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Mechanisms of Oral Staphylococcal Enterotoxin B-Induced Emeti

in the Monkey

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Introduction. Staphylococcal enterotoxin B (SEB), isolated from cultures of Staphylococcus aureus, produces symptoms of food poisoning in man and other primates (1-3). Purified SEB (> 99%) prepared by the method of Schantz et al. (4) induces vomiting and diarrhea in monkeys when given either orally or intravenously (iv) (1, 3). Although considerable effort has been expended on attempts to clarify the pathogenesis whereby oral administration of SEB induces vomiting, the physiological mechanisms still remain obscure.

When crude staphylococcal enterotoxin filtrates were administered iv or intraperitoneally (ip) to cats, Bayliss (5) concluded that emesis was induced through a peripheral action involving the vagal nerves. However, oral administration and intraventricular application of the enterotoxin filtrate failed to induce emesis. A subsequent study by Clark et al. (6) with a more purified enterotoxin confirmed the findings of Bayliss (5). Sugiyama and coworkers (7, 8) demonstrated that denervation of abdominal viscera by sympathectomy and abdominal vagotomy suppressed vomiting in monkeys after oral or iv challenge with SEB. They believed that a neural mechanism played a role in SEB-induced vomiting. Although these investigators showed that injection of enterotoxin into the fourth ventricle did not produce vomition, destruction of the area postrema gave complete protection from both oral and iv toxin administration. Thus, the question could be raised as to whether the vomiting reflex caused by oral SEB resulted from a direct action of absorbed enterotoxin on the vomiting center, indirectly by the action of some secondary humoral mediating substance released into the circulation, or from a neural stimulus to the vomiting center originating within the gastrointestinal tract.

Cross-circulated pairs of rhesus monkeys were chosen as an experimental model to investigate the mechanism for the emetic response to orally administered SEB. During cross-circulation, Evans blue dye was employed to evaluate the exchange and distribution of blood between the pair. Blood pH, Pco2, Po2, hematocrit, and plasma osmolality were determined at selected intervals throughout the acute study. SEB hemagglutinating antibody (HA) titers were followed subsequently for a 6-wk period in all monkeys (9).

Method. Healthy rhesus monkeys, Macaca mulatta, of both sexes, weighing 3.0-4.5 kg, were used. Members of each pair demonstrated negative SEB hemagglutination titers (9) prior to study and were of approximately equal weight. The animals were tranquilized with 20 mg of ketamine hydrochloride intramuscularly (im), premedicated with atropine (0.04 mg/kg, im), and anesthetized with halothane (0.5-1.5%) vaporized during oxygen inhalation. Unilateral femoral artery and vein catheters were placed in each monkey using Teflon vessel tips (Estracorporeal) to which medical silicon tubing was attached according to the method of Chapple et al. (10). Monkeys were heparinized immediately following surgery (200 units iv, 700 units subcutaneously) and restrained in chairs for recovery. Food and water were maintained ad lib.

Twenty-four hours after surgery, blood samples were taken for hematocrit determinations, and monkeys were cross-circulated by femoral artery to femoral vein intercon-
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Results. Monkeys that vomited after receiving SEB exhibited signs of uneasiness and abdominal discomfort prior to vomition and appeared listless and relatively unaware of usual environmental distractions following the vomition period. On the other hand, paired controls that did not vomit remained alert and aggressive. Vomiting occurred in all recipients of intragastric SEB (Table 1), but only in one of the monkeys receiving saline. This particular monkey in pair 1 (Table 1) that vomited at 191 min did so without typical premonitory or postvomiting signs. The mean time for the first episode of vomition was 112 min (range, 55–270 min). The mean frequency of vomiting was 3.4 times per oral SEB-recipient monkey over the 5-hr period of observation. Vomiting occurred in the iv SEB-challenged pair in both the saline control and toxin-challenged monkeys at 50 and 95 min, respectively. Although plasma concentrations of Evans blue decreased as a function of time in all monkeys, values were essentially the same for both paired monkeys at any given time (Table II), indicating that there was a complete mixing of blood between the two.

No significant changes were observed in plasma osmolality, blood pH, $P_{CO_2}$, $P_{O_2}$, or hematocrit values during the 5-hr period in any monkey (Fig. 1). Four of five oral SEB-recipient monkeys developed HA titers within 6 wk after toxin challenge, while none of their saline-recipient pairmates developed an antibody response to SEB (Table 1). In contrast, both monkeys from the iv-challenged pair developed titers by 6 wk postchallenge. The marked HA response in the SEB recipient of pair 4 at 2 wk was unusual.

<table>
<thead>
<tr>
<th>Pair</th>
<th>Vomition time (min post-SEB)</th>
<th>Maximum hemagglutination titer (wk post-SEB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>SEB recipient</td>
<td>NaCl control</td>
</tr>
<tr>
<td>1</td>
<td>55, 70, 80, 105</td>
<td>191</td>
</tr>
<tr>
<td>2</td>
<td>75, 85, 97, 110</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>95, 110</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>65, 80, 85, 100, 103, 130</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>270</td>
<td>0</td>
</tr>
<tr>
<td>iv</td>
<td>95</td>
<td>50, 60, 80</td>
</tr>
</tbody>
</table>
TABLE II. THE RATIO OF PLASMA EVANS BLUE CONCENTRATIONS EXPRESSED AS SALINE CONTROL/SEB RECIPIENT.

