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Perphenazine and Reserpine as Antiemetics for Staphylococcal Enterotoxin.* (25447)

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Vomiting is one of the most characteristic reactions in staphylococcal food poisoning. The only laboratory procedure now available for detection of the etiological agent, staphylococcal enterotoxin, is the provocation of emesis in monkeys or cats following suitable challenge(1). Recently a number of antiemetics have been reported effective against various types of emetic agents or which control vomiting in various disease states. No specific study seems to have been made on use of these agents as antiemetics against staphylococcal enterotoxin induced emesis. The present communication reports on effectiveness of 2 tranquilizing drugs in inhibiting emesis in monkeys fed enterotoxin. Antiemetics effective in dogs against one or more of the emetics whose site of action is the chemoreceptor trigger zone(2) were tested: chlorpromazine(3,4), perphenazine(5,6), and cyclizine lactate(7). Reserpine was included, although reports on its ability to protect against apomorphine induced vomiting in dogs are contradictory(7,8). Non-specific protection by depression of the medullary reticular emetic center was evaluated by determining change in sensitivity of monkeys to orally administered copper sulfate which evokes vomiting by peripheral action on the gastro-intestinal tract(9).

Materials and methods. Monkey feeding tests were essentially that previously described(10). *Macaca mulatta* were selected into groups of approximately equal sensitivities to enterotoxin. The paired groups whose response to the toxin was to be compared were alternated, wherever possible, as control and drug treated groups to compensate for variations in reactivities to enterotoxin. Complete control of this variable was impossible due to development of tolerance to the emetic. Each drug was tested in different sets of animals. Enterotoxins were partially purified preparations derived from S-6 and 196E strains of *Staphylococcus* and are antigenically different. Dosage was increased with each feeding of same animals to overcome development of immunity. For intravenous challenge an S-6 enterotoxin of high purity (90% purity) was used. Monkeys were injected intramuscularly with perphenazine (Trilafon) immediately before and with chlorpromazine (Thorazine) and cyclizine lactate (Marezine) 30 min before oral administration of enterotoxin. Since these animals seldom vomit within 1 hr of feeding enterotoxin, seda-

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tion was established during the latent period. Reserpine (Serpasil) was injected intravenously about 18 hr preceding challenge with toxin. Vomiting in response to oral copper sulfate was used as a measure of depression of the vomiting center by the antiemetics. Fasted monkeys were fed 50 ml of $CuSO_4 \cdot 5H_2O$ solutions at 2-fold increments beginning at 80 mg and animals observed for vomiting for 120 min.. None of 8 monkeys vomited to 80, about 60% to 160, and about 85% to 320 mg. The latter amount was used as threshold dose; animals not responding within 60 min at this level in control feedings were not used. The 320 mg threshold dose was, therefore, 2× the actual minimum dosage in about 60% of monkeys. This may not be too different with animals challenged with enterotoxin since the latency and number of vomiting episodes indicate that many control animals received more than a single threshold dose. Actual minimal emetic dose of copper sulfate was previously established for each animal in testing possible depression of the vomiting center by perphenazine. Dogs were also tried. Dogs of 5-12 kg body weight were injected intravenously with the highly purified enterotoxin. Animals were used only once and were observed for 5 hr. Perphenazine was injected intramuscularly immediately after intravenous injection of enterotoxin; chlorpromazine was administered intravenously about 30 min preceding challenge with enterotoxin.

Results. Treatment of monkeys with 1 mg/kg body weight of reserpine, intravenously, about 18 hr preceding intragastric challenge with enterotoxin resulted in 68% reduction of vomiting incidence (Table I). The emetic response was more severe in control animals; each reacting animal vomited 2.5 times/enterotoxin feeding, as compared to those in the drug treated group which vomited 1.9 times. The average prodromal interval for the drug treated group was 124 min as compared to the 99 min for controls. Reserpine injection immediately before feeding of enterotoxin had no protective effect. The delayed but long lasting protection against vomiting is in accord with depletion of serotonin (11) and catechol amines(12) from body storage sites by reserpine.

