GI Pharmacology of Polyethyleneimine II: Motor Activity in Anesthetized Dogs

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Abstract □ The effects of orally and intravenously administered doses of polyethyleneimine were observed in 18 chloralose–urethan-anesthetized dogs. Polyethyleneimine produced an initial augmentation of rhythmic segmenting gastric antral contractions, a copious flow of gastric mucus, increased segmenting and propulsive activities of the small and large intestines, and occasional micturition and defecation. The gastric corpus and fundic regions became relaxed and enlarged. These events were associated with the prompt appearance of retching. The retching response to oral administration could only be abolished by bilateral vagotomy or bilateral sympathectomy. The skeletal muscle component of retching was blocked by tubocurarine. Intravenous administration of chlorpromazine blocked the retching response and gastric corporal atonia to either intravenous or oral doses of polyethyleneimine. Either oral or intravenous administration of polyethyleneimine produced no detectable changes in the lead II ECG but was associated with marked transient reductions in both mean and pulsatile arterial blood pressures. These depressor effects showed clear tachyphylaxis. In all cases where GI effects were noted, respiration was augmented and erratic in a manner associated with the retching responses.

Keyphrases □ Polyethyleneimine—GI and cardiopulmonary effects, oral and intravenous administrations compared, dogs □ GI effects—polyethyleneimine, oral and intravenous administrations compared, dogs □ Cardiopulmonary effects—polyethyleneimine, oral and intravenous administrations compared, dogs □ Polyamines—polyethyleneimine, GI and cardiopulmonary effects, oral and intravenous administrations compared, dogs

The naturally occurring polyamines putrescine, spermidine, and spermine have attracted considerable attention with regard to their biological functions (1). However, there have been no studies to determine the effects of either the naturally occurring or synthetic polyethyleneimines on GI functions.

Recent experimental data indicated that a high molecular weight polyethyleneimine polymer produced delayed gastric emptying when orally administered to fasted rats (2). It might be possible to utilize this effect to prolong orally administered drug action or as an appetite suppressant (3). The objective of the present experiments was to determine those GI secreto-motor effects associated with the oral administration of the polyamine as well as any cardiopulmonary effects.

EXPERIMENTAL

Methods—Eighteen mongrel dogs of both sexes were used. Following an overnight fast with water *ad libitum*, all animals were anesthetized with a mixture of 5% α-chloralose dissolved in polyethylene glycol 200 and 50% urethan in 0.9% saline. Each animal was individually titrated to a surgical plane of anesthesia using this mixture. Anesthesia was maintained by individual subsequent administration of the same mixture.

In all cases, the following procedures were used. A tracheal cannula was inserted to ensure patency of the airway and to permit monitoring of respiratory frequency. The femoral artery was cannulated for the purpose of recording blood pressure. The ipsilateral vein was cannulated to permit either bolus or infusion administration of drugs. The standard lead II ECG was continuously monitored throughout each experiment.

The following specific procedures were employed depending on the design of the particular experiment. Motor activities of the small and large intestines were monitored by surgically placed intraluminal water-filled balloons. Gastric motor activity was monitored by oral intubation with a water-filled balloon. Gastric secretion was collected by continuous drainage from a Thomas cannula surgically placed into the corpus of the stomach, as previously described (4).

Biliary and pancreatic flows were monitored by drop counters connected *via* polyethylene cannulas to the extramural pancreatic and common bile ducts. Portal venous pressure was monitored *via* a cannula placed in a splenic vein. The vagus nerves were exposed cervically, and either the splanchnic nerves or the sympathetic chains were exposed at the T-9–T-10 level.

In some experiments, the vagi were sectioned and the peripheral ends were electrically stimulated using bipolar electrodes. Square-wave stimuli ranged from 12 to 30 v at 30 Hz with a 2-msec duration. Monitored parameters were recorded on a strip-chart recorder. Increases in GI motor activities were directly visualized and photographed so that the resulting pressure wave forms could be interpreted in terms of the type of activity represented.

Materials—The test agent employed was the high molecular weight (40,000–60,000) branched polyethyleneimine[1] (I) in saline, 10–200 mg/kg iv depending on the experiment. Tubocurarine (1.0 mg/kg) and 2.0 mg of chlorpromazine hydrochloride[2]/kg were also used.

RESULTS AND DISCUSSION

In all 18 dogs, the intravenous administration of I was immediately associated with a marked reduction in arterial blood pressure (Fig. 1). This response showed marked tachyphylaxis to an extent that it had disappeared completely by the beginning of the third trial in each dog (Fig. 1). Other than reflex changes in heart rate, the morphology of the ECG showed no suggestive changes. Respiratory rate was augmented with substantially complete tachyphylaxis by the beginning of the third dose.

