Study of Emetic Receptor Sites for Staphylococcal Enterotoxin in Monkeys.** (26855)

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(Introduced by G. M. Dack)
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A medullary emetic center (MEC) which lies in the tractus solitarius and its nucleus and the dorsolateral border of the lateral reticular formation is needed for vomiting with all emetics. This center is neither motor nor sensory, but coordinates the activities which constitute the emetic act. Each type of emetic has its own primary locus of action. The early vomiting following oral administration of copper sulfate results from gastrointestinal irritation(1). The centrally acting emetics such as apomorphine and Hydergine (equimixture of 3 dihydrogenated ergot alkaloids) initiate vomiting by acting at the chemoreceptor trigger zone (CTZ) which lies in, or is immediately adjacent to, the area postrema on the floor of the fourth ventricle (2).

Only cats have been used in the published reports on the site of emetic action of staphylococcal enterotoxin. Acute bilateral vagotomy greatly reduced the incidence of vomiting in cats injected intraperitoneally with crude enterotoxic filtrates, indicating peripheral sensory receptors in the viscera which transmit through the vagi(3). A review of the data resulted in an emphasis on a possible forebrain receptor site(1). Chronic ablation of the CTZ did not prevent emesis following intravenous injection of a highly purified staphylococcal enterotoxin, but chronic midbrain transection resulted in refractory animals(4).

Monkeys may differ from cats as to the receptor sites for emesis. Rhesus and cynomologus monkeys do not vomit in response to the emetics known to act on the CTZ of dogs and cats(5). Nevertheless, either vagotomy or CTZ ablation protects monkeys against the early emesis following a single total body X-irradiation of 1200 r(6), while CTZ destruction in cats does not confer protection against radiation emesis(7).

Two other observations suggested the present study on the vomiting response to staphylococcal enterotoxin of monkeys subjected to area postrema ablation or vagotomy. A higher incidence of vomiting occurs in monkeys being challenged with enterotoxin when the animals are administered a non-emetic dose of dihydroergotamine methanesulfonate (8), a drug closely related to Hydergine, which is a CTZ emetic in dogs(2). Perphenazine is a potent anti-emetic in dogs against apomorphine and Hydergine induced vomiting; the antiemesis may be due to the competition with the emetics for the receptor sites of the CTZ(9). This drug effectively inhibits enterotoxin evoked emesis in monkeys in dosages not significantly depressing the medullary emetic center(10).

Materials and methods. The sensitivity of *Macaca mulatta* to the emetic stimuli of 2 antigenically different enterotoxins (partially purified and lyophilized enterotoxins prepared from the 196E and S-6 strains of staphylococci*) and copper sulfate was determined preoperatively by feedings of the emetics in 50 ml of H_2O at weekly intervals. Only those monkeys vomiting within 1 hr with 320 mg $CuSO_4 \cdot 5H_2O$ and within 3 hr with 100 μg S-6 and 750 μg 196E enterotoxins were used. When indicated by the latency, additional feedings with ½ the previous dosage of copper sulfate were carried out to determine more precisely the minimal emetic dose. No animal vomited to 80 mg of this salt. The postoperative vomiting response to the 2 types of emetics was determined after a convalescent period of 2-4 weeks.

Monkeys vary considerably in their origi-

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nal susceptibility and develop resistance to enterotoxin. The post-operative challenging doses were estimated from previous experience in this laboratory on the increase of tolerance to enterotoxin and are approximations of the number of minimal emetic doses for a normal monkey. If an animal vomits 1 or 2 times at initial feeding of a given dosage of enterotoxin, it usually vomits when fed 2× the original dose at the next feeding. If it vomits 3-4 times, the minimal emetic dose at the second challenge is about the same as the first feeding; if 5 or more times, one-half of the initial dose will usually cause vomiting at the next feeding. A single feeding of a small dose of one serological type of enterotoxin does not significantly increase the resistance to the other enterotoxin, but a single large dose was assumed to increase the tolerance to the other enterotoxin 2-fold. The general validity of these assumptions in calculating the number of minimal emetic doses is indicated by monkeys which vomited in response to one enterotoxin but not to the other. In these instances emesis always occurred following challenge with the larger of the calculated doses.

CTZ ablation was accomplished by the method used on dogs(11), the bilateral lesions centering on the area postrema being made by suction. Bilateral vagotomy was performed under nembutal anesthesia. A midline incision was made from the xiphoid to the umbilicus and the esophageal hiatus widened. The esophagus was mobilized and the main trunks of the vagus transected as high as possible. The serosa around the esophagus was stripped and cut circularly to interrupt nerve fibers that may have been intact. Pyloroplasty by the method of Heineke-Mikulicz(12) was done to facilitate the emptying by the vagotomized stomach.