TABLE I. Antiemetic Activity of Reserpine (1 mg/kg, i.v., 18 Hr Previously) against Vomiting Provoked by Orally Administered Staphylococcal Enterotoxin and Copper Sulfate.

Emetic		No. vomiting/No. fed	
Enterotoxin strain	mg/monkey	Control	Drug treated
S-6	.1	3/6	0/6
"	.25	4/6	2/6
196E	1.5	6/6	1/6
"	2.5	4/6	2/6
"	.75	2/6	1/6
Totals		19/30	6/30
$CuSO_4 \cdot 5H_2O$	320	10/10	9/10

Reserpine (1 mg/kg) did not materially affect the incidence of vomiting in animals challenged with threshold level of copper sulfate, although latency was increased from 31 to 50 min. Of the 19 enterotoxin fed animals that vomited in the control group, 15 responded in less than 120 min and all except one vomited within 150 min. Since toxin fed animals are observed for 5 hr, delayed vomiting, if it occurred, would have been observed; thus, it is unlikely that the antiemetic action of reserpine is due to depression of the vomiting center. On the other hand, depression of the vomiting center could partly explain the resistance to enterotoxin following 2.5 mg/kg of reserpine (control 20/42 *vs.* 2/41 drug treated). With this drug dosage only 2/9 monkeys given threshold dose of copper sulfate vomited and these only after a latent period of over 2 hr as compared with the average of 30 min in control feeding.

Perphenazine reduced vomiting incidence in enterotoxin challenged animals to 8% of that in controls (Table II). The minimal effective dose of the antiemetic is about 50 μg/kg since 25 μg/kg did not have any protective effect. Average number of vomiting episodes and latent periods of animals of control and drug treated groups which reacted, were not appreciably different. Perphenazine was effective even when administered after feeding enterotoxin; 50 μg/kg, intravenously, protected against the toxin fed 45 min previously.

Inhibition by perphenazine of vomiting in monkeys fed enterotoxin is not due to depression of the vomiting center. Perphenazine reduced the copper sulfate stimulated emesis by only 22% with an increase in latency from 30

TABLE II. Antiemetic Activity of Perphenazine (50 μg/kg) against Vomiting Provoked by Orally Administered Staphylococcal Enterotoxin and Copper Sulfate.

Emetic		No. vomiting/No. fed		
Enterotoxin strain	mg/monkey	Control	Drug treated	Perphenazine
S-6	2.0	2/6	0/6	Immediate*
"	10.0	3/6	1/6	"
"	.05	4/6	1/6	"
"	.5	4/6	0/6	45 min. after†
196E	5.0	5/6	0/6	Immediate
"	1.5	5/6	0/5	"
"	3.0	2/5	0/6	45 min. after
	Totals	25/41	2/41	
$CuSO_4 \cdot 5H_2O$	160–320	9/9	7/9	60 min. before‡

* Intramusc., immediately before feeding enterotoxin.
† Intrav., 45 min. after feeding enterotoxin.
‡ Intramusc., 60 min. before feeding each animal predetermined threshold dose.

to 39 min. The average prodromal period in animals of control group which vomited after enterotoxin challenge was 129 min (16 of 25 vomiting within 120 min; 21 of 25 within 150 min). Thus, a proportionate increase of latency as with copper sulfate by perphenazine would give an average latency of 168 min; this is well within the 5 hr observation period for enterotoxin fed animals.

The results with chlorpromazine were erratic. In the great majority of experiments 2 mg/kg of drug was used. No protection was evident against the S-6 enterotoxin (14/23 of controls *vs.* 13/23 of chlorpromazine treated). Some antiemetic activity against 196E was obtained (27/50 of controls *vs.* 22/50 of drug treated), but most of the protective evidence was obtained with 2 sets of animals. Seven of 9 monkeys responding to 320 mg $CuSO_4 \cdot 5H_2O$ vomited when fed the same level of copper sulfate 90 min after intramuscular injection of 2 mg/kg of chlorpromazine. Latency was increased from 29 to 54 min.