Portal venous pressure promptly and consistently rose following intravenous administration of I (Fig. 1). These responses also showed tachyphylaxis (Fig. 1). All doses were consistently associated with transient decreases in extrahepatic bile flow. Pancreatic flow was not influenced by any dose employed. The entire small and large intestines exhibited increased motor activities. Gastric motor activity took the form of an initial augmentation followed by a marked depression within 2–3 min of I administration. In all 18 animals, exaggerated respiratory movements were noted; in some cases, they progressed to violent cyclic retching (Fig. 1), frequently accompanied by micturition and defecation. Fatalities in six dogs occurred at total cumulative intravenous dosages of 25–31 mg/kg.

The described effects also were noted in eight dogs when doses of 200 mg/kg were administered by gastric or duodenal intubation. The only differences were the latencies of the responses, which were uniformly longer (Fig. 2).

The fatal total oral dose in three dogs was estimated by average to be about 1.8 g/kg. The retching observed subsequent to oral administration could be abolished by bilateral cervical vagotomy. This procedure did not abolish the retching responses to intravenous administration.

The first task resulting from the previous observations was to determine the exact changes of gastric motor activity that resulted from I administration. In these four experiments, the intravenous dosage was 1 mg/kg and the intragastric dosage was reduced to 50 mg/kg.

Direct visualization of the stomach following I administration revealed that the gastric antrum, defined as the region below the incisura angularis, exhibited protracted, rhythmic, propulsive contractile activity, which

[1] PEI 600, Dow Chemical Co., Midland, Mich.

[2] Thorazine Injectable, Smith Kline and French Laboratories, Philadelphia, Pa.

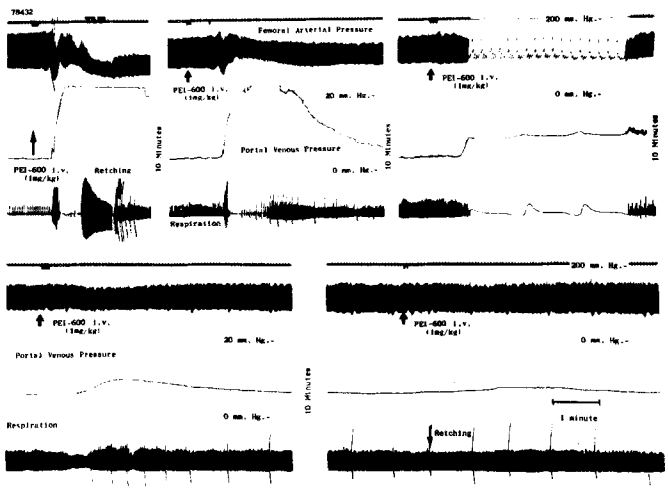

Figure 1—*Polygrams depicting the time course of femoral arterial pressure (top trace), portal venous pressure (center trace), and respiratory movements (bottom trace). The panels are ordered sequentially. This figure shows that tachyphylaxis was noticeably present with respect to all parameters upon the third intravenous administration of I (PEI-600). Tachyphylaxis was essentially complete following the fifth dose. The sharp spikes on the respiration trace show that retching movements were present during the entire period depicted.*

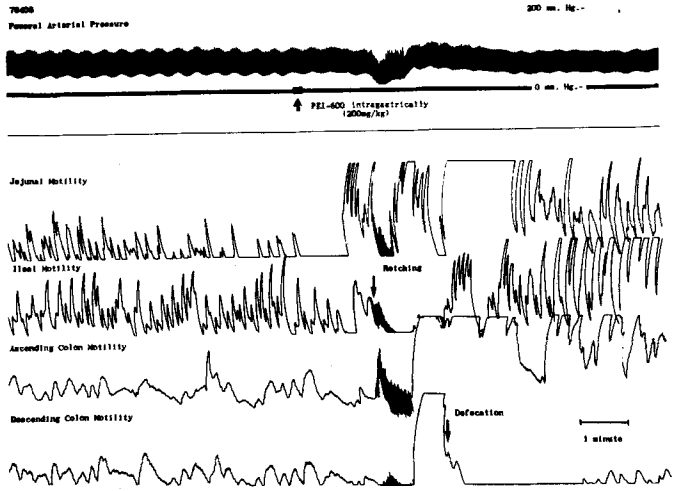

Figure 2—*Polygram depicting the time course of arterial blood pressure and jejunal, ileal, ascending colon, and descending colon motilities following the intragastric administration of 200 mg/kg of I (PEI-600). The latency of the responses was about 1.5 min. Arterial blood pressure was transiently affected. Jejunal, ileal, and ascending colonic movements underwent protracted augmentation. In this particular recording, the abrupt decrease in activity of the descending colon was associated with spontaneous defecation. The blurring of the motility traces was an artifact associated with gross retching movements.*

was coincident with increased propulsive activity of the small intestine. As additional drug was administered, a progressive enlargement of the corpus and fundic regions occurred to an extent that the total volume occupied by the stomach became greatly enlarged. During repeated administrations, exaggerated respiratory movements and retching appeared. Electrical stimulation of the peripheral vagi augmented the propulsive movements of both the gastric antrum and the small intestine.