<table>
<thead>
<tr>
<th>Pair</th>
<th>Hour</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.00</td>
<td>0.98</td>
<td>1.00</td>
<td>0.96</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.01</td>
<td>1.01</td>
<td>0.96</td>
<td>0.93</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.81</td>
<td>1.01</td>
<td>1.04</td>
<td>1.02</td>
<td>1.03</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.14</td>
<td>0.99</td>
<td>1.00</td>
<td>0.99</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.99</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SEB vomited consistently, while their cross-circulated pairmates generally remained unaffected. Apparently, the massive dose of oral SEB (1 mg/kg) received by one of each pair of monkeys failed to be absorbed in sufficient quantity to induce vomition in the saline pairmate by any possible mechanism.

The absence of an SEB hemagglutinin response in saline-fed pairmates of oral SEB recipients provided additional immunological evidence suggesting that during the 5-hr cross-circulation period, little, if any, fully antigenic SEB or SEB fragment was transferred from the challenged monkey. This observation lends further support to the hypothesis that SEB was not readily or completely absorbed from the gut. SEB antigen evidently does not enter the general circulation during the first 5-hr postadministration period. Some may have been absorbed at a later time in sufficient quantity to initiate an immunological response in the recipient monkey alone. In contrast, a small iv dose of SEB (5μg/kg) was capable of producing emesis and eliciting an SEB-antibody response in both members of a cross-circulated monkey pair, demonstrating the effectiveness of a minute quantity of SEB transmitted in the circulation.

Sugiyama and Hayama demonstrated that complete protection from emesis due to oral or iv challenge of SEB was obtained in monkeys through complete deafferentation of the abdominal viscera (7). Sympathectomy alone did not modify the emetic response, while vagotomy alone prevented vomition in animals subjected to oral challenge. These investigators also demonstrated that surgical destruction of the area postrema (chemoreceptor trigger zone [CTZ]) gave a complete refractoriness against oral and iv enterotoxin. However, injection of SEB into the fourth ventricle produced no vomition, suggesting that SEB in the spinal fluid did not act directly on the area postrema as a chemical mediator. Although these findings indicated that the mechanism of oral SEB-induced vomition was based on intact neural emesis reflexes, they offered no explanation as to why vagotomy alone failed to suppress emesis following the iv injection of SEB. The present studies do not define any anatomical
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site at which circulating SEB can stimulate an emetic response; they do, however, sug-

gest that initiation of vomition by orally administered SEB is within the gut.

Since emesis can also be induced by iv injection of SEB at a smaller dose, it is possible that SEB administered iv may circulate to neural receptors in the gastrointestinal tract to stimulate vomition. This assumption is supported by the fact that complete abdomi-
nal deafferentation abolished emesis in mon-
keys following either oral or iv challenge of SEB (7). Since radioactively labeled SEB

has been shown to accumulate in large quan-
tities in both kidney and liver after its iv administration (11, 12), it is also possible

that neural receptors in these organs might be stimulated by circulating SEB. No evidence

to support or reject this possibility was ob-
tained in the present study. It is theoretically

possible that after oral administration, mi-
nute quantities of SEB were absorbed into

the portal circulation and removed by hep-
tic cells. This possibility could lead to neural

receptor stimulation within the liver. Based

on available evidence, the likelihood of such

an event does not seem great; to be com-

patible with data of the present cross-circula-
tion study, the liver would have to be capable

of complete removal of SEB during a single

passage of the portal vein blood. Further,

Morris et al. demonstrated that there was no

significant difference in SEB distribution

after peripheral or portal venous routes of

administration (12). The data of Morris et al.

(12) were obtained with a large single injec-
tion of 151-tagged SEB. To rule out com-
pletely the possibility of hepatic accumula-
tion of SEB after oral administration, studies

using a more stable SEB tag or highly sensi-
tive analytical techniques for SEB assay will

be required.

When induced by other than psychic stimuli, vomition is generally thought to require either a centrally acting humoral stimulation of the CTZ, or a stimulation of unidentified neural receptors in the gastro-

intestinal tract with impulses traveling along

afferent autonomic fibers to the vomiting center. Orally administered SEB appears to

initiate a neural reflex arising within the gut

that leads to vomiting and intestinal hyper-
motility. The present study provided addi-
tional evidence that vomiting after oral SEB
does not involve any centrally acting hum-
oral stimulation.

Summary. Vomition is the most consistent

response to oral SEB challenge in the mon-
key. The technique of cross-circulation
clearly differentiates local neural phenome-
non from humoral mechanisms. Our results

support the theory that SEB-induced vom-
imation follows stimulation of local neural

receptors in the gut. The evidence indicates

no significant amount of enterotoxin ab-

sorption or stimulation of vomition by any

centrally acting humoral mechanism.

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2. Raj, H. D., and Bergdoll, M. S., J. Bacteriol. 98,


4. Schantz, E. J., Roessler, W. G., Wagman, J., Spero,

L., Dunnery, D. A., and Bergdoll, M. S., Bio-
chemistry 4, 1011 (1965).


6. Clark, W. G., Vanderhoof, G. F., and Borison,


7. Sugiyama, H., and Hayama, T., J. Infect. Dis. 115,

330 (1965).

8. Sugiyama, H., Chow, K. L., and Dragstedt, II,


10. Chapple, III, F. E., Crosbie, J. M., and Reisberg,


11. Normann, S. J., Jaeger, R. F., and Johnsey, R. T.,