Cyclizine (10 mg/kg) did not protect monkeys against enterotoxin. One experiment with each enterotoxin preparation showed 5/11 vomiting in control groups and 6/12 in drug treated groups.

The highly purified S-6 enterotoxin preparation provoked emesis in 10/10 dogs at 1 μg/kg body weight and in 8/14 at 0.5 μg/kg following intravenous injection. The initial vomiting usually occurred about 2 hrs after injection. Intragastric administration of 25 times the threshold intravenous dose did not cause vomiting. Intravenous chlorpromazine (2 mg/kg) given to dogs challenged with a threshold dose of enterotoxin (1 μg/kg) resulted in 58% protection (5/12). Intramuscular perphenazine (0.1 mg/kg) gave 50% protection (6/12) in dogs challenged with twice the minimal emetic dose. The chlorpromazine and perphenazine dosages used give 31 and 27% protection respectively, in dogs receiving threshold doses of intragastric copper sulfate(7). Thus, perphenazine seems to confer some protection to dogs challenged with enterotoxin; the chlorpromazine results are less suggestive. The limited supply of purified enterotoxin did not allow sufficient work for statistical analysis.

Discussion. Significant reduction of vomiting incidence in monkeys challenged with staphylococcal enterotoxin is obtained when reserpine or perphenazine is used as an antiemetic. Perphenazine is the more potent, but reserpine may be more effective than indicated by the data. Since many control animals fed for the first time vomited rapidly and repeatedly, these animals probably did not receive as much immunizing stimulus as did the drug treated animals. Thus, in the next feeding, sensitivities of animal groups to enterotoxin would be weighted against obtained good protective results. If previously unused animals had been used for all experiments, reserpine might have given better protection.

The number of emetic doses of enterotoxin against which reserpine or perphenazine are

effective was not established, but the nature of the feeding experiments and the results with dogs would suggest that the effectiveness of perphenazine would be limited to only a few times the threshold emetic doses.

At the dosages used, degree of tranquilization of monkeys was not correlated with antiemetic potency. Chlorpromazine produced the greatest tranquilizing effect yet gave only doubtful protection against the 196E enterotoxin, while perphenazine which resulted in least sedation, was effective against both enterotoxins. Whether the basic mechanisms of perphenazine and reserpine protection are the same, or whether the reserpine effect is specifically related to its ability to deplete the biogenic amines from the body binding sites remains to be investigated.

Whether either of the drugs giving protection in these experiments has clinical use remains to be determined. The delayed pharmacological effects of reserpine probably make it unsuitable, but perphenazine was effective even when administered 45 min after enterotoxin. Use of perphenazine in staphylococcal food poisoning outbreaks seems limited, since the majority of cases develop within a short period and recovery begins spontaneously after a few hours of acute symptoms. However, perphenazine may be of value in controlling vomiting caused by staphylococcal enterotoxin in pseudomembranous enterocolitis(13).

The basis of the antiemetic effect in dogs of drugs such as chlorpromazine and perphenazine against the chemoreceptor trigger zone (CTZ) acting emetics is not clear. It has been suggested that at dosage levels which only moderately depress the medullary emetic center the antiemetic activity may be the result of the competition of the antiemetic and the emetic for the receptors in the trigger zone (3,6). On the basis of this interpretation, the results reported here suggest the possibility that staphylococcal enterotoxin may be a CTZ acting emetic.

But monkeys are refractory to emetics such as Hydergine (equimixture of 3 dihydrogenated ergot alkaloids) known to act on CTZ of dogs and cats. This would indicate that CTZ for emesis in the monkey is non-functional from the standpoint of drug action(14). On the other hand, vomiting incidence increases significantly when subemetic dose of dihydroergotamine (related to Hydergine) is administered to monkeys challenged with enterotoxin(10). Moreover, the area postrema of monkeys, which is presumably the CTZ, plays some role in vomiting; its ablation results in elimination of early vomiting response which normally follows 1200 r x-irradiation (14,15).

