats were treated according to the protocol in Table 1. Food was removed 16 h before sacrifice on day 8. Half of each group (five to seven animals) were given 0.5 mg of epinephrine bitartrate subcutaneously 30 min before sacrifice. Blood was removed for glucose assay by cardiac puncture. Glucose was assayed by the hexokinase method. SEM, Standard error of the mean.

b Different from group I, \( P < 0.005 \).
tain tissues by B. pertussis (14, 17). However, this decrease was also demonstrable in the response to PGE, and fluoride, and hence is not an effect at the β-receptor level, but rather at some distal site (14, 17). In addition, our study with rat reticulocyte membranes and other studies (7, 13) show that this altered response is not generalized, but is restricted to certain tissues.

The other major studies proposing pertussis-induced β-adrenergic blockade were based on the initial observation that epinephrine-induced hyperglycemia is decreased or absent after B. pertussis treatment (20). It was subsequently discovered that pertussis-vaccinated animals have augmented glucose tolerance with increased peripheral utilization, enhanced glycogen deposition, and decreased free-fatty acid release in response to epinephrine (4). Children with clinical whooping cough also have a decreased blood sugar response to subcutaneous epinephrine (2). Despite the fact that each of these effects could also be explained by hyperinsulinemia, it was concluded that the phenomena were due to β blockade with loss of the β-
adrenergic antagonism to insulin action (4).

It is now known, however, that B. pertussis administration is associated with profound alterations in the response of insulin secretion to assorted secretagogues (5, 8, 19). Normally, epinephrine has a predominantly a-adrenergic effect, inhibiting insulin secretion and lowering circulating insulin levels (5, 8, 18, 19). In contrast, rats or mice given B. pertussis vaccine develop marked hyperinsulinemia and respond to epinephrine with a further increase in circulating insulin levels (5, 8, 18, 19). Perspertussis treatment also enhances insulin secretion in response to glucose, isoproterenol, isobutyl methylxanthine and arginine (8). Because our present results rule out generalized beta-adrenergic blockade, the effects on glucose metabolism induced by B. pertussis treatment are best explained by this enhanced insulin secretion. It is not yet clear whether the active component from B. pertussis exerts its effect directly on the beta cell of the pancreas or whether the hyperinsulinemia and enhanced secretion represent secondary responses. In any case, these data illustrate that adrenergic responses cannot be reliably interpreted simply on the basis of effects on blood glucose.

**LITERATURE CITED**