A gastric pH electrode was introduced orally into the stomachs of three dogs. The gastric pH rose continuously during 2 hr of intravenous I administration. At the end of 2 hr, the gastric pH was 8.0. A single gastric fistula dog was then prepared to determine the effect of intravenous I administration on gastric acid secretion following an overnight fast. The repeated intravenous administration of 1.0 mg/kg markedly increased the amount of gastric mucus produced during the 15-min collection periods. Tachyphylaxis was not noted.

The latter observations suggested that a sympathetic component might be responsible for the motor effects and the secretion of gastric mucus (4). Accordingly, in another series of four dogs, the sympathetic chains were surgically interrupted prior to intravenous I administration. Gastric enlargement was not seen in these animals, but they all had cyclic bouts of retching. One dog retched continuously. Retching was abolished in the four dogs by intravenous tubocurarine administration.

A final experimental series utilized two dogs and was designed to determine whether the prior intravenous administration of 2 mg/kg of chlorpromazine could modify the observed GI effects of oral or intravenous I. Oral dosages ranged from 50 to 200 mg/kg, and intravenous dosages ranged from 1.0 to 5.0 mg/kg. In no case was either retching or gastric enlargement observed.

During the first canine experiments, it was immediately noted that the classic physiological responses (5) included in the description of retching were produced by I. The 1.0-mg/kg iv dose appears to be near the threshold for this response because the responses were variable. One animal retched each time the drug was administered, while another dog retched unpredictably with no apparent relationship between total accumulated dosage and prediction of the response.

The responses themselves were of unpredictable duration. One dog might retch continuously during the entire experiment following the initial dosage while another retched for a short time following a dosage that happened to produce the response. At the higher intragastric and intravenous dosages, retching was more predictable and of longer duration.

An analysis of the retching responses indicates the following. The skeletal muscle component was blocked by curare, indicating that the classical voluntary skeletal muscle component of the response was present. The gastric response consisted of increased secretion of mucus, relaxation of the corpus and fundus, and marked antral contractions. Interruption of the sympathetic chains and sectioning of the splanchnic nerves abolished relaxation and increased mucus secretion. This response indicates that a characteristic sympathetic component of the retch reflex was present.

The immediate sympathetic and parasympathetic responses to gastric or duodenal administration could be blocked by bilateral vagotomy (Fig. 3), indicating that the reflex can be subserved by vagal afferent pathways (6). Furthermore, the observation that retching produced by intravenous administration of I could be blocked by prior treatment with chlorpromazine (7) indicates that the agent or one or more of its metabolites can directly stimulate the chemoreceptor trigger zone. Thus, the emetic action of I (emesis did occur in the nonfasted dog) appears to be similar to that of ipecac (7).

The most pronounced effects of I upon systems other than the GI are upon the circulation. The marked decreases in arterial pressure were

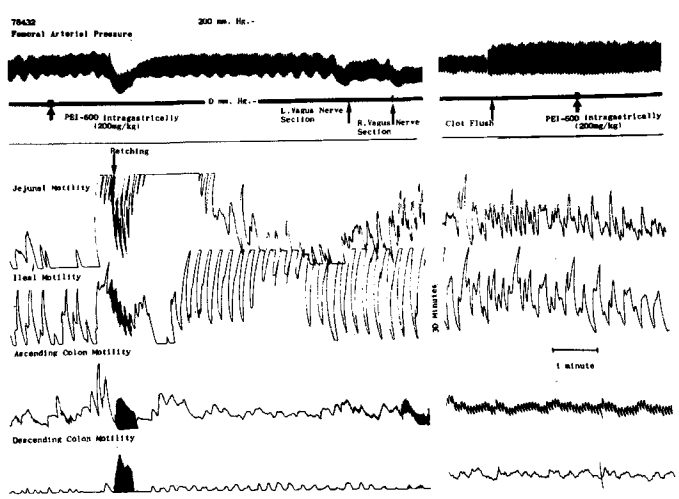

Figure 3—*Polygram depicting the time course of femoral arterial pressure and motor activities of the jejunum, ileum, and ascending and descending colons of a dog subsequent to the intragastric administrations of 200 mg/kg of I (PEI-600) prior to and following bilateral cervical vagotomy. The pressure artifacts produced by gross retching movements were absent following vagotomy.*

associated with reductions in pulse pressures and rises in portal venous pressures. These changes appeared to exhibit rapid and complete tachyphylaxis. The lack of apparent changes in the morphology of the lead II ECG indicates that the effects of the agent did not produce arrhythmias or ectopic foci of excitation. A determination of whether minor local changes in excitation occurred secondary to such effects as regional changes in myocardial circulation cannot be inferred from these limited data.