The above consideration suggests the need for determining the sensitivity of monkeys to enterotoxin following bilateral destruction of the area postrema. Preliminary experiments indicate that the area postrema of monkeys is an important site for emetic action of staphylococcal enterotoxin (to be published).

Summary. Perphenazine and reserpine have significant antiemetic activity against staphylococcal enterotoxin induced emesis in monkeys; chlorpromazine and cyclizine lactate have little or no protective activity. Perphenazine gave the best protection and was effective at 50 μg/kg, intravenously; it inhibited vomiting even when administered 45 min after the enterotoxin had been fed. Pretreatment of monkeys was necessary for protection with reserpine. The effective drug levels did not greatly depress the medullary vomiting center. Perphenazine had some protective effect in dogs being challenged with intravenous injection of enterotoxin.

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A-Norprogesterone, an Androgen Antagonist. (25448)

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Typical growth responses of accessory sex organs of the rat or the chick comb to androgen have been inhibited by several substances. These include progesterone(1,2), estrone(2, 3), estradiol(2,4), dehydroepiandrosterone (5), methylcholanthrene(6), 2-acetyl-7-oxo-1, 2,3,4,4a,4b,5,6,7,9,10,10a - dodecahydrophenanthrene(7,8), and 20-methyl-Δ^5-pregnene-3β,20-diol(9); all being steroids or steroid-like in configuration. Structural resemblance of such compounds to natural steroids could form a basis for hormonal antagonism. Recently a series of A-norsteroids was synthesized by Weisenborn and Applegate(10). We studied these steroidal homologs for hormonal and anti-hormonal activities. This report is concerned with A-norprogesterone, which possesses anti-androgenic properties.

Material and methods. Androgenic and anti-androgenic activities. Twenty-three day old male rats (Sprague Dawley strain) weighing approximately 50-60 g were castrated and divided into groups of 4 or 6 animals. Test compounds were dissolved or suspended in sesame oil. A-norprogesterone alone and in combination with testosterone propionate was administered subcutaneously once daily for 7 days starting on day of castration. Animals were sacrificed on day following last injection, and the ventral prostate, seminal vesicles, coagulating gland, levator ani muscle, adrenals, thymus and thyroids were removed and weighed. Intact immature male rats weighing approximately 100 g were subjected to experimental technics similar to those described for the castrated animal. In addition the pituitary, testis, spleen, kidney, heart and liver were removed and weighed. Two day old White Leghorn cockerels, in groups of 24, were injected subcutaneously with a single .5 mg dose of testosterone enanthate (Delatestryl) in sesame oil as in the method described by Dorfman(8). A-norprogesterone was dissolved in sesame oil in several concentrations, and .05 ml was applied on the comb once daily for 7 days starting on day of androgen treatment. The chicks were sacrificed on day following last inunction and the comb and body weights were recorded. *Uterotrophic and anti-estrogenic activities.* Immature female mice of the Swiss Albino strain weighing approximately 7 g were divided into groups of 5 animals. A-norprogesterone dissolved in sesame oil was administered subcutaneously once daily for 3 days, either alone or in combination with a daily dose of .033 μg of estradiol benzoate. Animals were sacrificed on day following last injection. The quantity of uterine luminal fluid was estimated. This fluid was then expressed and the uteri were weighed. *Progestational and anti-progestational activity.* Immature female rabbits in groups of 4 animals were employed in a modification of the McPhail method(11). The animals were given 3 intramuscular injections of 5 μg of estradiol benzoate on days 1, 3, and 5. From sixth day on, they received 5 daily intramuscular injections of progesterone, A-norprogesterone or both compounds. Animals were sacrificed on day following last injection