The animals were sacrificed after completion of the post-operative feeding tests. The vagotomized animals were dissected to ascertain grossly the completeness of vagotomy. The lesions in the brain stem were localized by a study of serial histological sections stained by Masson trichrome method.

Results. Monkeys with the area postrema destroyed are highly tolerant of the emetic stimulus of staphylococcus enterotoxin, this resistance being effective against both serological types of the toxin (Table I).

TABLE I. Vomiting Response to Orally Administered Staphylococcal Enterotoxin of Area Postrema Ablated Rhesus Monkeys.

Monkey No.	Enterotoxin dosage and response*
498	Resistant to 20 MED 196E and 20 MED S-6
550	" " 20 " " " 30 " S-6
628	" " 35 " " but not to 60 MED S-6
627	" " 25 " " and 50 MED S-6
787	" " 70 " " " 100 " "
791	" " 90 " " " 120 " "
795	" " 100 " " " 100 " "

* Challenging dose in numbers of minimal emetic doses (MED) if animal had not been surgically treated. Estimated from preoperative responses and corrected for avg increase in resistance resulting from previous enterotoxin feedings.

Area postrema ablation increased the tolerance of $CuSO_4$ to some degree; 2-3-fold greater dosage than preoperatively was usually required to obtain vomiting within the same latency. These results indicate that the MEC is still functional. In none of the animals was there histological evidence that the experimental lesion extended into the region of the vomiting center.

Some interference with the dorsal sensory nucleus of the vagus was found in some of the animals. However, there was no correlation between this extension of the lesion and resistance to staphylococcal enterotoxin. Fig. 1 shows lesions produced in 4 of the animals listed in Table I.

Monkey 628 is especially informative. There was negligible damage to the sensory nucleus of the vagus, but the outline of the area postrema on one side was intact. The resistance of this animal to 35 minimal doses, but not to 60, correlates well with the altered histology of the remaining area postrema. The area postrema must, therefore, be an important site for enterotoxin induced emesis.

Monkeys subjected to vagotomy showed increased tolerance of the emetic stimulus of both serological types of staphylococcal enterotoxin (Table II). Complete protection was not obtained in all animals, but other animals did not vomit in response to the feed-

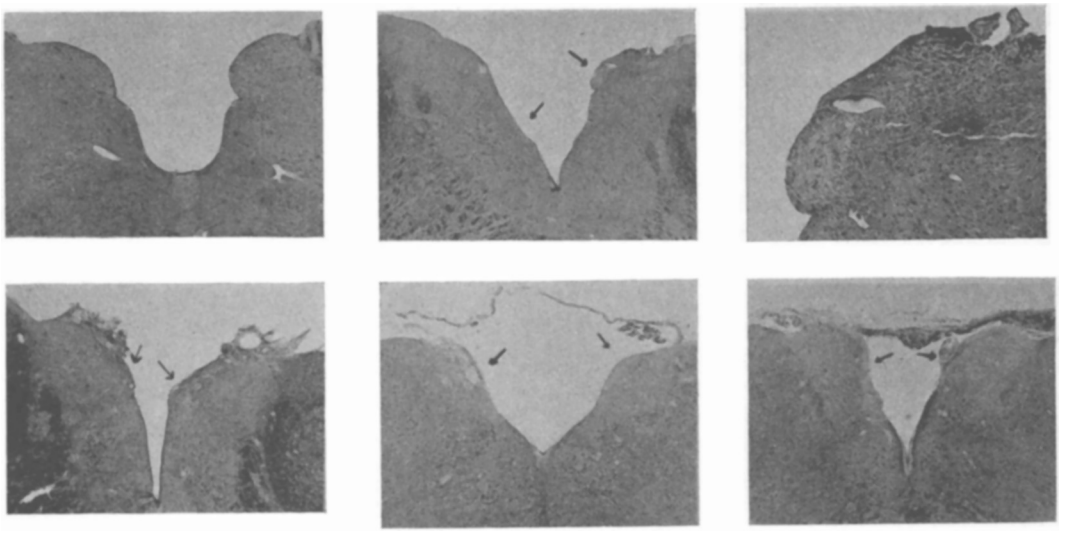

FIG. 1. Sections of medulla at level of area postrema. Left to right, top row: normal animal, monkey # 628, and enlargement of area postrema on right side of #628, showing extensive deterioration of normal structure. Bottom row: monkey #627, 787, and 795. See text for feeding results.

ing of up to 100 minimal emetic doses. The resistance to orally administered copper sulfate was raised so that an increase in dosage to about 2 times the preoperative level was required to obtain emesis or the latency with the equivalent dose was 2 or 3 times longer.