Experiments with lower dosages or apparently ineffective 1.0-mg/kg iv and 50-mg/kg intragastric dosages indicated that the individual components of the retch response (increased frequency of respiration, gastric mucus secretion, antral contractions, and corporal-fundal atonia) were invariably present but of such small intensity that the composite response was decidedly less than that constituting frank retching.

A similar set of sequelae is also present in humans who report feelings of nausea but who do not visibly retch. It is impossible to make this judgment with dogs, particularly anesthetized dogs. But these observations furnish an adequate physiological basis to suspect that the gastric or intravenous administration of dosages that are insufficient to produce frank retching might be accompanied by sensations of nausea.

It is concluded that the high molecular weight, branched polyethyleneimine, whether administered to dogs by gastric intubation or intravenously, produces an emetic response similar to ipecac in that it can act reflexly or by direct stimulation of the chemoreceptor trigger zone.

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New Compounds: Potential Antituberculous Agents I: Alkylaryl 4-Arylformamidinothiosemicarbazones

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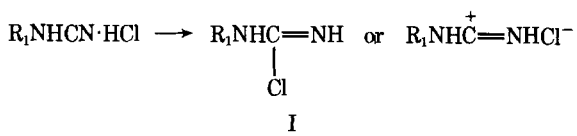
Abstract □ A series of alkylaryl 4-arylformamidinothiosemicarbazones was synthesized for evaluation as antituberculous agents. The synthesis was effected by the condensation of different arylcyanamides with various thiosemicarbazones. The required intermediates also are described.

Keyphrases □ Thiosemicarbazones, various—synthesized for evaluation as antituberculous agents □ Antituberculous agents, potential—various thiosemicarbazones synthesized

Interest in the synthesis and biological evaluation of thiosemicarbazone derivatives was renewed by the fact that tibione (*p*-acetylaminobenzaldehyde thiosemicarbazone) (1–3) possesses antituberculous activity. Many reports (4–13) discussed the change of activity due to variations in the structure of the parent compound.

DISCUSSION

The present report deals with the synthesis of acetophenone 4-arylformamidinothiosemicarbazones (V*a*–V*m*, Table I) and 1-acetonaphthone 4-arylformamidinothiosemicarbazones (VI*a*–VI*m*, Table II) by the condensation of corresponding arylcyanamide hydrochlorides (I, Scheme I) with the appropriate thiosemicarbazones (II or III, Scheme II). Although the monosulfides (IV) could not be isolated, this intermediate stage was confirmed previously in analogous reactions (14–17).



Scheme I

Precursors (I) were obtained by the desulfurization of related thioureas by lead hydroxide and conversion to related hydrochlorides (18). The synthesized thiosemicarbazones (Tables I and II) are white crystalline compounds soluble in polar solvents. Since these compounds could not be crystallized without decomposition, purification was achieved by repeated washings with several solvents. It was hoped that these potential antituberculous agents would have low toxicity to normal cells and have a good chemotherapeutic index[1].

EXPERIMENTAL[2]

Phenylcyanamide (18)—A mixture of phenylthiourea (15.2 g, 0.1 mole) dissolved in sodium hydroxide (3 g in 200 ml of water) and freshly prepared lead hydroxide (24.12 g, 0.1 mole) was heated on a water bath for 4 hr. It then was cooled and filtered, and the filtrate was acidified with acetic acid. The fluffy precipitate of phenylcyanamide was dissolved in ether and dried by adding anhydrous sodium sulfate. The hydrochloride of phenylcyanamide was prepared by passing dry hydrogen chloride gas through the ethereal solution.

By adopting a similar procedure, the following cyanamides were prepared: 2-methylphenyl, 3-methylphenyl, 4-methylphenyl, 2-methoxyphenyl, 4-methoxyphenyl, 4-ethoxyphenyl, 2-chlorophenyl, 3-chlorophenyl, 4-chlorophenyl, 4-bromophenyl, 2,5-dimethylphenyl, and 1-naphthyl.

Acetophenone Thiosemicarbazone (II) (12, 13)—Acetophenone (12.0 g, 0.1 mole) was dissolved in ethanol (50%, 100 ml) and acetic acid (2.0 ml), and thiosemicarbazide (9.10 g, 0.1 mole) was added. The solution was warmed with occasional swirling until the thiosemicarbazide dissolved, and then the solution was refluxed for 1 hr. After cooling, crys-

[1] These compounds have been submitted for testing, and the results will be reported elsewhere.

[2] Melting points were determined with a Kofler hot-stage apparatus and are uncorrected.