Discussion. Surgical destruction of the area postrema of rhesus monkeys resulted in complete refractoriness of the monkeys to feeding of staphylococcal enterotoxin. This induced resistance was effective against both antigenic types of enterotoxin used. The responsiveness of these monkeys to orally administered copper sulfate and the study of the brain section showed that the vomiting center and motor components of the vomiting reflex were functional.

Vagotomy at the level of the diaphragm gave complete resistance in some monkeys but not in others. This may be due to incomplete vagotomy, although no macroscopically intact fiber was evident. The partial protection in monkeys 629 and 634 and the protection against the very high challenging doses in the last 3 animals of Table II would favor this explanation. These results with enterotoxin would then parallel those obtained in the refractoriness of monkeys to X-irradiation induced emesis following either vagotomy or CTZ ablation(6).

The proximity of the dorsal sensory nucleus of the vagus to the area postrema and the similarity of the protection achieved by area postrema destruction or vagotomy make difficult the proof for existence of 2 emetic receptor sites for enterotoxin in the monkey. The histological evidence indicates that CTZ ablation without significant encroachment of the lesion into the dorsal sensory nucleus confers effective protection against enterotoxin induced vomiting. X-irradiation-induced emesis was not eliminated in a monkey with severe damage to the dorsal sensory nucleus without appreciable damage to the area postrema(6). However, vagal element or pre-

TABLE II. Vomiting Response to Orally Administered Staphylococcal Enterotoxin of Chronically Vagotomized Monkeys.

Monkey No.	Enterotoxin dosage and response*
577	Resistant to 20 MED 196E and 20 MED S-6
629	" " 15 " " but not to 40 MED S-6
630	" " 20 " S-6 but not to 30 MED 196E
634	" " 25 " 196E and 40 MED S-6
794	" " 50 " " " 100 " "
796	" " 80 " " " 100 " "
865	" " 70 " " " 70 " "

* See footnote of Table I.

reticular structures necessary for vomiting following X-irradiation or oral administration of enterotoxin may traverse the area postrema of the monkey. On the other hand, if complete protection results from complete vagotomy at the diaphragm level, then the termination near the ala cinerea of the thoraco-, lumbo-, and sacro-bulbar tracts(13) would not be important in enterotoxin induced vomiting in rhesus monkeys.

However, the incomplete protection in some of the vagotomized monkeys may be significant. One of 8 cats with bilateral vagotomy performed supradiaphragmatically at a level where the recurrent laryngeal nerves are also cut still vomited after intraperitoneal injection of enterotoxic filtrate(2). If the incomplete protection of monkeys is not due to partial vagotomy, the importance of the integrity of the CTZ for the vomiting stimulated by enterotoxin is enhanced. In either case, monkeys would differ from cats, since CTZ-destroyed cats still vomited to intravenous enterotoxin(4). Whether the parenteral injections of enterotoxin into cats as compared to the intragastric route used in the monkeys accounts for the difference is unknown. The ineffectiveness of CTZ ablation in cats as contrasted to the protection in dogs against the emesis resulting from X-irradiation(7) and nitrogen mustard(14) further suggests species differences. A forebrain site for emesis due to enterotoxin as demonstrated in cats(4) would seem to be lacking in monkeys, unless it serves a facilitatory function.

Summary. Bilateral destruction of the area postrema on the floor of the fourth ventricle (chemoreceptor trigger zone) makes rhesus monkeys completely refractory to the emetic action of staphylococcal enterotoxin. The surgically induced resistance is effective against the 2 antigenic types of enterotoxin used. Vagotomy at the level of the diaphragm gave protection in all animals, but in some protection was not complete. The partial protection against enterotoxin-induced emesis in some of the vagotomized monkeys may be due to incompleteness of the vagotomy. If this interpretation of the results is correct, a comomn basis of emesis prevention following staphylococcal enterotoxin feedings may account for the similarity of vomiting response of the area postrema ablated and vagotomized animals. A species difference between cats and rhesus monkeys in the importance of the area postrema region for enterotoxin stimulated vomiting is indicated.

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